



**A.S.D. Government Degree College for Women**  
**An Autonomous Institution**

Jagannaickpur, Kakinada, Andhra Pradesh-533002  
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**INTERNAL QUALITY ASSURANCE CELL**

**3.4.3 Number of research papers published per teacher in the Journals as notified on UGC CARE list during the last five years**

శ్రీ విద్యా ప్రవర్ధనాం

**NUMBER OF RESEARCH PAPERS  
PUBLISHED  
(2018-2019)**

### 3.4.3 Number of research papers published per teacher in 2018-2019

S.No	Title of paper	Name of the author/s	Department of the teacher	Name of journal
1	Upper unidomination Number and upper total Unidomination Number of a 3-Regularized Wheel	Dr V.Ananthalakshmi	Mathematics	International journal of Computer Applications
2	Iron-Catalyzed Aerobic oxidative cleavage and Construction of C-N bonds: A Facile method for Synthesis of 2,4,6Trisubstitued Pyridines	D Chenna Rao	Chemistry	Asian Journal of Organic Chemistry (AJOC)
3	The Optimum solution of degenerate Transportation problem	M.Madhavi	Mathematics	IOSR Journal of Engineering
4	A New Algorithm for initial basic feasible solution of Transportation problem	M.Madhavi	Mathematics	International Journal of Engineering science Invention
5	Upper Unidomination Number of a path	Dr V.Ananthalakshmi	Mathematics	International Journal of Research and Analytical Reviews (IJRAR)
6	Baby Kamble's The prisons we broke : Breaking free :Impacted by Dr.B R.Ambedkar	Dr P.Shanthi	English	International Journal of English Language , Literature in Humanities Indexed
7	Total Unidominating Functions and Total Unidomination Number of a 3-Regularized Wheel	Dr V.Ananthalakshmi	Mathematics	International Journal of Current Engineering and Scientific Research (IJCEsr)
8	Reproductive ecology of carpet weeds species of Molluginaceae	Dr M.Sulakshana	Botany	Advances in pollen spore research
9	Floral biology and pollination of carpet weeds, <i>Glinus lotoides</i> L. and <i>Glinus oppositifolius</i> (L.) Aug. DC. (Molluginaceae)	Dr M.Sulakshana	Botany	Anales de Biologia
10	Pollination ecology of three ecologically valuable carpetweed herbs, <i>Mollugo cerviana</i> , <i>M. nudicaulis</i> and <i>M. pentaphylla</i> (Molluginaceae)	Dr M.Sulakshana	Botany	Journal of threatened taxa

11	Pollination ecology of the species <i>Mollugo cerviana</i> (L.) ser. (Molluginaceae)	Dr M.Sulakshana	Botany	Transylvanian Review of Systematical and Ecological Research
12	Balancing the Pond Environment Through Probiotics For Enchancing Environmental Conditions In A Shrimp Farm to achieve Improved Margins	M.Vasantha Lakshmi	Zoology	Journal of Emerging Technologies and Innovative Research (JETIR)
13	Benefits of Renewable Energy Sources to the Environment	R.Sashikala	Physics	Research Review International Journal of Multidisciplinary
14	Benefits of Renewable Energy Sources to the Environment	K. Venkateswara Rao	Physics	Research Review International Journal of Multidisciplinary
15	Benefits of Renewable Energy Sources to the Environment	G.Sridevi	Physics	Research review international journal of multidisciplinary
16	Parasitic corepods in Carangid fishesd from ManginapudiCoastal Waters Machilipatna, A. p., India	M.Vasantha Lakshmi	Zoology	Journal of Emerging Technologies and Innovative Research (JETIR)

# Upper Unidomination Number and Upper Total Unidomination Number of a 3-Regularized Wheel

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## ABSTRACT

The concept of unidominating function and total unidominating functions are introduced in [5] and [6] respectively. Minimal unidominating function and upper unidomination number are introduced in [7] and minimal total unidominating function and upper total unidomination number are introduced in [8]. The unidomination number and total unidomination number of a 3-regularized wheel are obtained in [9], [10]. In this paper the authors study the minimal unidominating functions, minimal total unidominating functions of a 3-regularized wheel and determined its upper unidomination number, upper total unidomination number. Further the number of minimal unidominating functions, minimal total unidominating functions with maximum weight are found.

## Keywords

Wheel, 3-regularized wheel, minimal unidominating function, minimal total unidominating function, upper unidomination number, upper total unidomination number.

## 1. INTRODUCTION

Graph Theory plays an important role in several areas of computer science such as artificial intelligence, formal languages, computer graphics etc. An important branch of graph theory is domination and its properties have been widely studied by T.W.Haynes and others in [1,2]. Hedetniemi [3] introduced the concept of dominating function which has many applications. Zelinka, B [4] has given some remarks on domination in cubic graphs. In [5], [6] the concepts of unidominating function and total unidominating function are introduced by the authors. The concept of minimal unidominating function and upper unidomination number are introduced by the authors in [7] and minimal total unidominating function and upper total unidomination are introduced in [8]. In [9], [10] the unidomination number and total unidomination number of a 3-regularized wheel are obtained. In this paper the upper unidomination number and upper total unidomination number of a 3-Regularized Wheel are found. Further the number of minimal unidominating functions and minimal total unidominating functions with maximum weight for this graph are obtained and the results obtained are illustrated.

## 2. DEFINITIONS

In this section the concepts of unidominating function, minimal unidominating function, upper unidomination number, total unidominating function, minimal total unidominating function, and upper total unidomination number are defined as follows.

*Definition 2.1:* Let  $G(V, E)$  be a graph. A function

$f: V \rightarrow \{0,1\}$  is said to be an unidominating function

$$\text{if } \sum_{u \in N[v]} f(u) \geq 1 \quad \forall v \in V \text{ and } f(v) = 1,$$

$$\sum_{u \in N[v]} f(u) = 1 \quad \forall v \in V \text{ and } f(v) = 0.$$

where  $N[v]$  is the closed neighbourhood of the vertex  $v$ .

*Definition 2.2:* Let  $G(V, E)$  be a graph. An unidominating function  $f: V \rightarrow \{0,1\}$  is called a minimal unidominating function if for all  $g < f$ ,  $g$  is not an unidominating function.

*Definition 2.3:* The upper unidomination number of a graph  $G(V, E)$  is defined as  $\max \{f(V) / f \text{ is a minimal unidominating function}\}$ ,

$$\text{where } f(V) = \sum_{u \in V} f(u).$$

The upper unidomination number of  $G$  is denoted by  $\Gamma_u(G)$ .

*Definition 2.4:* Let  $G(V, E)$  be a connected graph. A function  $f: V \rightarrow \{0,1\}$  is said to be a total unidominating function, if

$$\sum_{u \in N(v)} f(u) \geq 1 \quad \forall v \in V \text{ and } f(v) = 1,$$

$$\sum_{u \in N(v)} f(u) = 1 \quad \forall v \in V \text{ and } f(v) = 0,$$

where  $N(v)$  is the open neighbourhood of the vertex  $v$ .

*Definition 2.5:* Let  $G(V, E)$  be a connected graph. A total unidominating function  $f: V \rightarrow \{0,1\}$  is called a minimal total unidominating function if for all  $g < f$ ,  $g$  is not a total unidominating function.

*Definition 2.6:* The upper total unidomination number of a connected graph  $G(V, E)$  is defined as  $\max \{f(V) / f \text{ is a minimal total unidominating function}\}$ .

It is denoted by  $\Gamma_{t_u}(G)$ .

*Definition 2.7:* A 3-regularized wheel is defined as "A graph formed from  $W_{1,n}$  by replacing the center of  $W_{1,n}$  by a cycle  $C_n$  and each of the remaining  $n$  vertices in  $W_{1,n}$  are replaced by cycles  $C_3$ ".

## 3. UPPER UNIDOMINATION NUMBER OF A 3-REGULARIZED WHEEL

In this section the upper unidomination number of a 3-regularised wheel and the number of minimal unidominating functions with maximum weight are obtained.

**Theorem 3.1:** The upper unidomination number of a 3-regularized wheel formed from  $W_{1,n}$  is

$$\begin{cases} \frac{5n}{2} & \text{if } n \text{ is even,} \\ \left\lfloor \frac{5n}{2} \right\rfloor - 1 & \text{if } n \text{ is odd.} \end{cases}$$

**Proof:** Let  $W_{1,n}$  be a wheel and  $C_n$  be the cycle replacing the center of  $W_{1,n}$  and  $C_1^j, C_2^j, \dots, C_3^j$  are the cycles replacing the  $n$  vertices in  $W_{1,n}$  respectively.

Let  $u_1, u_2, \dots, u_n$  be the vertices in  $C_n$ , and  $v_1, v_2, \dots, v_n$  be the vertices in  $C_1^j, C_2^j, \dots, C_3^j$  respectively which are adjacent to  $u_1, u_2, \dots, u_n$  respectively. Let  $w_1, w_2, w_3, w_4, \dots, w_{2n-1}, w_{2n}$  be the remaining vertices in  $C_1^j, C_2^j, \dots, C_3^j$  respectively.

$$\text{Here } d(u_i) = d(v_i) = d(w_{2i}) = d(w_{2i-1}) = 3$$

for  $i = 1, 2, \dots, n$ .

The upper unidomination number of this 3-regularized wheel is found in the following two cases.

**Case I:** Let  $n$  be an even number.

Define a function  $f: V \rightarrow \{0,1\}$  by

$$f(u) = \begin{cases} 1 & \text{when } u = u_i, i = 1, 2, \dots, n, u = v_j, w_{2j-1}, w_{2j} \\ & \text{when } j \text{ is an odd number,} \\ 0 & \text{otherwise.} \end{cases}$$

**Sub case 1:** Let  $i$  be an even number.

$$\text{Then } \sum_{u \in N[u_i]} f(u) = f(u_{i-1}) + f(u_i) + f(u_{i+1}) + f(v_i) = 1 + 1 + 1 + 0 = 3 > 1,$$

$$\sum_{u \in N[v_i]} f(u) = f(u_i) + f(v_i) + f(w_{2i-1}) + f(w_{2i}) = 1 + 0 + 0 + 0 = 1,$$

$$\sum_{u \in N[w_{2i-1}]} f(u) = f(w_{2i-1}) + f(v_i) + f(w_{2i-2}) + f(w_{2i}) = 0 + 0 + 1 + 0 = 1,$$

$$\sum_{u \in N[w_{2i}]} f(u) = f(w_{2i}) + f(v_i) + f(w_{2i-1}) + f(w_{2i+1}) = 0 + 0 + 0 + 1 = 1.$$

**Sub case 2:** Let  $i$  be an odd number.

$$\text{Then } \sum_{u \in N[u_i]} f(u) = f(u_{i-1}) + f(u_i) + f(u_{i+1}) + f(v_i) = 1 + 1 + 1 + 1 = 4 > 1,$$

$$\sum_{u \in N[v_i]} f(u) = f(u_i) + f(v_i) + f(w_{2i-1}) + f(w_{2i}) = 1 + 1 + 1 + 1 = 4 > 1,$$

$$\sum_{u \in N[w_{2i-1}]} f(u) = f(w_{2i-1}) + f(v_i) + f(w_{2i-2}) + f(w_{2i}) = 1 + 1 + 0 + 1 = 3 > 1,$$

$$\sum_{u \in N[w_{2i}]} f(u) = f(w_{2i}) + f(v_i) + f(w_{2i-1}) + f(w_{2i+1}) = 1 + 1 + 1 + 0 = 3 > 1,$$

Therefore  $f$  is a unidominating function.

$$\begin{aligned} \text{Now } f(V) &= \sum_{u \in V} f(u) = \sum_{i=1}^n [f(u_i) + f(v_i)] \\ &+ \sum_{i=1}^{2n} f(w_i) = n + \frac{n}{2} + n = \frac{5n}{2}. \end{aligned}$$

Now we check for the minimality of  $f$ .

Now we define a function  $g$  such that  $g < f$ , and show that  $g$  is not a unidominating function for all possibilities of defining  $g$ .

**Case (i):** Define a function  $g: V \rightarrow \{0,1\}$  by

$$g(u) = f(u) \text{ for all } u \in V, u \neq u_k, u_{k+1} \\ \text{for some } k \in \{1, 2, \dots, n\}$$

$$\text{and } g(u_k) = g(u_{k+1}) = 0.$$

Then obviously  $g < f$ .

**Sub case 1:** Let  $k$  be even.

$$\text{Then } g(v_k) = g(w_{2k-1}) = g(w_{2k}) = 0.$$

$$\begin{aligned} \text{But } \sum_{u \in N[v_k]} g(u) &= g(u_k) + g(v_k) + g(w_{2k-1}) + g(w_{2k}) \\ &= 0 + 0 + 0 + 0 = 0 \neq 1. \end{aligned}$$

**Sub case 2:** Let  $k$  be odd. Then  $k+1$  is even and hence

$$g(v_{k+1}) = g(w_{2k+1}) = g(w_{2k+2}) = 0.$$

$$\begin{aligned} \text{But } \sum_{u \in N[v_{k+1}]} g(u) &= g(u_{k+1}) + g(v_{k+1}) + g(w_{2k+1}) \\ &+ g(w_{2k+2}) = 0 \neq 1. \end{aligned}$$

Therefore it follows that  $g$  is not a unidominating function.

**Case (ii):** Define another function  $g_1: V \rightarrow \{0,1\}$  by

$$g_1(u) = f(u) \text{ for all } u \in V, u \neq w_{2k-1}, w_{2k} \\ \text{for some } k \in \{1, 2, \dots, n\}, k \text{ is odd,}$$

$$\text{and so } g_1(w_{2k-1}) = g_1(w_{2k}) = 0$$

Since  $k$  is odd,  $k-1$  is even so that

$$g_1(v_{k-1}) = g_1(w_{2k-3}) = g_1(w_{2k-2}) = 0.$$

$$\begin{aligned} \text{Now } \sum_{u \in N[w_{2i-1}]} g_1(u) &= g_1(w_{2k-3}) + g_1(w_{2k-2}) + g_1(w_{2k-1}) \\ &+ g_1(v_{k-1}) = 0 \neq 1 \end{aligned}$$

Therefore  $g_1$  is not a unidominating function.

**Case (iii):** Define another function  $g_2: V \rightarrow \{0,1\}$  by

$$g_2(u) = f(u) \text{ for all } u \in V, u \neq v_k \\ \text{for some } k \in \{1, 2, \dots, n\}, k \text{ is odd,}$$

$$\text{And so } g_2(v_k) = 0.$$

$$\begin{aligned} \text{Now } \sum_{u \in N[v_k]} g_2(u) &= g_2(u_k) + g_2(v_k) + g_2(w_{2k-1}) \\ &+ g_2(w_{2k}) = 1 + 0 + 1 + 1 = 3 \neq 1. \end{aligned}$$

Therefore  $g_2$  is not a unidominating function.

**Case (iv):** Define another function  $g_3: V \rightarrow \{0,1\}$  by

$$g_3(u) = f(u) \text{ for all } u \in V, u \neq v_k, w_{2k-1}, w_{2k}$$

for some  $k \in \{1, 2, \dots, n\}$ ,  $k$  is odd,

and so  $g_3(v_k) = 0, g_3(w_{2k-1}) = g_3(w_{2k}) = 0$ .

Then by the same argument for  $g_1$  in Case (ii), it follows that  $g_3$  is not a unidominating function.

Since all these functions are defined arbitrarily and as there is no other possibility of defining a function  $< f$ , it follows that  $f$  is a minimal unidominating function.

Thus  $\Gamma_u(3\text{-regularized wheel}) \geq \frac{5n}{2} \dots (1)$

Consider a minimal unidominating function  $f$  of a 3-regularised wheel. Then it must satisfy the following conditions, otherwise it cannot be a minimal unidominating function.

1. If  $f(u_i) = 1$  for all  $i \in \{1, 2, \dots, n\}$  then all  $v_i$ 's should not be assigned 1 and no two consecutive  $v_i$ 's can have functional value 1.
2. If  $f(u_i) = 1$  and  $f(v_i) = 0$  then  $f(w_{2i-1}) = f(w_{2i}) = 0$ .
3. If  $f(v_i) = 1$  then either  $f(w_{2i-1}) = f(w_{2i}) = 1$  or  $f(w_{2i-1}) = f(w_{2i}) = 0$ .
4. If  $f(u_i) = 0$  and any one of  $f(u_{i-1})$  or  $f(u_{i+1})$  is 1 then  $f(v_i)$  must be 0 and any one of  $f(w_{2i-1}), f(w_{2i})$  must be 1.

Otherwise if  $f(u_{i-1}) = f(u_i) = f(u_{i+1}) = 0$  then  $f(v_i)$  must be 1 and  $f(w_{2i-1}), f(w_{2i})$  are 0.

Let  $f$  be a minimal unidominating function of a 3-regularized wheel.

Let  $k$  be the number of  $u_i$ 's which have functional value 0. Then for each such  $i$ ,

$$f(v_i) + f(w_{2i-1}) + f(w_{2i}) = 1.$$

Therefore  $\sum [f(v_i) + f(w_{2i-1}) + f(w_{2i})] = k$  for these  $k$  sets of vertices

$(v_i, w_{2i-1}, w_{2i})$ , where  $i$  is such that  $f(u_i) = 0$ .

Suppose that the number of consecutive  $u_i$ 's which have functional value 1 are  $k_1, k_2, \dots, k_p$  respectively.

Then  $k_1 + k_2 + \dots + k_p = n - k$ ,

$$1 \leq k_1, k_2, \dots, k_p \leq n - k, p \leq \frac{k}{2} < k.$$

Then we have

$$\sum [f(v_i) + f(w_{2i-1}) + f(w_{2i})] \leq \frac{k_1}{2} + \frac{k_1}{2} + \frac{k_1}{2} = \frac{3k_1}{2}$$

if  $k_1$  is even,

$$= \frac{k_1 + 1}{2} + \frac{k_1 + 1}{2} + \frac{k_1 + 1}{2} \leq \frac{3(k_1 + 1)}{2} \text{ if } k_1 \text{ is odd.}$$

Similar is the case for  $k_2, k_3, \dots, k_p$ .

Without loss of generality assume that  $k_1, k_2, \dots, k_r$  are even and  $k_{r+1}, k_{r+2}, \dots, k_p$  are odd. Then

$$\begin{aligned} f(V) &= \sum_{i=1}^n f(u_i) + \sum_{k_1} [f(v_i) + f(w_{2i-1}) + f(w_{2i})] + \dots \\ &\quad + \sum_{k_2} [f(v_i) + f(w_{2i-1}) + f(w_{2i})] \\ &\quad + \sum_{k_p} [f(v_i) + f(w_{2i-1}) + f(w_{2i})] \\ &\leq n - k + \frac{3(k_1 + k_2 + \dots + k_r)}{2} \\ &\quad + \frac{3(k_{r+1} + 1 + k_{r+2} + 1 + \dots + k_p + 1)}{2} + k \\ &= n + \frac{3(n - k + p - r)}{2} = \frac{5n}{2} - \frac{3(k + r - p)}{2} \leq \frac{5n}{2} \end{aligned}$$

Therefore for any minimal unidominating function  $f$  of a 3-regularized wheel  $f(V) \leq \frac{5n}{2}$ .

Thus  $\max(f(V)/f \text{ is a minimal unidominating function}) \leq \frac{5n}{2}$ .

That is  $\Gamma_u(3\text{-regularized wheel}) \leq \frac{5n}{2} \dots (2)$

Therefore from the inequalities (1) and (2) we have

$$\Gamma_u(3\text{-regularized wheel}) = \frac{5n}{2}, \text{ when } n \text{ is even.}$$

**Case 2:** Let  $n$  be an odd number.

Define a function  $f: V \rightarrow \{0, 1\}$  by

$$f(u) = \begin{cases} 1 & \text{for } u = u_i, i = 1, 2, \dots, n-2, \\ 1 & \text{for } u = v_j, w_{2j-1} \text{ when } j \text{ is an odd number, } j \neq n, \\ 1 & \text{for } u = w_{2n-1}, \\ 1 & \text{for } u = w_{2j} \text{ when } j \text{ is an odd number,} \\ 0 & \text{otherwise.} \end{cases}$$

This function is similar to the function  $f$  defined in Case 1 except for the vertices  $u_{n-1}, u_n, v_n, w_{2n-3}, w_{2n-1}$ .

We can check the condition of unidominating function in the closed neighbourhood of the above vertices and see that  $f$  is a unidominating function.

Now we check for the minimality of  $f$ .

Define a function  $g: V \rightarrow \{0, 1\}$  by

$$g(u) = f(u) \text{ for all } u \in V, u \neq w_{2n-3}.$$

Then obviously  $g < f$  and

$$\begin{aligned} \sum_{u \in N[w_{2n-3}]} g(u) &= g(w_{2n-3}) + g(w_{2n-2}) + g(w_{2n-1}) \\ &\quad + g(v_{n-1}) = 0 + 0 + 0 + 0 = 0 \neq 1 \end{aligned}$$

Therefore  $g$  is not a unidominating function.

For all possibilities of defining a function  $g < f$ , we can see that  $g$  is not a unidominating function.

Therefore  $f$  is a minimal unidominating function.

$$\text{Now } f(V) = \sum_{u \in V} f(u)$$

$$= \sum_{i=1}^n [f(u_i) + f(v_i) + f(w_{2i-1}) + f(w_{2i})]$$

$$= (n-2) + \frac{n-1}{2} + \frac{n-1}{2} + 1 + \frac{n-1}{2} + 1$$

$$= \frac{5n-3}{2} = \frac{5n-1}{2} - 1 = \left\lfloor \frac{5n}{2} \right\rfloor - 1.$$

Hence it follows that

$$\Gamma_u(3\text{-regularized wheel}) \geq \left\lfloor \frac{5n}{2} \right\rfloor - 1 \text{ --- (1)}$$

If  $f$  is a minimal unidominating function then in Case 1 we have proved in general that  $f(V) \leq n + \frac{3(n-k+p-r)}{2}$ . That is we have not taken in to consideration that  $n$  is even.

$$\text{Therefore } f(V) \leq n + \frac{3(n-k+p-r)}{2}$$

$$= n + \frac{3((n-1) - (k+r-p-1))}{2}$$

$$= \frac{5n-3}{2} - \frac{3(k+r-p-1)}{2} \leq \frac{5n-3}{2} = \left\lfloor \frac{5n}{2} \right\rfloor - 1.$$

Thus for any minimal unidominating function  $f$ ,

$$f(V) \leq \left\lfloor \frac{5n}{2} \right\rfloor - 1.$$

Hence it follows that

$$\Gamma_u(3\text{-regularized wheel}) \leq \left\lfloor \frac{5n}{2} \right\rfloor - 1 \text{ --- (2)}$$

Therefore from the inequalities (1) and (2) we have

$$\Gamma_u(3\text{-regularized wheel}) = \left\lfloor \frac{5n}{2} \right\rfloor - 1. \blacksquare$$

**Theorem 3.2:** The number of minimal unidominating functions with maximum weight of a 3-regularized wheel is  $\begin{cases} 2 & \text{when } n \text{ is even,} \\ n & \text{when } n \text{ is odd.} \end{cases}$

**Proof:** Consider a 3-regularized wheel formed from a wheel  $W_{1,n}$ .

**Case 1:** Let  $n$  be an even number.

Consider the minimal unidominating function  $f$  with maximum weight  $\frac{5n}{2}$  given in Case 1 of Theorem 3.1. Then the functional values of  $f$  are

$$f(u_i) = 1 \forall i \in \{1, 2, \dots, n\}$$

$$f(v_i) = f(w_{2i-1}) = f(w_{2i}) = 1 \text{ for } i \text{ is odd,}$$

$$f(v_i) = f(w_{2i-1}) = f(w_{2i}) = 0 \text{ for } i \text{ is even.}$$

Represent

$$f(u_i), f(v_i), f(w_{2i-1}), f(w_{2i}) \text{ as } \begin{cases} 1 & \text{when } f(v_i) = f(w_{2i-1}) = f(w_{2i}) = 1 \\ & \text{and } f(u_i) = 1, \\ 0 & \text{when } f(v_i) = f(w_{2i-1}) = f(w_{2i}) = 0 \\ & \text{and } f(u_i) = 1, \end{cases}$$

Therefore the values of  $(u_i, v_i, w_{2i-1}, w_{2i})$ ,  $i = 1, 2, \dots, n$  are represented by

$$1010 \text{ --- } 10 \text{ circularly.}$$

Take  $a = 10$ . Then the functional values of  $f$  are in the pattern of  $aa \dots a$  circularly. These can be arranged in only one way. By the rotation of these values after the first rotation we obtain the same function  $f$ .

Therefore there are two minimal unidominating functions with maximum weight  $\frac{5n}{2}$ .

Now we test whether there are any other minimal unidominating functions of weight  $\frac{5n}{2}$ .

Let  $f$  be any minimal unidominating function of a 3-regularized wheel. Then we have proved in Case 1 of Theorem 3.1 that

$$f(V) \leq n + \frac{3(n-k+p-r)}{2}.$$

If  $k = 0$  we get  $r = 1$ ,  $p = 1$  so that  $f(V) = \frac{5n}{2}$ .

That is this function coincides with one of the above said two minimal unidominating functions.

If  $k = 1$  then  $f$  cannot be a unidominating function.

Suppose  $k \geq 2$ . Then  $r \geq 0$ ,  $p \geq 1$ , and so

$k + r - p > 0$ . Then

$$f(V) \leq \frac{5n}{2} - \frac{3(k+r-p)}{2} < \frac{5n}{2}.$$

Then  $f$  has no maximum weight.

Therefore there is no other minimal unidominating function with maximum weight  $\frac{5n}{2}$ .

**Case 2:** Let  $n$  be an odd number.

Let  $f$  be a minimal unidominating function defined in Case 2 of Theorem 3.1. As in Case 1 by rotating the functional values of the vertices taking  $u_i, v_i, w_{2i-1}, w_{2i}$  as one unit we get  $n$  minimal unidominating functions.

We now verify that if there is any other minimal unidominating function with maximum weight  $\frac{5n}{2}$ .

Let  $f$  be a minimal unidominating function of a 3-regularized wheel. Then we have proved in Case 2 of Theorem 3.1 that

$$f(V) \leq \frac{5n-3}{2} - \frac{3(k+r-p-1)}{2}.$$

If  $k = 0$  and 1 then  $f$  can not be a minimal unidominating function. Therefore  $k$  must be greater than or equal to 2.

If  $k = 2$  then  $p = 1, r = 0$ . Now  $f(V) = \frac{5n-3}{2} = \left\lfloor \frac{5n}{2} \right\rfloor - 1$ .

If  $k > 2$  then  $k + r - p - 1 > 0$ , so that

$$f(V) \leq \frac{5n-3}{2} - \frac{3(k+r-p-1)}{2} < \frac{5n-3}{2} = \left\lfloor \frac{5n}{2} \right\rfloor - 1.$$

Therefore there is no other minimal unidominating function with maximum weight.  $\blacksquare$

#### 4. UPPER TOTAL UNIDOMINATION NUMBER OF A 3-REGULARIZED WHEEL

In this section the upper total unidomination number of a 3-regularized wheel and the number of minimal total unidominating functions with maximum weight are obtained.

**Theorem 4.1:** The upper total unidomination number of a 3-regularized wheel formed from  $W_{1,n}$  is

$$\Gamma_{tu}(3\text{-regularized wheel}) = 3n.$$

**Proof:** Let  $W_{1,n}$  be a wheel. Consider the 3-regularized wheel formed from  $W_{1,n}$  which is defined in 2.7.

Define a function  $f: V \rightarrow \{0,1\}$  by

$$f(u) = \begin{cases} 1 & \text{for } u = v_i, w_{2i-1}, w_{2i}, i = 1, 2, \dots, n, \\ 0 & \text{for } u = u_i, i = 1, 2, \dots, n. \end{cases}$$

Now for  $i = 1, 2, \dots, n$  we have

$$\sum_{u \in N(v_i)} f(u) = f(u_{i-1}) + f(u_{i+1}) + f(v_i) = 0 + 0 + 1 = 1.$$

$$\sum_{u \in N(u_i)} f(u) = f(u_i) + f(w_{2i-1}) + f(w_{2i}) = 0 + 1 + 1 = 2.$$

$$\sum_{u \in N(w_{2i-1})} f(u) = f(v_i) + f(w_{2i-2}) + f(w_{2i}) = 1 + 1 + 1 = 3.$$

$$\sum_{u \in N(w_{2i})} f(u) = f(v_i) + f(w_{2i-1}) + f(w_{2i+1}) = 1 + 1 + 1 = 3.$$

Therefore  $f$  is a total unidominating function.

Now we check for the minimality of  $f$ .

Now we define a function  $g$  such that  $g < f$ , and show that  $g$  is not a total unidominating function for all possibilities of defining  $g$ .

Define a function  $g: V \rightarrow \{0,1\}$  by

$$g(u) = f(u) \forall u \in V, \text{ for } u \neq v_k, \\ k \in \{1, 2, \dots, n\}, \text{ and } g(v_k) = 0.$$

Obviously  $g < f$ .

$$\text{Now } \sum_{u \in N(u_k)} g(u) = g(u_{k-1}) + g(u_{k+1}) + g(v_k) \\ = 0 + 0 + 0 = 0 \neq 1.$$

Therefore  $g$  is not a total unidominating function.

Define another function  $h: V \rightarrow \{0,1\}$  by

$$h(u) = f(u) \forall u \in V, \text{ for } u \neq w_{2k-1}, \\ k \in \{1, 2, \dots, n\}, \text{ and } h(w_{2k-1}) = 0.$$

Obviously  $h < f$ .

$$\text{Now } \sum_{u \in N(w_{2k-1})} h(u) = h(w_{2k}) + h(w_{2k-2}) + h(v_k) \\ = 1 + 1 + 1 = 3 \neq 1.$$

Therefore  $h$  is not a total unidominating function.

Define another function  $h_1: V \rightarrow \{0,1\}$  by

$$h_1(u) = f(u) \forall u \in V, u \neq w_{2k-1}, w_{2k}, k \in \{1, 2, \dots, n\}, \\ \text{and } h_1(w_{2k-1}) = 0, h_1(w_{2k}) = 0.$$

Obviously  $h_1 < f$ .

$$\text{Now } \sum_{u \in N(v_i)} h_1(u) = h_1(u_k) + h_1(w_{2k-1}) + h_1(w_{2k}) \\ = 0 + 0 + 0 = 0 \neq 1.$$

Therefore  $h_1$  is not a total unidominating function.

Since  $g, h, h_1$  are defined arbitrarily, and there is no other possibility of defining a total unidominating function  $< f$  it follows that  $f$  is a minimal total unidominating function.

$$\text{Now } f(V) = \sum_{i=1}^n [f(u_i) + f(v_i) + f(w_{2i-1}) + f(w_{2i})] \\ = 0 + n + n + n = 3n.$$

Therefore  $\Gamma_{tu}(3\text{-regularized wheel}) \geq 3n \dots (1)$

Let  $f$  be a minimal total unidominating function.

Then  $f(u_i) = f(v_i) = f(w_{2i-1}) = f(w_{2i}) = 1$  is possible only for at most two consecutive  $i$ 's.

Let  $k_1$  be the number of  $u_i$ 's such that

$f(u_i) = 0$  and any one of  $f(u_{i-1}), f(u_{i+1})$  is 1 and  $k_2$  be the number of  $u_i$ 's such that  $f(u_i) = 0$  and

$$f(u_{i-1}) = f(u_{i+1}) = 0$$

$$\text{Then } \sum_{i=(k_1+k_2)}^n [f(v_i) + f(w_{2i-1}) + f(w_{2i})] \\ \leq 3 \left( \frac{2(n - (k_1 + k_2))}{3} \right)$$

$$\text{and } f(V) \leq n - (k_1 + k_2) + 2(n - (k_1 + k_2)) + k_1 + 3k_2 \\ = 3n - 2k_1 \leq 3n.$$

Since  $f$  is arbitrary, it follows that  $\Gamma_{tu}(3\text{-regularized wheel}) \leq 3n \dots (2)$

Thus from the inequalities (1) and (2) we have

$$\Gamma_{tu}(3\text{-regularized wheel}) = 3n. \blacksquare$$

**Theorem 4.2:** The number of minimal total unidominating functions of a 3-regularized wheel with maximum weight is

$$\begin{cases} 4 & \text{when } n \equiv 0 \pmod{3}, \\ 1 & \text{when } n \equiv 1, 2 \pmod{3}. \end{cases}$$

**Proof:** Consider the minimal total unidominating function  $f$  with maximum weight  $3n$  given by

$$f(u) = \begin{cases} 1 & \text{for } u = v_i, w_{2i-1}, w_{2i}, i = 1, 2, \dots, n, \\ 0 & \text{for } u = u_i, i = 1, 2, \dots, n. \end{cases}$$

By rotating the functional values of  $f$  taking  $u_i, v_i, w_{2i-1}, w_{2i}$  as one group we get the same function. Therefore there is one and only one minimal total unidominating function with maximum weight  $3n$ .

Now we investigate for some other minimal total unidominating functions with maximum weight  $3n$ .

Let  $f$  be a minimal total unidominating function. Then we have proved in Theorem 4.1 that  $f(V) \leq 3n - 2k_1$ .

If  $k_1 > 0$  then  $f(V) \leq 3n - 2k_1 < 3n$ . Therefore  $f$  is not a function with maximum weight.

If  $k_1 = 0$  then  $k_2 = n$  or  $k_2 = 0$ .

Suppose  $k_2 = n$ . Then  $f(V) = 3n$  and this function coincides with the above said function.

Suppose  $k_2 = 0$ . Then  $f(u_i) = 1 \forall i = 1, 2, \dots, n$ .

Based on the condition



$f(u_i) = f(v_i) = f(w_{2i-1}) = f(w_{2i}) = 1$  is possible only for at most two consecutive  $i$ 's we get another minimal total unidominating function with weight  $3n$  when  $n \equiv 0 \pmod{3}$ .

This function is defined by

$$f(u) = \begin{cases} 1 & \text{for } u = u_i, i = 1, 2, \dots, n \text{ and } u = v_i, w_{2i-1}, w_{2i}, i \equiv 1, 2 \pmod{3}, \\ 0 & \text{otherwise.} \end{cases}$$

By rotating the functional values of  $f$  taking  $u_i, v_i, w_{2i-1}, w_{2i}$  as one group we get two other minimal total unidominating functions with maximum weight. Therefore there are  $1+3=4$  minimal total unidominating functions with maximum weight  $3n$  when  $n \equiv 0 \pmod{3}$ .

When  $n \equiv 1, 2 \pmod{3}$  the total unidominating functions obtained has weight  $< 3n$  and hence there is no other function with maximum weight.

Hence the number of minimal total unidominating functions with maximum weight  $3n$  is 4 when  $n \equiv 0 \pmod{3}$  and 1 when  $n \equiv 1, 2 \pmod{3}$ . ■

## 5. ILLUSTRATIONS

In this section the examples for upper unidominating functions and upper total unidominating functions are given.

**Example 5.1:** Let  $n = 8$ .  
Clearly  $8 \equiv 2 \pmod{3}$ .

The functional values of a minimal unidominating function defined in Case 1 of Theorem 3.1 are denoted at the corresponding vertices in Fig. 1.

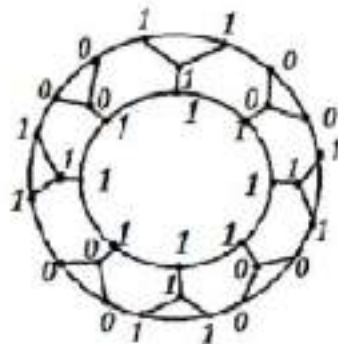


Fig. 1 3-regularized wheel formed from  $W_{1,8}$

Upper unidomination number of 3-regularized wheel formed from  $W_{1,8}$  is

$$\Gamma_u(3\text{-regularized wheel}) \text{ is } \frac{5n}{2} = 20.$$

There are 2 minimal unidominating functions with maximum weight 20. ■

**Example 5.2:** Let  $n = 15$ .  
Clearly  $15 \equiv 0 \pmod{3}$ .

The functional values of a minimal unidominating function defined in Case 2 of

Theorem 3.1 are denoted at the corresponding vertices in Fig. 2

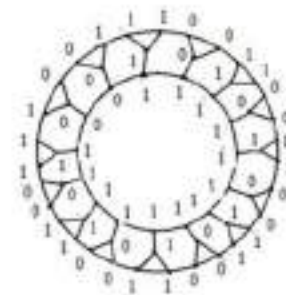


Fig. 2 3-regularized wheel formed from  $W_{1,15}$

Upper unidomination number of 3-regularized wheel formed from  $W_{1,15}$  is

$$\Gamma_u(3\text{-regularized wheel}) = \left\lfloor \frac{5n}{2} \right\rfloor - 1 = 37 - 1 = 36$$

There are 15 minimal unidominating functions with maximum weight 36. ■

**Example 5.3:** Let  $n = 8$ .

The functional values of the minimal total unidominating function defined in Theorem 4.1 of a 3-regularized wheel formed from  $W_{1,8}$  are given at the corresponding vertices in Fig. 3

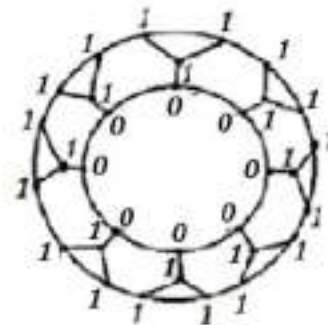


Fig. 3 3-regularized wheel formed from  $W_{1,8}$

Upper total unidomination number of this 3-regularized wheel is 24.

There is only one total unidominating function with maximum weight. ■

## 6. CONCLUSION

3-regularized wheel formed from  $W_{1,n}$  is new concept introduced by the authors and in this paper the upper unidomination number of a 3-regularized wheel and number of minimal unidominating functions with maximum weight is calculated based on  $n$  is even or odd. Further, the number of minimal total unidominating functions with maximum weight is based on whether  $n \equiv 0 \pmod{3}$  or otherwise. This gives scope for further study on this new graph and various domination parameters can be enumerated.

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# ASIAN JOURNAL OF ORGANIC CHEMISTRY

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## Accepted Article

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**Authors:** Gopalaiah Kovuru, Chenna Rao Devarapalli, Kuldeep Mahiya, and Ankit Tiwari

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## Iron-Catalyzed Aerobic Oxidative Cleavage and Construction of C-N Bonds: A Facile Method for Synthesis of 2,4,6-Trisubstituted Pyridines

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### Graphical Abstract



### ABSTRACT

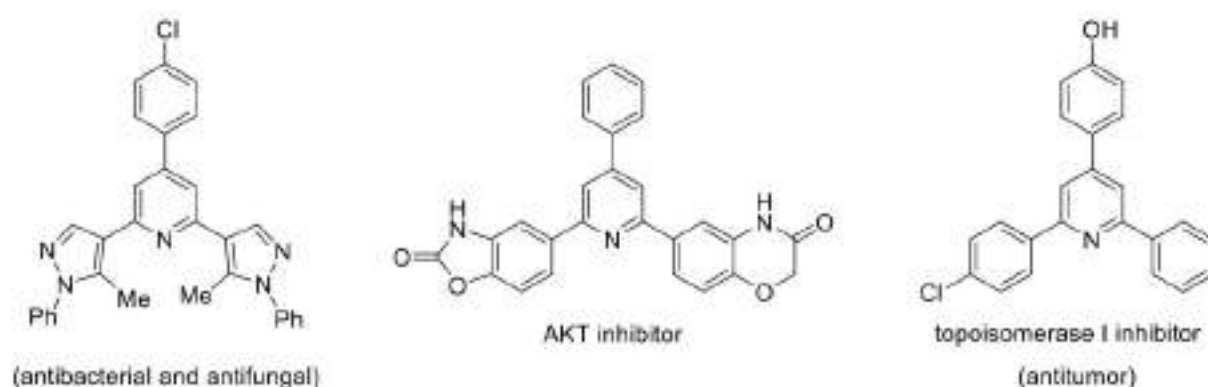
Abundant and inexpensive iron, readily catalyzed the oxidative tandem reactions of arylalkylketones and benzylamines using molecular oxygen as the green oxidant in one-pot solvent-free conditions. 2,4,6-Triaryl pyridines containing a wide variety of functional groups are synthesized from easily available starting materials. The synthetic importance of this reaction protocol has been demonstrated by preparing topoisomerase I and II inhibitors, and other valuable pyridine derivatives. Key intermediates are isolated and a plausible mechanism has been proposed.

## Introduction

Transition metal catalyzed oxidative C-N bond cleavage and formation of C-N bonds have attracted much attention because of their potential applications in organic synthesis.<sup>1</sup> In recent years, various transition metal catalysts such as palladium, ruthenium and rhodium based systems have been developed for oxidative transformations in which stoichiometric or large amounts of inorganic or organic oxidants were employed. However, molecular oxygen is an ideal oxidant because of its abundance and low cost, and generates at best only water as the by-product.<sup>2</sup> Recently, researchers have made impressive advances in the iron-catalyzed oxidative transformation of C-H bonds owing to their economic viability and environmentally friendly features.<sup>3</sup> In this perspective, efficient iron-catalyzed aerobic oxidative annulation reactions for synthesis of nitrogen-heterocycles have been developed by our group<sup>4</sup> and others.<sup>5</sup>

Pyridine represents an important and abundant class of *N*-heterocycles found in bioactive natural products and synthetic pharmaceuticals.<sup>6</sup> In particular, 2,4,6-triarylpyridine (Kröhnke pyridine) derivatives have been implicated in a large spectrum of biological activities including anticancer, antidepressant, antifungal, antibacterial, and antitumor activities (Figure 1).<sup>7</sup> In addition, 2,4,6-triarylpyridines have been exploited as chemosensors,<sup>8</sup> ligands,<sup>9</sup> photosensitizers,<sup>10</sup> and synthetic intermediates for the direct synthesis of therapeutic drugs, herbicides, insecticides, and surfactants.<sup>11</sup> Despite their diverse applications, however, only a limited number of synthetic routes are available to access these scaffolds. Most of the reported methods relied on the classical cyclocondensation reaction of an aldehyde, an enolizable ketone and an ammonium salt as the nitrogen source using various catalysts.<sup>12</sup> Recently, some new approaches were developed by employing various starting materials, which include cyclocondensation of acetophenone oxime/oxime acetates with aldehydes<sup>13</sup> or acetophenones

with benzyl halides<sup>14</sup>, iodine-mediated catabolism and reconstruction process of amino acids with ketones,<sup>15</sup> oxidative cyclization of ketones with benzylamines using Cu(OTf)<sub>2</sub> or HOTf or iodine catalysts,<sup>16</sup> and photoredox catalysis of amines with aryl ketones.<sup>17</sup> Although the above reactions are conducive for construction of 2,4,6-triarylpyridines, however, continuous attempts are being made for more economical and practical methods to access these heterocycles.




**Figure 1:** Representative examples of bioactive 2,4,6-triarylpyridine compounds

In continuation of our studies on the development of sustainable and environmentally benign methods for synthesis of azaheterocycles,<sup>4,18</sup> herein we report an efficient iron-catalyzed aerobic oxidative annulation of arylalkylketones with benzylamines for the synthesis of 2,4,6-trisubstituted pyridine derivatives. This annulation reaction proceeds with inexpensive iron salt as catalyst and molecular oxygen as sole oxidant which makes the transformation more practicable and highly sustainable.

## Results and Discussion

We began our study by attempting the reaction of acetophenone (**1a**, 2.0 mmol) with benzylamine (**2a**, 1.2 mmol) in chlorobenzene at 110 °C under air atmosphere, and the results are summarized in Table 1. Initially, when the reaction was conducted in the absence of catalyst, no desired product **3a** was observed (Table 1, entry 1). Next, several iron salts were screened for the reaction under similar reaction conditions. Iron catalysts such as  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $\text{Fe}_2\text{O}_3$ ,  $\text{Fe}(\text{acac})_3$ , and  $\text{Fe}(\text{NO}_3)_3$  were proved to be ineffective for this transformation (Table 1, entries 2-5). Pleasingly, the product **3a** was obtained in 17% and 26% yields when  $\text{FeO}$  and  $\text{Fe}(\text{OAc})_2$  were used (Table 1, entries 6 and 7). The reaction yields were further improved to 38%, 41%, and 55% when 10 mol% of  $\text{FeCl}_3$ ,  $\text{FeF}_2$  and  $\text{FeCl}_2$ , respectively, were employed (Table 1, entries 8-10). Among the various iron catalysts examined,  $\text{FeBr}_2$  was found to be the most effective, and it is resulted the formation of **3a** in 74% yield (Table 1, entry 11). We then surveyed the reaction in toluene and polar solvents such as DMSO and DMA. The reaction was effective in toluene, but ineffective either in DMSO or DMA (Table 1, entries 12-14). To our delight, in the absence of solvent, the reaction was accelerated and yield of the desired product was increased to 85% (Table 1, entry 15). Further, on performing the reaction under molecular oxygen atmosphere using  $\text{FeBr}_2$  catalyst resulted in the formation of desired product **3a** in 93% yield (Table 1, entry 16). The reaction temperature is an important factor for yield of the product. The yield of **3a** decreased to 79% when the reaction was carried out at 90 °C (Table 1, entry 17). Thus, the conditions observed in entry 16 of Table 1 were used as optimal reaction conditions.

**Table 1.** Screening of reaction conditions for Fe-catalyzed oxidative annulation<sup>a</sup>


The reaction scheme shows acetophenone (1a) reacting with benzylamine (2a) in the presence of an iron catalyst to produce 2,4,6-triphenylpyridine (3a). The structures are color-coded: 1a is blue, 2a is red, and 3a is a combination of blue and red.

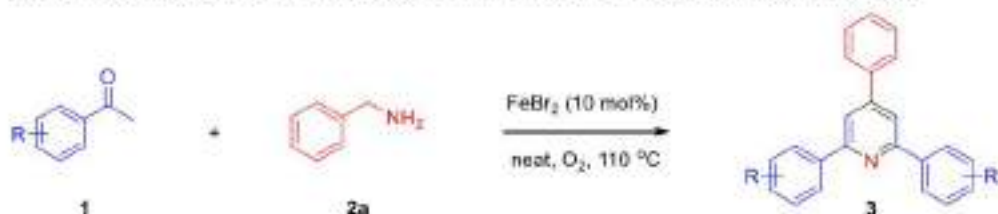
Entry	Catalyst	Solvent	Time (h)	Yield (%) <sup>b</sup>
1	--	chlorobenzene	18	0
2	FeSO <sub>4</sub> ·7H <sub>2</sub> O	chlorobenzene	18	trace
3	Fe <sub>2</sub> O <sub>3</sub>	chlorobenzene	18	trace
4	Fe(acac) <sub>3</sub>	chlorobenzene	18	trace
5	Fe(NO <sub>3</sub> ) <sub>3</sub>	chlorobenzene	18	trace
6	FeO	chlorobenzene	18	17
7	Fe(OAc) <sub>2</sub>	chlorobenzene	18	26
8	FeCl <sub>3</sub>	chlorobenzene	18	38
9	FeF <sub>2</sub>	chlorobenzene	18	41
10	FeCl <sub>2</sub>	chlorobenzene	18	55
11	FeBr <sub>2</sub>	chlorobenzene	18	74
12	FeBr <sub>2</sub>	toluene	18	61
13	FeBr <sub>2</sub>	DMSO	18	trace
14	FeBr <sub>2</sub>	DMA	18	trace
15 <sup>c</sup>	FeBr <sub>2</sub>	--	12	85
16 <sup>c,d</sup>	FeBr <sub>2</sub>	--	10	93
17 <sup>c,d,e</sup>	FeBr <sub>2</sub>	--	18	79






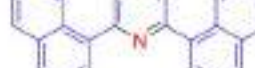


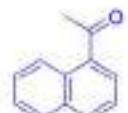

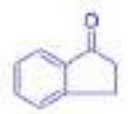

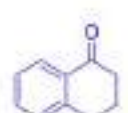



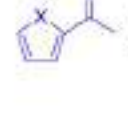

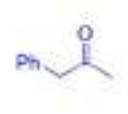

<sup>a</sup> Reactions were performed using ketone **1a** (2.0 mmol), amine **2a** (1.2 mmol), with iron catalyst (10 mol%) in 2 mL solvent at 110 °C under air atmosphere, unless otherwise specified. <sup>b</sup> Isolated yields. <sup>c</sup> Reaction was carried out in neat condition.

<sup>d</sup> Reaction was performed under molecular oxygen (O<sub>2</sub> balloon). <sup>e</sup> Reaction was carried out at 90 °C.



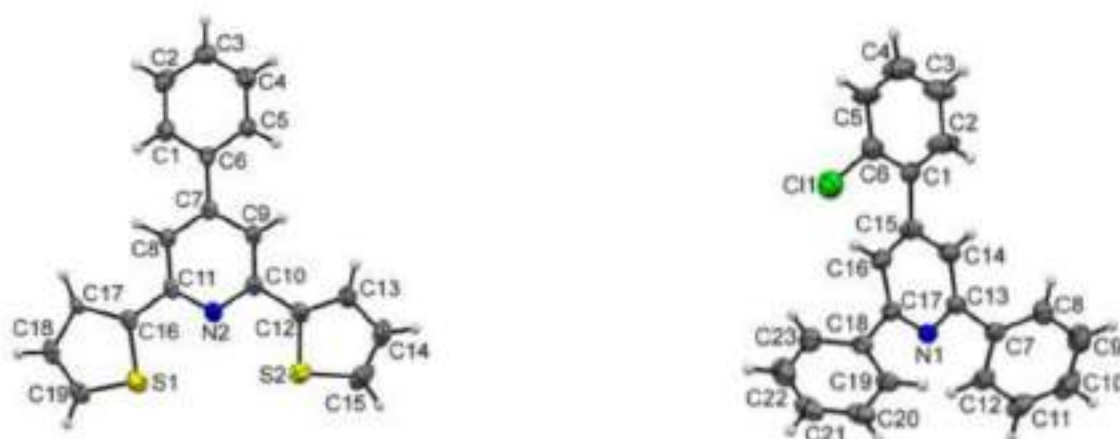
Having the optimized reaction conditions in hand, we next explored the substrate scope with a variety of substituted arylalkylketones (Table 2). In all cases, arylmethylketones bearing electron-donating as well as electron-withdrawing groups on the aromatic rings were smoothly transformed to the corresponding 2,4,6-triarylpyridines with good to excellent yields (Table 2, entries 1-8). However, the *ortho*-substituted arylmethylketones gave slightly lower yields than that of the *meta* and *para*-substituted ketones due to the steric effect (Table 2, entries 4 vs 2 and 3, and entry 7 vs 6). It is noteworthy that the halo-substituted arylmethylketones tolerated well, leading to halo-substituted 2,4,6-triarylpyridines (Table 2, entries 6-8), which could be further applied in conventional Pd-catalyzed cross-coupling reactions. Furthermore, the naphthyl-substituted ketone viz., 1-acetonaphthone was also compatible under the reaction conditions, affording the desired product **3j** in 81% yield (Table 2, entry 9). Besides these substrates, the catalytic system was equally effective for arylcycloalkylketones such as 1-indanone and  $\alpha$ -tetralone, giving the corresponding products in high yields (Table 2, entries 10 and 11). In addition, heteroaryl methyl ketones, namely, 2-acetylthiophene and 2-acetylfuran were also good substrates to provide 4-phenyl-2,6-di(2-thienyl)pyridine (**3m**) and 2,6-di(2-furyl)-4-phenylpyridine (**3n**), respectively in 82% and 67% yields, respectively (Table 2, entries 12 and 13). However, phenylacetone gave the desired product in trace amount (Table 2, entry 14).

**Table 2.** Iron-catalyzed aerobic oxidative annulation of a range of ketones **1** with **2a**<sup>a</sup>


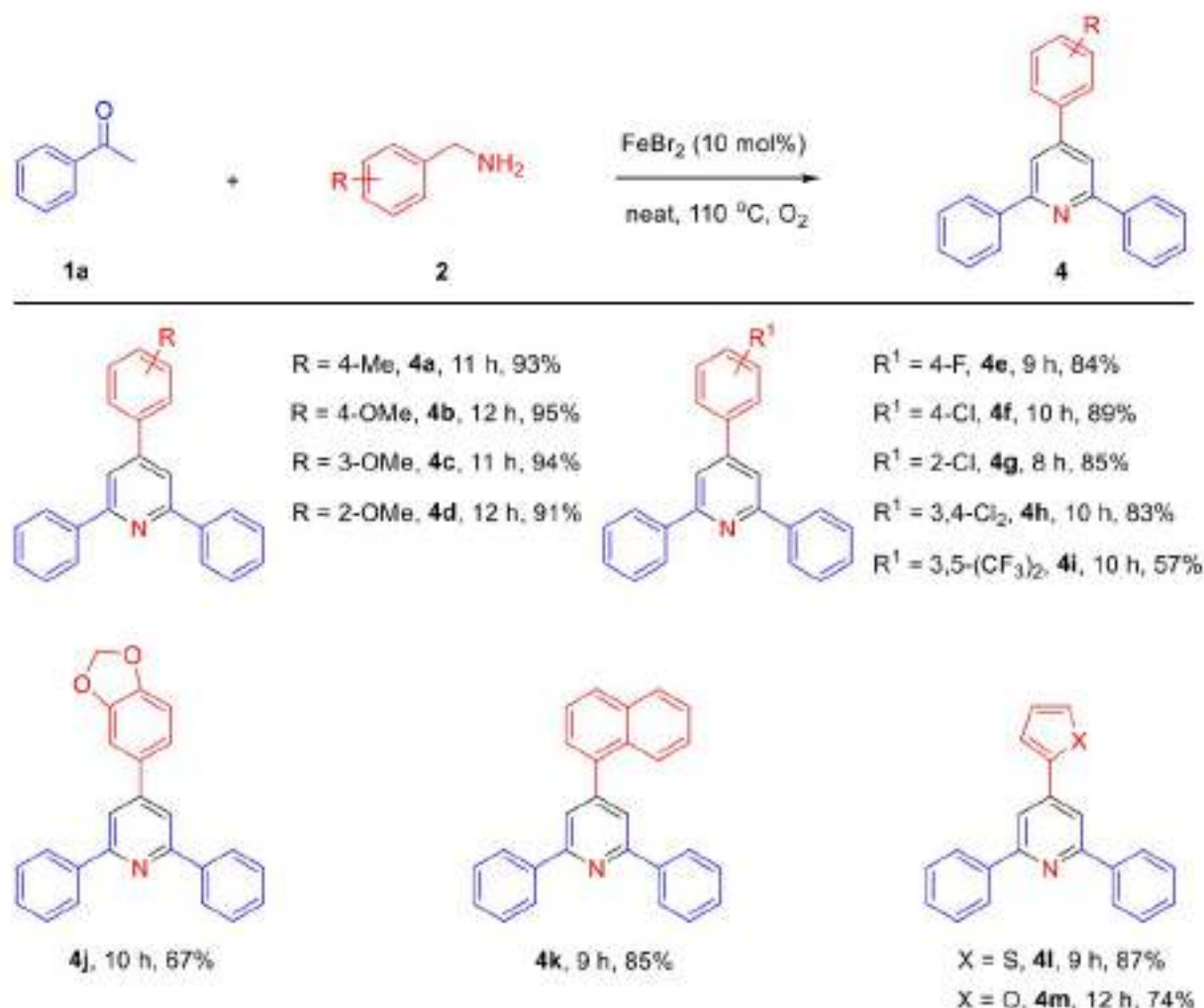
Entry	Ketone (1)	Time (h)	Product (3)	Yield (%) <sup>b</sup>	
1	R = 4-Me	10		<b>3b</b> 92	
2	R = 4-OMe	12		<b>3c</b> 90	
3	R = 3-OMe	13		<b>3d</b> 89	
4	R = 2-OMe	12		<b>3e</b> 85	
5	R = 4-N(CH <sub>3</sub> ) <sub>2</sub>	11		<b>3f</b> 79	
6	R = 4-Cl	8		<b>3g</b> 94	
7	R = 2-Cl	10		<b>3h</b> 87	
8	R = 4-Br	7		<b>3i</b> 96	
9		9		<b>3j</b> 81	
10		10		<b>3k</b> 78	
11		12		<b>3l</b> 73	
12		X = S	9		<b>3m</b> 82
13		X = O	13		<b>3n</b> 67
14		24		<b>3o</b> trace	

<sup>a</sup> Reactions were performed using ketone **1** (2.0 mmol), amine **2a** (1.2 mmol), with FeBr<sub>2</sub> (10 mol%) in the oxygen atmosphere (O<sub>2</sub> balloon) at 110 °C under neat conditions. <sup>b</sup> Isolated yields.

The iron-catalyzed aerobic oxidative annulation was further extended to a broad variety of substituted benzylamines **2** (Scheme 1). Benzylamines bearing electron-donating groups such as  $-Me$ ,  $-OMe$ , and  $-OCH_2O-$ , and electron-withdrawing groups such as  $-F$ , and  $-Cl$  on the phenyl ring, reacted well with acetophenone to produce the desired products in 67-95% yields (**4a-4h** and **4j**). Substitutions at the *para*, *meta* and *ortho*-positions of phenyl ring did not show much variation on the reaction yields (**4b-4d**, **4f** and **4g**). However, benzylamine containing strong electron-withdrawing group such as  $-CF_3$  functionality at *meta* positions gave 57% yield of the desired product (**4i**). On the other hand, the fused ring system, 1-naphthylmethylamine was annulated well with acetophenone to afford 4-(1-naphthyl)-2,6-diphenylpyridine (**4k**) in 85% yield. Furthermore, heteroarylmethanamines viz., 2-thiophenemethylamine and furfurylamine were also reacted with **1a** to give the corresponding pyridine derivatives in 87% and 74% yields, respectively (**4l** and **4m**). All the products were characterized by  $^1H$  and  $^{13}C$  NMR spectra, and HRMS. In addition to the spectroscopic analysis, the structure of 2,4,6-trisubstituted pyridines was further confirmed by studying the single crystal X-ray diffraction analysis of **3m** and **4g** (Figure 2).

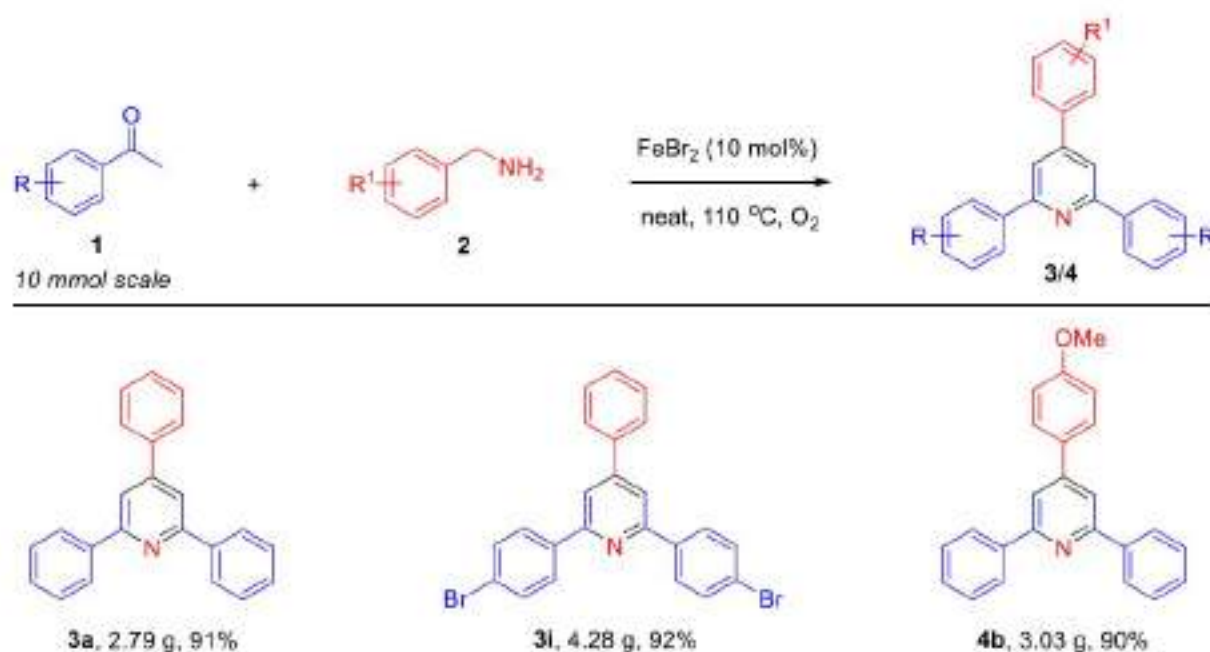


**Figure 2.** ORTEP drawing of compounds **3m** (CCDC 1838029, left) and **4g** (CCDC 1838030, right): The ellipsoids are drawn at 30% thermal probability level (see SI for details).

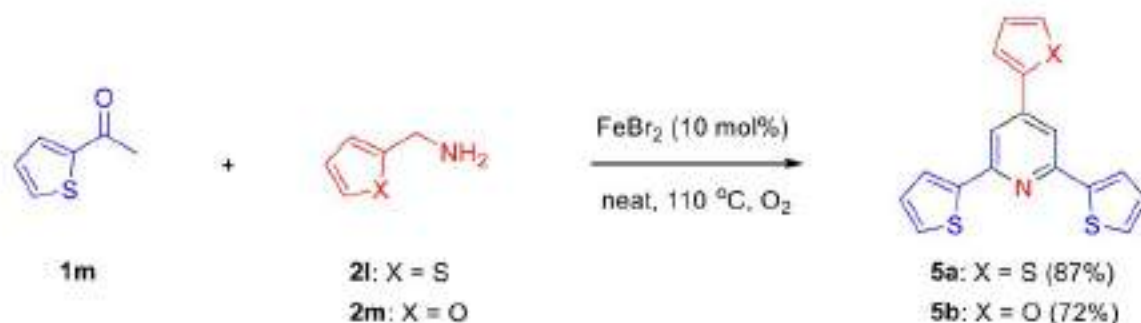
**Scheme 1.** Iron-catalyzed aerobic oxidative annulation of **1a** with a range of amines **2**<sup>a,b</sup>

<sup>a</sup> Reactions were performed using ketone **1a** (2.0 mmol), amine **2** (1.2 mmol), with  $\text{FeBr}_2$  (10 mol%) in the oxygen atmosphere ( $\text{O}_2$  balloon) at  $110\text{ }^\circ\text{C}$  under neat conditions. <sup>b</sup> isolated yields.

To examine the practicability and robustness of the synthesis, 2,4,6-triarylpyridines were successfully scaled up to 10 mmol and isolated 2.79 g of **3a** with 91% yield under the optimal reaction conditions. Gram-scale synthesis of 2,4,6-trisubstituted pyridines **3i** and **4b** was also performed (Scheme 2).

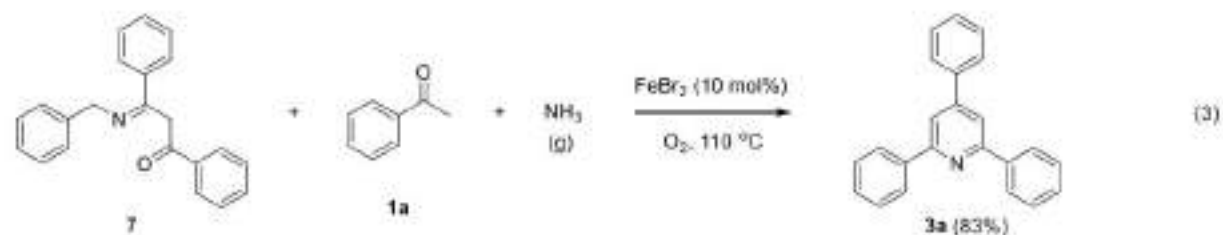
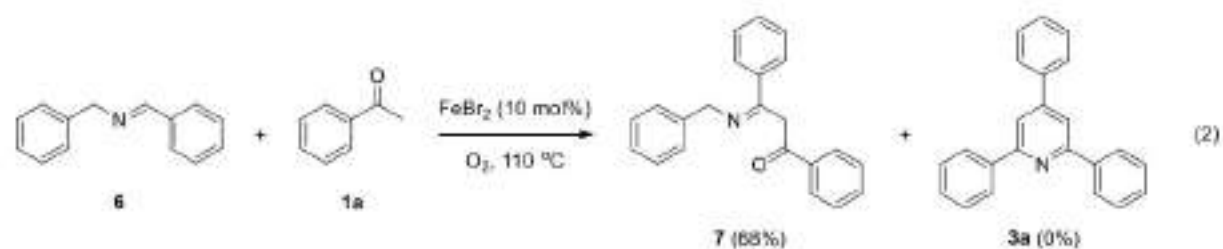
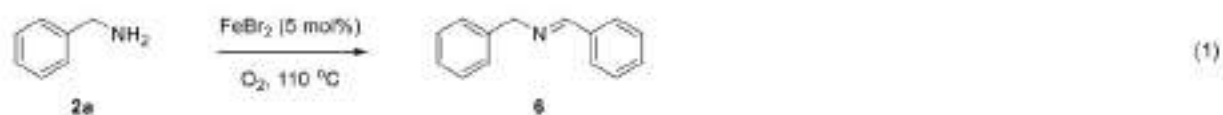
**Scheme 2.** Gram-Scale Synthesis of 2,4,6-Trisubstituted Pyridines **3a**, **3i** and **4b**

To demonstrate the synthetic utility of the present reaction protocol, several valuable 2,4,6-trisubstituted pyridine compounds have been prepared. For example, 2,4,6-tri(2-thienyl)pyridine (**5a**) and 4-(2-furyl)-2,6-di(2-thienyl)pyridine (**5b**), which are reported as excellent inhibitors of topoisomerase I and II activities,<sup>19</sup> can be easily synthesized by the oxidative annulation of 2-acetylthiophene (**1m**) with 2-thiophenemethylamine (**2l**) or with furfurylamine (**2m**) respectively, under the optimal reaction conditions (Scheme 3). In addition, many of the synthesized pyridine compounds, namely 7-phenyl-5,6,8,9-tetrahydrodibenzo[c,h]acridine (**3l**) and 4-(4-methylphenyl)-2,6-diphenylpyridine (**4a**) are used as precursors for the preparation of organic electroluminescent devices<sup>20</sup>; 2,6-bis(4-bromophenyl)-4-phenylpyridine (**3i**) and 4-phenyl-2,6-di(2-thienyl)pyridine (**3m**) are employed as key precursors for the synthesis of photoluminescence polymer sensors.<sup>21</sup>

**Scheme 3.** Synthesis of Topoisomerase Inhibitors

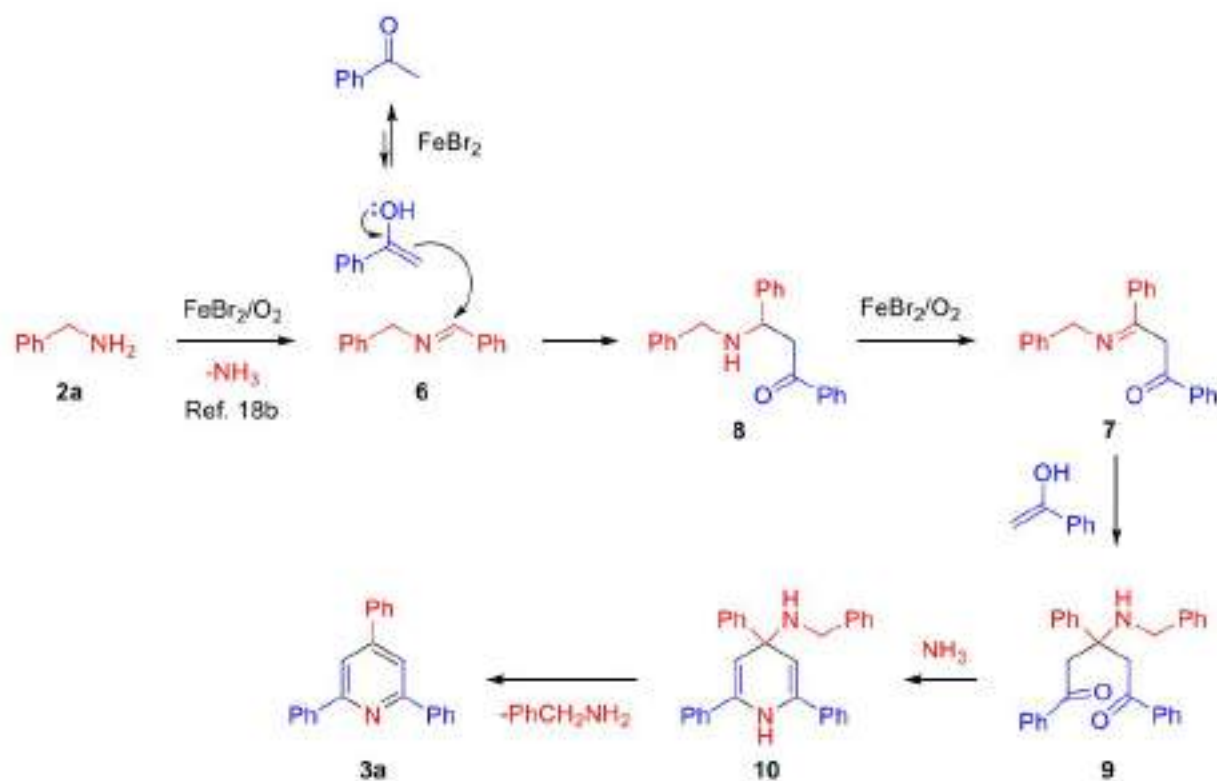
Some control experiments were performed to explore the possible mechanism for this oxidative annulation. Initially, when benzylamine (**2a**) was treated with iron(II) bromide and molecular oxygen, resulted the formation of homo-coupled imine **6** in quantitative yield (Scheme 4, eq 1). In another experiment, the isolated imine **6** was treated with acetophenone (**1a**, 1 equiv) under standard conditions, fortunately, a compound, 3-(benzylimino)-1,3-diphenyl-1-propanone (**7**) was formed in 68% yield, without the production of **3a** (Scheme 4, eq 2). The desired product **3a** could be successfully obtained by the reaction of keto-imine **7** with acetophenone (**1a**, 1 equiv) in the presence of gaseous ammonia under the standard conditions (Scheme 4, eq 3). This indicates that the compound **7** is one of the key intermediates of the transformation.

## Scheme 4. Control Experiments



On the basis of the above experimental results, a plausible mechanism for the formation of 2,4,6-triarylpyridines has been illustrated in Scheme 5. The first step involves the oxidative self-condensation of benzylamine (**2a**) in the presence of iron(II) bromide catalyst and molecular oxygen, leading to *N*-benzylbenzaldimine (**6**) with the generation of ammonia.<sup>18b</sup> Subsequently, the imine **6** reacts with enol of acetophenone to produce the amino-ketone (**8**), which on further oxidation gives imino-ketone intermediate (**7**). Next, the addition of second enol of acetophenone to **7** leads to the formation of diketone **9**. The intermolecular condensation of **9** with ammonia gives the cyclized intermediate **10**, which is then aromatized to produce triarylpyridine **3a**.

## Scheme 5. Plausible Reaction Mechanism



## Conclusions

In conclusion, we have developed an efficient and practicable method for the oxidative tandem C-C and C-N bonds formation by direct annulation of readily available arylalkylketones with benzylamines/heteroaryl methanamines to provide 2,4,6-trisubstituted pyridines. The reaction was proceeded with wide functional group tolerance using inexpensive  $\text{FeBr}_2$  as catalyst and molecular oxygen as green oxidant. Additionally, to highlight the advantages of the reaction protocol, several important 2,4,6-triaryl pyridine derivatives have been synthesized. Some key



intermediates were isolated to establish a plausible mechanistic route for this oxidative annulation reaction.

## Experimental Section

Unless mentioned specifically, all starting materials as well as solvents were procured commercially and performed directly without further purification. Reactions were conducted in an oven-dried schlenk tubes and round-bottom flasks. Melting points were recorded on a Büchi melting point apparatus and were uncorrected.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on 400 MHz and 100 MHz spectrometers respectively. Chemical shifts (in ppm) were recorded with respect to tetramethylsilane ( $\delta = 0$  ppm) internal reference standard in  $\text{CDCl}_3$  at room temperature. Proton coupling patterns were labeled as singlet (s), doublet (d), doublet of doublet (dd), triplet (t), multiplet (m). Mass of the samples were recorded with High-resolution mass spectra (HRMS) equipped with ESI source and a TOF detector. Column chromatography was performed on silica gel (100–200 mesh ASTM) using ethyl acetate/hexane as eluents. Thin-layer chromatography (TLC) was done on  $4 \times 15$  cm plates with a layer thickness of 0.2 mm.

### General Procedure for Synthesis of 2,4,6-Trisubstituted Pyridines

Benzylamines (1.2 mmol), arylalkylketones (2.0 mmol) and iron(II) bromide (10 mol%) were added into an oven-dried 10 mL Schlenk tube equipped with an oxygen balloon. The tube was immersed in silicon oil bath placed magnetic stirrer and heated at  $110^\circ\text{C}$  with constant magnetic stirring. The progress of the reaction was monitored with TLC at regular intervals of time. After completion of the reaction, the mixture was cooled to room temperature, diluted with  $\text{CH}_2\text{Cl}_2$  and dried over silica gel. The dried-up products were purified by silica gel (100–200 mesh) column chromatography employing hexane/ EtOAc as eluents.

### Characterization of products

**2,4,6-Triphenylpyridine (3a).** Yield: 93%, white solid, Melting point: 129-130 °C (Lit.: 130-132 °C)<sup>22</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.21-8.19 (m, 4H), 7.87 (s, 2H), 7.74-7.72 (m, 2H), 7.53-7.49 (m, 6H), 7.46-7.44 (m, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 157.4, 150.1, 139.5, 138.9, 129.1, 129.0, 128.9, 128.7, 127.1, 127.0, 117.1; HRMS (ESI, m/z): calcd for C<sub>23</sub>H<sub>17</sub>N [M + H]<sup>+</sup> 308.1426, found 308.1429.

**4-Pheny-2,6-bis(4-tolyl)pyridine (3b).** Yield: 92%, white solid, Melting point: 149-150 °C (Lit.: 148-150 °C)<sup>22</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.10 (d, *J* = 8.4 Hz, 4H), 7.84 (s, 2H), 7.75-7.73 (m, 2H), 7.54-7.45 (m, 3H), 7.32 (d, *J* = 8.4 Hz, 4H), 2.43 (s, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 157.3, 149.9, 139.2, 138.9, 136.8, 129.4, 129.0, 128.8, 127.2, 126.9, 116.5, 21.3; HRMS (ESI, m/z): calcd for C<sub>25</sub>H<sub>21</sub>N [M + H]<sup>+</sup> 336.1747, found 336.1748.

**2,6-Bis(4-methoxyphenyl)-4-phenylpyridine (3c).** Yield: 90%, white solid, Melting point: 124-125 °C (Lit.: 125-127 °C)<sup>22</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.17 (d, *J* = 9.2 Hz, 4H), 7.77 (s, 2H), 7.74-7.72 (m, 2H), 7.54-7.45 (m, 3H), 7.04 (d, *J* = 9.2 Hz, 4H), 3.88 (s, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 160.4, 156.9, 149.9,

139.2, 132.2, 128.9, 128.8, 128.3, 127.1, 115.6, 113.9, 55.3; HRMS (ESI, m/z): calcd for  $C_{25}H_{21}NO_2$  [M + H]<sup>+</sup> 368.1645, found 368.1647.

**2,6-Bis(3-methoxyphenyl)-4-phenylpyridine (3d).** Yield: 89%, white solid, Melting point: 75-76 °C (Lit.: 76-78 °C)<sup>22</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.88 (s, 2H), 7.82 (s, 2H), 7.78 (d, *J* = 7.2 Hz, 2H), 7.67 (d, *J* = 8.0 Hz, 2H), 7.47-7.37 (m, 5H), 6.99-6.96 (m, 2H), 3.83 (s, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 159.5, 156.4, 149.4, 140.4, 138.2, 129.1, 128.9, 128.5, 126.6, 118.9, 116.5, 114.0, 112.2, 54.6; HRMS (ESI, m/z): calcd for  $C_{25}H_{21}NO_2$  [M + H]<sup>+</sup> 368.1645, found 368.1647.

**2,6-Bis(2-methoxyphenyl)-4-phenylpyridine (3e).** Yield: 85%, white solid, Melting point: 151-152 °C (Lit.: 152-153 °C)<sup>22</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.99 (s, 2H), 7.97-7.94 (m, 2H), 7.74-7.72 (m, 2H), 7.53-7.36 (m, 5H), 7.13-7.09 (m, 2H), 7.03 (d, *J* = 7.6 Hz, 2H), 3.90 (s, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 157.1, 155.9, 147.7, 139.5, 131.6, 129.7, 129.6, 128.9, 127.4, 121.4, 121.3, 121.1, 111.4, 55.8; HRMS (ESI, m/z): calcd for  $C_{25}H_{21}NO_2$  [M + H]<sup>+</sup> 368.1645, found 368.1649.

**2,6-Bis(4-*N,N*-Dimethylphenyl)-4-phenylpyridine (3f).** Yield: 79%, reddish brown solid, Melting point: 133-134 °C (Lit.: 136-135 °C)<sup>26</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.13 (d, *J* = 9.2 Hz, 4H), 7.75-7.73 (m, 2H), 7.69 (s, 2H), 7.53-7.44 (m, 3H), 6.83 (d, *J* = 9.2 Hz, 4H), 3.03 (s, 12H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 157.1,

150.9, 149.5, 139.8, 128.9, 128.5, 127.9, 127.8, 127.1, 114.4, 112.2, 40.5; HRMS (ESI, m/z): calcd for  $C_{27}H_{27}N_2$  [M + H]<sup>+</sup> 394.2278, found 394.2286.

**2,6-Bis(4-chlorophenyl)-4-phenylpyridine (3g).** Yield: 94%, white solid, Melting point: 176-177 °C (Lit.: 178-180 °C)<sup>22</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.13 (dt, *J* = 9.2, 2.4 Hz, 4H), 7.86 (s, 2H), 7.74-7.72 (m, 2H), 7.56-7.52 (m, 3H), 7.51-7.47 (m, 4H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 156.3, 150.6, 138.7, 137.7, 135.3, 129.2, 128.9, 128.3, 127.1, 117.1; HRMS (ESI, m/z): calcd for  $C_{23}H_{15}NCl_2$  [M + H]<sup>+</sup> 376.0654, found 376.0659.

**2,6-Bis(2-chlorophenyl)-4-phenylpyridine (3h).** Yield: 87%, Pale yellow solid, Melting point: 146-147 °C (Lit.: 147-149 °C)<sup>22</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.87 (s, 2H), 7.75-7.72 (m, 4H), 7.52-7.42 (m, 5H), 7.39-7.33 (m, 4H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 157.1, 148.3, 139.2, 138.2, 132.3, 131.8, 130.1, 129.6, 129.1, 127.2, 127.0, 121.5; HRMS (ESI, m/z): calcd for  $C_{23}H_{15}NCl_2$  [M + H]<sup>+</sup> 376.0654, found 376.0635.

**2,6-Bis(4-bromophenyl)-4-phenylpyridine (3i).** Yield: 96%, white solid, Melting point: 192-193 °C (Lit.: 194-196 °C)<sup>22</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.07 (d, *J* = 8.4 Hz, 4H), 7.87 (s, 2H), 7.74-7.72 (d, *J* = 7.2 Hz, 2H), 7.64 (d, *J* = 8.4 Hz, 4H), 7.57-7.48 (m, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 156.4, 150.6, 138.6, 138.2,

131.9, 129.2, 128.6, 127.2, 123.6, 117.2; HRMS (ESI, m/z): calcd for  $C_{23}H_{15}NBr_2$   $[M + H]^+$  463.9644, found 463.9645.

**2,6-Di(naphthalen-1-yl)-4-phenylpyridine (3j).** Yield: 81%, yellow oil,  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  8.29-8.27 (m, 2H), 7.91 (d,  $J = 7.6$  Hz, 4H), 7.86 (s, 2H), 7.78-7.75 (m, 4H), 7.59-7.55 (m, 2H), 7.53-7.42 (m, 7H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  159.6, 149.0, 138.7, 138.2, 133.9, 131.3, 129.2, 128.9, 128.4, 127.7, 127.2, 126.4, 125.8, 125.7, 125.4, 121.5; HRMS (ESI, m/z): calcd for  $C_{31}H_{21}N$   $[M + H]^+$  408.1747, found 408.1761.

**Phenyl-10H,12H-diindeno[1,2-b:2',1'-e]pyridine (3k).** Yield: 78%, yellow solid, Melting point: 291-292 °C (Lit.: 293-295 °C)<sup>27</sup>;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  8.29 (d,  $J = 7.6$  Hz, 2H), 7.57-7.55 (m, 4H), 7.53-7.48 (m, 4H), 7.42-7.38 (m, 3H), 3.84 (s, 4H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  160.1, 144.0, 143.3, 141.3, 137.3, 133.4, 128.8, 128.2, 128.2, 127.1, 124.9, 121.1, 33.9; HRMS (ESI, m/z): calcd for  $C_{25}H_{17}N$   $[M + H]^+$  332.1434, found 332.1442.

**7-Phenyl-5,6,8,9-tetrahydrodibenzo[*c,h*]acridine (3l).** Yield: 73%, yellow solid, Melting point: 161-162 °C (Lit.: 160-162 °C)<sup>22</sup>;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  8.57 (d,  $J = 8.0$  Hz, 2H), 7.50-7.46 (m, 2H), 7.43-7.38 (m, 3H), 7.36-7.26 (m, 3H), 7.19 (d,  $J = 7.6$  Hz, 3H), 2.84-2.80 (m, 4H), 2.66-2.62 (m, 4H);  $^{13}C$  NMR (100 MHz,

CDCl<sub>3</sub>)  $\delta$  150.0, 147.4, 137.9, 137.8, 135.3, 128.7, 128.6, 128.6, 128.5, 127.5, 127.4, 126.9, 125.3, 28.1, 25.8; HRMS (ESI, m/z): calcd for C<sub>27</sub>H<sub>21</sub>N [M + H]<sup>+</sup> 360.1747, found 360.1760.

**4-Phenyl-2,6-di(thiophen-2-yl)pyridine (3m).** Yield: 82%, white solid, Melting point: 107-108 °C (Lit.: 108-110 °C)<sup>22</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.72-7.69 (m, 6H), 7.55-7.48 (m, 3H), 7.43-7.42 (m, 2H), 7.15-7.13 (m, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  152.6, 150.1, 144.9, 138.5, 129.1, 127.9, 127.8, 127.1, 124.8, 115.1; HRMS (ESI, m/z): calcd for C<sub>19</sub>H<sub>13</sub>NS<sub>2</sub> [M + H]<sup>+</sup> 320.0562, found 320.0543.

**2,6-Di(furan-2-yl)-4-phenylpyridine (3n).** Yield: 67%, white solid, Melting point: 102-103 °C (Lit.: 102-104 °C)<sup>22</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.83 (s, 2H), 7.78 (d, *J* = 7.2 Hz, 2H), 7.59-7.58 (m, 2H), 7.55-7.47 (m, 3H), 7.24 (d, *J* = 3.6 Hz, 2H), 6.59 (dd, *J* = 3.2, 1.6 Hz, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  153.7, 149.6, 143.3, 138.3, 129.1, 128.9, 126.9, 114.7, 112.0, 109.1; HRMS (ESI, m/z): calcd for C<sub>19</sub>H<sub>13</sub>NO<sub>2</sub> [M + H]<sup>+</sup> 288.1019, found 288.1022.

**4-(4-Methylphenyl)-2,6-diphenylpyridine (4a).** Yield: 93%, white solid, Melting point: 108-109 °C (Lit.: 109-111 °C)<sup>22</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.19 (d, *J* = 7.6 Hz, 4H), 7.84 (s, 2H), 7.61 (d, *J* = 8.4 Hz, 2H), 7.51-7.48 (m, 4H), 7.44-7.40 (m, 2H), 7.29 (d, *J* = 7.6 Hz, 2H), 2.39 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$

157.3, 149.9, 139.6, 138.9, 135.9, 129.8, 128.9, 128.6, 127.1, 126.9, 116.8, 21.2;

HRMS (ESI, m/z): calcd for  $C_{24}H_{19}N$   $[M + H]^+$  322.1590, found 322.1609.

**4-(4-Methoxyphenyl)-2,6-diphenylpyridine (4b).** Yield: 95%, pale yellow solid, Melting point: 101-102 °C (Lit.: 103-105 °C)<sup>22</sup>;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  8.26 (d,  $J = 7.6$  Hz, 4H), 7.88 (s, 2H), 7.71 (d,  $J = 8.8$  Hz, 2H), 7.57 (t,  $J = 7.6$  Hz, 4H), 7.51-7.48 (m, 2H), 7.06 (d,  $J = 8.4$  Hz, 2H), 3.86 (s, 3H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  160.3, 157.3, 149.4, 139.6, 131.0, 128.9, 128.6, 128.2, 127.0, 116.4, 114.4, 55.2; HRMS (ESI, m/z): calcd for  $C_{24}H_{19}NO$   $[M + H]^+$  338.1539, found 338.1541.

**4-(3-Methoxyphenyl)-2,6-diphenylpyridine (4c).** Yield: 94%, pale yellow solid, Melting point: 124-125 °C (Lit.: 124-127 °C)<sup>23</sup>;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  8.21-8.19 (m, 4H), 7.88 (s, 2H), 7.54-7.50 (m, 5H), 7.46-7.42 (m, 3H), 7.33 (d,  $J = 7.6$  Hz, 1H), 7.02 (dd,  $J = 7.6, 2.4$  Hz, 1H), 3.90 (s, 3H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  160.1, 157.5, 150.1, 140.5, 139.5, 130.2, 129.0, 128.7, 127.1, 119.6, 117.2, 114.2, 112.9, 55.4; HRMS (ESI, m/z): calcd for  $C_{24}H_{19}NO$   $[M + H]^+$  338.1539, found 338.1545.

**4-(2-Methoxyphenyl)-2,6-diphenylpyridine (4d).** Yield: 91%, white solid, Melting point: 119-120 °C (Lit.: 120-121 °C)<sup>24</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.18 (d, *J* = 6.8 Hz, 4H), 7.86 (s, 2H), 7.52-7.48 (m, 5H), 7.47-7.41 (m, 3H), 7.12-7.05 (m, 2H), 3.87 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 156.7, 156.6, 147.9, 139.8, 130.5, 130.0, 128.8, 128.6, 128.4, 127.1, 121.0, 119.7, 111.4, 55.7; HRMS (ESI, *m/z*): calcd for C<sub>24</sub>H<sub>19</sub>NO [M + H]<sup>+</sup> 338.1539, found 338.1548.

**4-(4-Fluorophenyl)-2,6-diphenylpyridine (4e).** Yield: 84%, yellow solid, Melting point: 136-137 °C (Lit.: 138-140 °C)<sup>22</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.19 (d, *J* = 8.4 Hz, 4H), 7.82 (s, 2H), 7.69 (dd, *J* = 8.4, 5.6 Hz, 2H), 7.53-7.49 (m, 4H), 7.46-7.42 (m, 2H), 7.23-7.18 (m, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 163.3 (d, <sup>1</sup>*J*<sub>CF</sub> = 247.9 Hz), 157.5, 149.1, 139.4, 135.1, 129.1, 128.9 (d, <sup>3</sup>*J*<sub>CF</sub> = 8.6 Hz), 128.7, 127.1, 116.9, 116.1 (d, <sup>2</sup>*J*<sub>CF</sub> = 21.9 Hz); HRMS (ESI, *m/z*): calcd for C<sub>23</sub>H<sub>16</sub>NF [M + H]<sup>+</sup> 326.1340, found 326.1364.

**4-(4-Chlorophenyl)-2,6-diphenylpyridine (4f).** Yield: 89%, yellow solid, Melting point: 111-112 °C (Lit.: 112-114 °C)<sup>22</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.19 (d, *J* = 6.8 Hz, 4H), 7.84 (s, 2H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.54-7.49 (m, 6H), 7.47-7.44 (m, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 157.6, 148.9, 139.4, 137.4, 135.2, 129.3,



129.2, 128.7, 128.4, 127.1, 116.8; HRMS (ESI, m/z): calcd for  $C_{23}H_{16}NCl$   $[M + H]^+$  342.1044, found 342.1066.

**4-(2-Chlorophenyl)-2,6-diphenylpyridine (4g).** Yield: 85%, white solid,  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  8.19 (d,  $J = 8.4$  Hz, 4H), 7.78 (s, 2H), 7.56-7.49 (m, 5H), 7.46-7.42 (m, 3H) 7.40-7.36 (m, 2H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  156.9, 148.5, 139.3, 138.5, 132.3, 130.9, 130.3, 129.7, 129.1, 128.7, 127.2, 127.1, 119.5; HRMS (ESI, m/z): calcd for  $C_{23}H_{16}NCl$   $[M + H]^+$  342.1044, found 342.1066.

**4-(3,4-Dichlorophenyl)-2,6-diphenylpyridine (4h).** Yield: 83%, white solid, Melting point: 95-96 °C;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  8.19 (d,  $J = 8.0$  Hz, 4H), 7.82-7.80 (m, 3H), 7.60-7.56 (m, 2H), 7.55-7.43 (m, 6H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  157.8, 147.7, 139.1, 138.9, 133.9, 133.4, 131.1, 129.3, 129.0, 128.8, 127.1, 126.4, 116.6; HRMS (ESI, m/z): calcd for  $C_{23}H_{15}NCl_2$   $[M + H]^+$  376.0654, found 376.0622.

**4-(3,5-Bis(trifluoro)phenyl)-2,6-diphenylpyridine (4i).** Yield: 57%, white solid, Melting point: 149-150 °C;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  8.22-8.19 (m, 4H), 8.14 (s, 2H), 7.99 (s, 1H), 7.84 (s, 2H), 7.56-7.52 (m, 4H), 7.49-7.46 (m, 2H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  158.1, 147.2, 141.4, 138.8, 132.7, 132.4, 129.5, 129.3, 128.8,

128.7, 127.3, 127.1, 124.5, 122.6, 121.8, 116.8; HRMS (ESI, m/z): calcd for  $C_{25}H_{15}NF_6$  [M + H]<sup>+</sup> 444.1181, found 444.1193.

**4-(1,3-Benzodioxol-5-yl)-2,6-diphenylpyridine (4j).** Yield: 67%, pale yellow solid, Melting point: 151-152 °C (Lit.: 152-153 °C)<sup>25</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.21-8.18 (m, 4H), 7.82 (s, 2H), 7.54-7.49 (m, 4H), 7.47-7.43 (m, 2H), 7.27-7.23 (m, 2H), 6.96 (d, *J* = 7.6 Hz, 1H), 6.06 (s, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 157.6, 149.8, 148.6, 139.7, 133.2, 129.1, 128.8, 127.2, 121.2, 116.9, 108.9, 107.6, 101.6; HRMS (ESI, m/z): calcd for  $C_{24}H_{17}NO_2$  [M + H]<sup>+</sup> 352.1332, found 352.1336.

**4-(1-Naphthaylenyl)-2,6-diphenylpyridine (4k).** Yield: 85%, white crystalline solid, Melting point: 133-134 °C (Lit.: 134-135 °C)<sup>24</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.22 (d, *J* = 6.8 Hz, 4H), 7.96 (d, *J* = 8.4 Hz, 2H), 7.85 (s, 2H), 7.61-7.57 (m, 2H), 7.55-7.49 (m, 7H), 7.46-7.43 (m, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 156.9, 150.2, 139.4, 138.0, 133.8, 130.9, 129.1, 128.8, 128.7, 128.5, 127.1, 126.7, 126.6, 126.2, 125.4, 120.1; HRMS (ESI, m/z): calcd for  $C_{27}H_{19}N$  [M + H]<sup>+</sup> 358.1590, found 358.1592.

**2,6-Diphenyl-4-(thiophen-2-yl)pyridine (4l).** Yield: 87%, white solid, Melting point: 162-163 °C (Lit.: 162-164 °C)<sup>22</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.29-8.26 (m, 4H), 7.94 (s, 2H), 7.67-7.66 (m, 1H), 7.64-7.59 (m, 4H), 7.57-7.53 (m, 2H), 7.49-7.48 (m, 1H), 7.24-7.22 (m, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 157.5, 142.8, 141.8, 139.2, 129.1, 128.6, 128.3, 127.0, 126.8, 125.2, 115.2; HRMS (ESI, m/z): calcd for C<sub>21</sub>H<sub>15</sub>NS [M + H]<sup>+</sup> 314.0448, found 314.1000.

**4-(Furan-2-yl)-2,6-diphenylpyridine (4m).** Yield: 74%, white solid, Melting point: 168-169 °C (Lit.: 170-171 °C)<sup>24</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.21-8.18 (m, 4H), 7.94 (s, 2H), 7.59 (d, *J* = 2.0 Hz, 1H), 7.54-7.49 (m, 4H), 7.47-7.42 (m, 2H), 6.99-6.98 (m, 1H), 6.58 (dd, *J* = 3.6, 2.0 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 157.5, 151.9, 143.6, 139.4, 139.0, 129.1, 128.7, 127.0, 112.9, 112.1, 108.5; HRMS (ESI, m/z): calcd for C<sub>21</sub>H<sub>15</sub>NO [M + H]<sup>+</sup> 298.1226, found 298.1227.

**2,4,6-Tri(thiophen-2-yl)pyridine (5a).** Yield: 87%, white solid, Melting point: 128-129 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.70-7.69 (m, 2H), 7.66 (s, 2H), 7.58-7.57 (m, 1H), 7.43-7.42 (m, 3H), 7.18-7.13 (m, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 152.7, 144.6, 142.8, 141.3, 128.3, 127.9, 127.9, 127.0, 125.4, 124.9, 113.2; HRMS (ESI, m/z): calcd for C<sub>17</sub>H<sub>11</sub>NS<sub>3</sub> [M + H]<sup>+</sup> 326.0126, found 326.0121.

**4-(Furan-2-yl)-2,6-di(thiophen-2-yl)pyridine (5b).** Yield: 72%, white solid, Melting point: 121-122 °C (Lit.: 120-122 °C)<sup>19b</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.71 (s, 2H), 7.69-7.68 (m, 2H), 7.57 (d, *J* = 2.0 Hz, 1H), 7.42-7.40 (m, 2H), 7.13-7.11 (m, 2H), 6.94 (d, *J* = 3.2 Hz, 1H), 6.55 (dd, *J* = 3.2, 1.6 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 152.6, 151.4, 144.8, 143.7, 143.7, 138.9, 127.9, 127.8, 124.8, 112.1, 110.9, 108.8; HRMS (ESI, *m/z*): calcd for C<sub>17</sub>H<sub>11</sub>NOS<sub>2</sub> [M + H]<sup>+</sup> 310.0355, found 310.0357.

***N*-Benzylidenebenzylamine (6).** Yield: 89%, yellow oil,<sup>18b</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.29 (s, 1H), 7.69-7.68 (m, 2H), 7.32-7.29 (m, 3H), 7.26-7.24 (m, 4H), 7.19-7.13 (m, 1H), 4.73 (s, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 161.9, 139.2, 136.1, 130.7, 128.5, 128.4, 128.2, 127.9, 126.9.

**3-(Benzylimino)-1,3-diphenylpropan-1-one (7).** Yield: 68%, white semi solid; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.02-8.00 (m, 4H), 7.65-7.60 (m, 2H), 7.53-7.47 (m, 3H), 7.44-7.37 (m, 6H), 4.31 (s, 2H), 4.08 (s, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 192.9, 144.8, 135.4, 135.3, 133.9, 132.7, 130.5, 128.9, 128.7, 128.5, 128.4, 128.4, 128.3, 125.7, 121.9, 60.9, 59.3; HRMS (ESI, *m/z*): calcd for C<sub>22</sub>H<sub>19</sub>NO [M + H]<sup>+</sup> 314.1539, found 314.1535.

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## The Optimum Solution of Degenerate Transportation Problem

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**Abstract:** The Solution of a Transportation Problem is obtained in two phases. The first phase is finding the initial basic feasible solution by using various methods. The optimal solution is obtained either by using stepping stone method or by MODI method in the second phase. Here we proposed the MODI method with modifications to solve the degenerate transportation problem. This is also illustrated with numerical example.

**Key Words:** Transportation problem, degeneracy, difference cost, optimum solution.

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### I. INTRODUCTION:

The Transportation model is a special case of Linear programming models, widely used in the areas of inventory control, employment scheduling, aggregate planning and personal assignment among other. It can be solved by the regular simplex method. Due to its special structure of the model, the stepping stone method (Charnes and Cooper, 1954) was developed for the efficiency reason. While the simplex method is not suitable for the transportation problem, especially for those large scale transportation problems.

Other research results can be found from Ford and Fulkerson (1956), Balinski and Gomory (1964), Muller-Merback (1966), Grigoriadis and Walker (1968), Glover et al (1974), Shafiq and Goyal (1988) and Arsham and Kahn (1989). A brief review on this area was presented by Gass (1990).

The stepping stone method is very popularly used to solve transportation problem. It is a technique for moving from an initial feasible solution to an optimal solution by evaluating all non-basic cells that are empty cells. It adopts the path tracing approach to evaluate an empty cell. Another way to evaluate empty cells is the modification distribution method (MODI). MODI method is similar to the stepping stone method.

One serious problem of the stepping stone method is the degeneracy, that is too few basic cells in a feasible solution. Some researchers carried out to solve degeneracy problem (Goyal 1984 and Shafiq and Goyal, 1988).

The simplex degeneracy doesn't cause any serious difficulty, but it can cause computational problem in transportation technique. In stepping stone method it will not be possible to make closed paths for each and every vacant cell and hence net evaluations of all the vacant cells cannot be calculated. If MODI method is applied, it will not be possible to find all the dual variables  $u_i, v_j$ , since the number of allocated cells and their  $c_{ij}$  values is not enough. It is thus necessary to identify a degenerate transportation problem and take appropriate steps to avoid computational difficulty. Degeneracy can occur in the initial solution or during some subsequent iteration.

**Degeneracy in the initial solution:** Normally, while finding the initial solution, any allocation made either satisfies supply or demand, but not both. If however, both supply and demand are satisfied simultaneously row as well as columns are cancelled simultaneously and the number of allocations become less than  $m + n - 1$ .

#### Degeneracy during subsequent iteration:

Sometimes even if the starting feasible solution is non-degenerate, degeneracy may develop later at some subsequent iteration. This happens when the selection of the entering variable (least value in the closed loop that has been assigned a negative sign), causes two or more current basic variables to become zero.

#### Optimality test:

The optimality test for given Basic feasible solution of the transportation problem may be summarized as follows;

- i) If all  $d_{ij} \geq 0$  solution under test is optimal
- ii) Alternate optimal solution exists, if none is negative but any is zero.

- iii) Solution under test is not optimal, if any  $d_{ij}$  is negative, then further improvement is required. Where  $d_{ij} = c_{ij} - u_i - v_j$

**MODI's Algorithm:**

1. First, construct a transportation table entering the origin capacities  $a_i$ , the destination requirements  $b_j$  and the costs  $c_{ij}$ .
2. Find an initial basic feasible solution.
3. For all the basic variables  $x_{ij}$ , solve the system of equation  $c_{ij} + (u_i + v_j) = 0$  for all  $i, j$  for which cell  $(i, j)$  is in the basis, starting initially with some  $u_i = -c_{ij}$  and entering successfully the values of  $u_i, v_j$  in the transportation table.
4. Compute the cost differences  $d_{ij} = c_{ij} - u_i - v_j$
5. Apply the optimality test, if atleast one  $d_{ij} < 0$  (negative) select the variable  $x_{rs}$  (having the most negative  $d_{rs}$ ) to enter the basis.
6. Let the variables  $x_{rs}$  enter the basis. Allocate an unknown quantity  $\theta$  to the cell  $(r, s)$ . Then construct a loop that starts and ends at the cell  $(r, s)$  and connects some of the basic cells. The amount  $\theta$  is added to and subtracted from the transition cells of the loop in such a manner that availabilities and requirements remain satisfied.
7. Assign the largest possible value to  $\theta$  in such a way that the value of atleast one basic variable becomes zero and other basic variables remain non negative. The basic cell, whose allocation has been made zero, will leave the basis.
8. Now return to step 3 and then repeat the process until an optimum basic feasible solution is obtained.

In MODI method, if degeneracy occurs, assign  $\epsilon$  to the empty cell. To resolve the degeneracy, allocate an extremely small amount of goods to one or more of the empty cells so that a number of occupied cells becomes  $m + n - 1$ . The cell containing this extremely small allocation is considered to be an occupied cell, subject to the following assumptions

- i)  $\epsilon < x_{ij}, x_{ij} > 0$ , ii)  $x_{ij} + \epsilon = x_{ij} - \epsilon$  iii)  $\epsilon + 0 = \epsilon$

**Proposed Method:**

1. In the case of degeneracy, we face the problem in two steps of MODI 's algorithm:
  - i) In finding the dual variables  $u_i, v_j$  successively in the transportation table. In such a case we consider  $u_i = -c_{ij}, v_j = 0$  for the basic cell.
  - ii) Construct the loop, starts at the most negative cell and ends with the same cell connecting some basic cells in such a way that at-least one of the basic cell should leave the basis in the MODI's algorithm.

**Numerical Example:**

**Table 1**

$S_i/D_j$	$D_1$	$D_2$	$D_3$	$D_4$	$D_5$	source
$S_1$	4	7	3	8	2	4
$S_2$	1	4	7	3	8	7
$S_3$	7	2	4	7	7	9
$S_4$	4	8	2	4	7	2
Destination	8	3	7	2	2	

Step1: The initial feasible solution by North - West corner rule is in the following table. Degeneracy occurs in the initial stage. Now apply Proposed method to find the optimal solution. Non -basic cells are denoted by (.)

**Table 2**

$S_i/D_j$	$D_1$	$D_2$	$D_3$	$D_4$	$D_5$	$u_i$
$S_1$	4(4)	7(0)	3(-1)	8(1)	2(-2)	-4
$S_2$	1(4)	4(3)	7(6)	3(-1)	8(7)	-1
$S_3$	7(3)	2(-5)	4(7)	7(2)	7(3)	-4
$S_4$	4(-3)	8(-2)	2(-5)	4(-6)	7(2)	-7
$v_j$	0	-3	0	-3	0	

The most negative cost difference is in the cell (4,4) Now construct the loop by using the cells (4,4),(4,5),(3,5) and (3,4) and  $\theta = \min(2,2) = 2$

$S_i/D_j$	$D_1$	$D_2$	$D_3$	$D_4$	$D_5$	$u_i$
$S_1$	4(4)	7(0)	3(-1)	8(4)	2(-5)	-4
$S_2$	1(4)	4(3)	7(6)	3(2)	8(4)	-1
$S_3$	7(3)	2(-5)	4(7)	7(3)	7(2)	-4
$S_4$	4(0)	8(1)	2(-2)	4(2)	7(0)	-4
$v_j$	0	-3	0	0	-3	

The most negative cost difference is in the cell (3,2), construct the loop by using the cells (3,2),(3,5),(1,5),(1,1),(2,1),(2,2) and (3,2).  $\theta = \min(2,4,3) = 2$

$S_i/D_j$	$D_1$	$D_2$	$D_3$	$D_4$	$D_5$	$u_i$
$S_1$	4(2)	7(0)	3(-6)	8(4)	2(2)	-4
$S_2$	1(6)	4(1)	7(1)	3(2)	8(9)	-1
$S_3$	7(8)	2(2)	4(7)	7(8)	7(10)	1
$S_4$	4(0)	8(1)	2(-7)	4(2)	7(5)	-4
$v_j$	0	-3	-5	0	2	

The most negative difference is in the cell (4,3), construct the closed loop (4,3),(4,4),(3,4) and (4,3).  $\theta = \min(2,4, ) = 2$

$S_i/D_j$	$D_1$	$D_2$	$D_3$	$D_4$	$D_5$	$u_i$
$S_1$	4(2)	7(0)	3(-6)	8(-4)	2(2)	-4
$S_2$	1(6)	4(1)	7(1)	3(-6)	8(9)	-1
$S_3$	7(8)	2(2)	4(5)	7(2)	7(10)	1
$S_4$	4(7)	8(8)	2(2)	4(-1)	7(5)	3
$v_j$	0	-3	-5	-8	2	

The most negative is in the cell (1,4) and construct the loop with the cells (1,4),(1,1),(2,1),(2,2),(3,2),(3,3) and (1,4).  $\theta = \min(2,1,5) = 1$

$S_i/D_j$	$D_1$	$D_2$	$D_3$	$D_4$	$D_5$	$u_i$
$S_1$	4(1)	7(6)	3(1)	8(2)	2(2)	-4
$S_2$	1(7)	4(6)	7(7)	3(0)	8(9)	-1
$S_3$	7(2)	2(3)	4(4)	7(2)	7(4)	-5
$S_4$	4(1)	8(8)	2(2)	4(-1)	7(6)	-3
$v_j$	0	3	1	-2	2	

The most negative is in the cell (4,4). Construct the closed loop with the cells (4,4),(4,3),(3,3),(3,4) and (4,4).  $\theta = \min(2,2) = 2$

$S_i/D_j$	$D_1$	$D_2$	$D_3$	$D_4$	$D_5$	$u_i$
$S_1$	4(1)	7(6)	3(1)	8(3)	2(2)	-4
$S_2$	1(7)	4(6)	7(7)	3(1)	8(9)	-1
$S_3$	7(2)	2(3)	4(6)	7(1)	7(4)	-5
$S_4$	4(1)	8(8)	2(0)	4(2)	7(6)	-3
$v_j$	0	3	1	-1	2	

Since all non basic cells have non negative cost differences . The optimal solution is  $x_{11} = 1, x_{13} = 1, x_{15} = 2, x_{21} = 7, x_{22} = 3, x_{33} = 6, x_{43} = 0, x_{44} = 2$   
The total cost is 56.

## II. CONCLUSION:

In MODI method if degeneracy occurs, then we take the small number  $\epsilon$  to avoid the two problems finding the  $u_i, v_j$  values and construction of loop for the most negative cost difference. The suggested method

ensures improvement of the solution or recognition of its optimality, thereby avoiding unnecessary iterations that resulting in shifting of the  $\epsilon$  from an independent cell to another.

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## A New Algorithm for initial basic feasible solution of Transportation Problem

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**Abstract:** In literature, there are several algorithms for initial basic feasible solution of transportation problem. In this paper, a new algorithm is proposed for solving the transportation problem almost nearer to the optimal solution. To illustrate the proposed algorithm a numerical example is solved and the results are compared with the results of existing approaches. This approach is easy to understand and to apply on real life transportation problems for the decision makers.

**Key words:** Transportation problem, transportation cost, initial basic feasible solution.

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### I. Introduction

The very interesting class of "allocation Methods" which is applied to a lot of very practical problems generally called "Transportation Problems". Transportation model was first introduced by F.L. Hitchcock in 1941[5]. The transportation problems can be modeled as standard linear programming problem, which can be solved by the simplex method. Later on, it was further improved by T.C. Koopmans in 1949 and G.B. Dantzig in 1951. Charnes and Cooper[6] developed the stepping stone method which provides an alternative way of determining the simplex method information. An initial basic feasible solution (IBFS) for the transportation problem can be obtained by using the north-west corner rule, row minima, column minima, least cost entry method or Vogel's approximation method [3]. The modified distribution method is useful for finding the optimal solution for the transportation problem.

**Definition:** The transportation problem is to transport various amounts of single homogeneous commodities that are initially stored at various sources, to different destinations in such a way that the total transportation cost is minimum.

#### Requirements Assumption:

Each source has a fixed supply of units where this entire supply must be distributed to the destination (we let  $a_i$  denote the number of units being supplied by the source  $i, i = 1$  to  $m$ )

Similarly, each destination has a fixed demand for units, where this entire demand must be received from the source. (We let  $b_j$  denote the number of units being received by destination  $j, j = 1$  to  $n$ ).

#### Cost Assumption:

The cost of distributing units from any particular source to any particular destination is directly proportional to the number of units distributed.

The Necessary and Sufficient condition for a Transportation problem to have feasible solution is  $\sum_{i=1}^m a_i = \sum_{j=1}^n b_j$ .

#### Mathematical Formation of Transportation problem:

Let  $a_i$  = Quantity of product available at source  $i$

$b_j$  = quantity of product required at destination  $j$

$c_{ij}$  = Cost of transporting one unit of product from source  $i$  to destination  $j$

$x_{ij}$  = Quantity of product transported from source  $i$  to destination  $j$

Then the transportation model would be in the form as follows:

Minimize  $Z = \sum_{i=1}^m \sum_{j=1}^n c_{ij} x_{ij}$

Subject to  $\sum_{j=1}^n x_{ij} = a_i \quad i = 1$  to  $m$

$$\sum_{i=1}^m x_{ij} = b_j \quad j = 1$$
 to  $n$ 
$$\forall i, j \quad x_{ij} \geq 0$$

**Formation of Transportation Table:**

$S_i/D_j$	$D_1$	$D_2$	.....	$D_n$	Availability
$S_1$	$c_{11}(x_{11})$	$c_{12}(x_{12})$	.....	$c_{1n}(x_{1n})$	$a_1$
$S_2$	$c_{21}(x_{21})$	$c_{22}(x_{22})$	.....	$c_{2n}(x_{2n})$	$a_2$
.....	.....	.....	.....	.....	.....
$S_m$	$c_{m1}(x_{m1})$	$c_{m2}(x_{m2})$	.....	$c_{mn}(x_{mn})$	$a_m$
Demand	$b_1$	$b_2$	.....	$b_n$	$\sum_{i=1}^m a_i = \sum_{j=1}^n b_j$

**Algorithm for Transportation Method:**

The Transportation Problem can be solved by the following steps:

Step1: Formulate the given problem in the matrix form.

Step 2: Obtain an Initial feasible solution.

Step3: Test the optimality of Initial solution.

Step4: Update the solution accordingly and repeat Step 3 until the most feasible solution is reached.

**Methodology for transportation problem:**

The different methods for finding the initial basic feasible solution are:

1. Northwest Corner method
2. Least cost method
3. Vogel's approximation method
4. Row Minimum Method
5. Column Minimum Method

The Vogel's approximation method is an iterative method depends on penalty costs, which gives the initial basic feasible solution nearer to optimal solution.

**Advantages of proposed method:**

The proposed method has the following advantages:

- i) In the proposed method linear programming techniques are not used.
- ii) The proposed method is not an iterative method.
- iii) It is easy to understand and to apply.
- iv) The proposed method takes less time to compute.

The proposed (ADS)method to find the initial feasible solution is:

Step 1: construct the transportation table from the given Transportation Problem.

Step2: Check whether the Transportation Problem is balanced or not, if not make it balance.

Step3: Select the minimum and next to minimum cost in each row. If the minimum value repeated, then select it again as the next to minimum value. Sometimes the next to minimum value repeats, in such a case select the cell which has maximum allocation. As well as, check all the columns, if in any one of the columns the cells are not selected then select minimum and next to minimum of the corresponding column the column then select the minimum and next to minimum cost in the corresponding column.

Step4: For each selected cell, Compute the sum of supply and demand of the of theselected cells in the corresponding row and column excluding that cell and then divide with total number of selected cells in the corresponding row and column excluding that cell. Let it call as Average Demand and Supply (ADS).

Step5: Now choose the minimum average demand and supply (ADS) cell and Allocate the maximum possible to the ADS cell. If ADS is repeated twice then select the cell which has least cost in between them.

Step6: There may arise the following cases: Case i): minimum  $(a_i, b_j) = a_i$ , then allocate  $x_{ij} = a_i$ , and cross the  $i^{\text{th}}$  row and reduce  $b_j$  by  $(b_j - a_i)$ . Go to step 5. Case ii) minimum  $(a_i, b_j) = b_j$ , allocate then  $x_{ij} = b_j$ , and cross the  $j^{\text{th}}$  row and reduce  $a_i$  by  $(a_i - b_j)$ . Go to step 5. Case iii) minimum  $a_i = b_j$ , then allocate  $x_{ij} = b_j$ , and cross either the  $[j]$  row or  $i^{\text{th}}$  row but not both. Go to step 5

Step7: Repeat step 5 and step 6 until all the demands are satisfied and all the Supplies are exhausted.

Step 8: Calculate the total cost of the transportation table

**Table 1**

$S_i/D_j$	$D_1$	$D_2$	$D_3$	$D_4$	source
$S_1$	3	1	7	4	300
$S_2$	2	6	5	9	400
$S_3$	8	3	3	2	500
Destination	250	350	400	200	

**Solution:**

Step2: the Given Transportation table is balanced.

Step3: the minimum and next to minimum cost in each row are shown in the following table in each row.

**Table 1**

$S_i/D_j$	$D_1$	$D_2$	$D_3$	$D_4$	source
$S_1$	3	1	7	4	300
$S_2$	2	6	5	9	400
$S_3$	8	3	3	2	500
Destination	250	350	400	200	

Step4: Calculate the ADS for each selected cell. The corresponding ADS are 650,700,675,650,675 and 850 respectively. Among these 650 is the minimum ADS, which occur for two cells, select the minimum cost cell, in the corresponding row. 1 is minimumCost. Allocate  $\min(300,350)=300$  to the corresponding cell. Delete the Row and reduce the corresponding column demand by 50. Repeat the allocation procedure for ADS cells until all the demands are satisfied and supplies are exhausted. The allocation transportation table is presented in table 2.

**Table 2**

$S_i/D_j$	$D_1$	$D_2$	$D_3$	$D_4$
$S_1$	3	1(300)	7	4
$S_2$	2(250)	6	5(150)	9
$S_3$	8	3(50)	3(250)	2(200)

The total Transportation cost is

$$1 \times 300 + 2 \times 250 + 5 \times 150 + 3 \times 50 + 3 \times 250 + 2 \times 200 = 2850$$

The optimal solution=2850.

## II. Result Analysis:

**Table 3**

Method	Total Transportation cost
North-West corner Rule	4400
Row Minimum Method	2850
Column Minimum Method	3600
Least Cost Method	2900
Vogel's approximation Method	2850
Proposed MCDT Method	2850
Optimum solution	2850

As observed from Table 3, The proposed ADS method provides comparatively a better initial basic feasible solution than the result obtained by the traditional algorithms which are either optimal or near to optimal.

## III. Conclusion:

The proposed method is very easy to understand and to apply to for transportation problems with large data. Since it is not an iterative method like Vogel's approximation, computational cost is very less and also it is easy to write computer program for this proposed method.

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# UPPER UNIDOMINATION NUMBER OF A PATH

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**Abstract :** The concept of unidominating function is introduced and the unidominating function of a path is studied in [6]. The concept of minimal unidominating function and upper unidomination number are introduced in [7]. In this paper the authors study the minimal unidominating functions of a path and determined its upper unidomination number. Further the number of minimal unidominating functions with maximum weight is found.

**IndexTerms - Path, unidominating function, minimal unidominating function, upper unidomination number.**

## I. INTRODUCTION

Graph theory is one of the most developing branches of Mathematics with wide applications to various branches of Science and Technology. Theory of domination in graphs introduced by Berg[1] and Ore [2] is a rapidly growing area of research in Graph Theory. Domination and its properties have been extensively studied by T.W.Haynes and others in [3, 4].

Hedetniemi et.al.[ 5 ] introduced the concept of dominating function and it is attracted by many researchers because of its applications. The concept of unidominating function is introduced by the authors and the unidominating functions of a path are studied in [6]. The concept of minimal unidominating function and upper unidomination number are introduced in [7].

In this paper minimal unidominating functions of a path are studied and the upper unidomination number of a path is found. Also the result on the number of minimal unidominating functions with maximum weight are obtained. Further the results obtained are illustrated.

## II. MINIMAL UNIDOMINATING FUNCTIONS AND UPPER UNIDOMINATION NUMBER:

The concepts of unidominating function, minimal unidominating function, upper unidomination number are defined as follows:

**Definition 2.1:** Let  $G(V, E)$  be a graph. A function  $f:V \rightarrow \{0,1\}$  is said to be a unidominating function

$$\text{if } \sum_{u \in N[v]} f(u) \geq 1 \quad \forall v \in V \text{ and } f(v) = 1,$$

$$\sum_{u \in N[v]} f(u) = 1 \quad \forall v \in V \text{ and } f(v) = 0.$$

where  $N[v]$  is the closed neighbourhood of the vertex  $v$ .

**Definition 2.2:** Let  $f$  and  $g$  be functions from  $V$  to  $\{0,1\}$ . We say that  $f < g$

if  $f(u) \leq g(u) \quad \forall u \in V$ , with strict inequality for at least one vertex  $u$ .

**Definition 2.3:** A unidominating function  $f:V \rightarrow \{0,1\}$  is called a minimal unidominating function if for all  $g < f$ ,  $g$  is not a unidominating function.

**Definition 2.4:** The upper unidomination number of a graph  $G(V, E)$  is defined as  $\max \{f(V) / f \text{ is a minimal unidominating function}\}$ ,

$$\text{where } f(V) = \sum_{u \in V} f(u).$$

The upper unidomination number of a graph  $G$  is denoted by  $\Gamma_u(G)$ .

## III. UPPER UNIDOMINATION NUMBER OF A PATH

In this section the upper unidomination number of a path and the number of minimal unidominating functions with maximum weight of a path are found.

**Theorem 3.1:** The upper unidomination number of a path  $P_n$  is  $\lfloor \frac{2n}{3} \rfloor$ .

**Proof:** Let  $P_n$  be a path with vertex set  $V = \{v_1, v_2, \dots, v_n\}$ .

To find upper unidomination number of  $P_n$ , the following five cases arise.

**Case 1:** Let  $n \equiv 0 \pmod{5}$ .

Define a function  $f:V \rightarrow \{0,1\}$  by

$$f(v_i) = \begin{cases} 1 & \text{for } i \equiv 2,3,4 \pmod{5}, \\ 0 & \text{for } i \equiv 0,1 \pmod{5}. \end{cases}$$

Now we check the condition of unidominating function at every vertex.

**Sub case 1:** Let  $i \equiv 0 \pmod{5}$  and  $i \neq n$ . Then  $f(v_i) = 0$ .

$$\text{Now } \sum_{u \in N[v_i]} f(u) = f(v_{i-1}) + f(v_i) + f(v_{i+1}) = 1 + 0 + 0 = 1.$$



$$\text{For } i = n, \sum_{u \in N(v_n)} f(u) = f(v_{n-1}) + f(v_n) = 1 + 0 = 1.$$

**Sub case 2:** Let  $i \equiv 1 \pmod{5}$  and  $i \neq 1$ . Then  $f(v_i) = 0$ .

$$\text{Now } \sum_{u \in N(v_i)} f(u) = f(v_{i-1}) + f(v_i) + f(v_{i+1}) = 0 + 0 + 1 = 1.$$

$$\text{For } i = 1, \sum_{u \in N(v_1)} f(u) = f(v_1) + f(v_2) = 0 + 1 = 1.$$

**Sub case 3:** Let  $i \equiv 2 \pmod{5}$ . Then  $f(v_i) = 1$ .

$$\text{Now } \sum_{u \in N(v_i)} f(u) = f(v_{i-1}) + f(v_i) + f(v_{i+1}) = 0 + 1 + 1 = 2 > 1.$$

**Sub case 4:** Let  $i \equiv 3 \pmod{5}$ . Then  $f(v_i) = 1$ .

$$\text{Now } \sum_{u \in N(v_i)} f(u) = f(v_{i-1}) + f(v_i) + f(v_{i+1}) = 1 + 1 + 1 = 3 > 1.$$

**Sub case 5:** Let  $i \equiv 4 \pmod{5}$ . Then  $f(v_i) = 1$ .

$$\text{Now } \sum_{u \in N(v_i)} f(u) = f(v_{i-1}) + f(v_i) + f(v_{i+1}) = 1 + 1 + 0 = 2 > 1.$$

$$\text{Since } \sum_{u \in N(v_i)} f(u) \geq 1 \text{ when } f(v_i) = 1 \text{ and } \sum_{u \in N(v_i)} f(u) = 1 \text{ when } f(v_i) = 0,$$

it follows that  $f$  is a unidominating function.

Now we check for the minimality of  $f$ .

Define a function  $g: V \rightarrow \{0,1\}$  by

$$g(v_i) = f(v_i) \forall v_i \in V, i \neq k, k \equiv 2 \pmod{5} \text{ and } g(v_k) = 0.$$

Then by the definition of  $f$  and  $g$  it is obvious that  $g < f$ .

Suppose  $k = 2$ . Then

$$\sum_{u \in N(v_1)} g(u) = g(v_1) + g(v_2) = 0 + 0 = 0 \neq 1.$$

Suppose  $k \neq 2$ . Then

$$\sum_{u \in N(v_{k-1})} g(u) = g(v_{k-2}) + g(v_{k-1}) + g(v_k) = 0 + 0 + 0 = 0 \neq 1.$$

Since  $k \equiv 2 \pmod{5}$ ,  $k-1 \equiv 1 \pmod{5}$ . Then  $g(v_{k-1}) = f(v_{k-1}) = 0$ .

Again  $g(v_i) = f(v_i) = 0$ .

That is  $\sum_{u \in N(v_i)} g(u) \neq 1$  for which  $g(v) = 0$ .

This contradicts the definition of unidominating function.

Therefore  $g$  is not a unidominating function.

Similarly when  $k \equiv 3, 4 \pmod{5}$ , then also it can be shown that  $g$  is not a unidominating function.

Since  $g$  is defined arbitrarily, it follows that there exists no  $g < f$  such that  $g$  is a unidominating function. Hence for all possibilities of defining a function  $g < f$ , it can be seen that  $g$  is not a unidominating function.

Hence  $f$  is a minimal unidominating function.

$$\text{Now } \sum_{u \in V} f(u) = \underbrace{0 + 1 + 1 + 1 + 0}_{\text{first 5 vertices}} + \dots + \underbrace{0 + 1 + 1 + 1 + 0}_{\text{last 5 vertices}} = 3 \cdot \frac{n}{5} = \frac{3n}{5}.$$

$$\text{Therefore } \Gamma_u(P_n) \geq \frac{3n}{5} \text{ --- (1)}$$

If  $f$  is a minimal unidominating function of  $P_n$ , then it can be seen that amongst five consecutive vertices in  $P_n$  at most three consecutive vertices can have functional value 1 and at least two vertices must have functional value 0.

Therefore sum of the functional values of five consecutive vertices is less than or equal to 3. That is

$$\sum_{i=1}^5 f(v_i) \leq 3, \quad \sum_{i=6}^{10} f(v_i) \leq 3, \quad \dots, \quad \sum_{i=n-4}^n f(v_i) \leq 3.$$

$$\text{Therefore } \sum_{u \in V} f(u) = \sum_{i=1}^5 f(v_i) + \sum_{i=6}^{10} f(v_i) + \dots + \sum_{i=n-4}^n f(v_i) \leq \underbrace{3 + 3 + \dots + 3}_{\frac{n}{5}\text{-times}} \leq \frac{3n}{5}.$$

This is true for any minimal unidominating function.

Therefore  $\max\{f(V)/f \text{ is a minimal unidominating function}\} \leq \frac{3n}{5}$ .

$$\text{That is } \Gamma_u(P_n) \leq \frac{3n}{5} \text{ --- (2)}$$

$$\text{Thus from the inequalities (1) and (2), } \Gamma_u(P_n) = \frac{3n}{5} = \left\lceil \frac{3n}{5} \right\rceil.$$

**Case 2:** Let  $n \equiv 1 \pmod{5}$ .

Define a function  $f: V \rightarrow \{0,1\}$  by

$$f(v_i) = \begin{cases} 1 & \text{for } i \equiv 2, 3, 4 \pmod{5}, i \neq n-2, \\ 0 & \text{for } i \equiv 0, 1 \pmod{5}, i \neq n. \end{cases}$$

and  $f(v_{n-2}) = 0, f(v_n) = 1$ . Then this function is defined similarly as the function  $f$  defined in Case 1 except for the vertices  $v_{n-2}$  and  $v_n$ . So we check the condition of undominating function in the closed neighbourhood of  $v_{n-2}, v_{n-2}, v_{n-1}$ , and  $v_n$ .

$$\sum_{u \in N[v_{n-3}]} f(u) = f(v_{n-4}) + f(v_{n-3}) + f(v_{n-2}) = 1 + 1 + 0 = 2 > 1.$$

$$\sum_{u \in N[v_{n-2}]} f(u) = f(v_{n-3}) + f(v_{n-2}) + f(v_{n-1}) = 1 + 0 + 0 = 1.$$

$$\sum_{u \in N[v_{n-1}]} f(u) = f(v_{n-2}) + f(v_{n-1}) + f(v_n) = 0 + 0 + 1 = 1.$$

$$\sum_{u \in N[v_n]} f(u) = f(v_{n-1}) + f(v_n) = 0 + 1 = 1.$$

Since  $\sum_{u \in N[v_i]} f(u) \geq 1$  when  $f(v_i) = 1$  and  $\sum_{u \in N[v_i]} f(u) = 1$  when  $f(v_i) = 0$ ,

it follows that  $f$  is a undominating function.

Now we check for the minimality of  $f$ .

Define a function  $g: V \rightarrow \{0,1\}$  by

$$g(v_i) = f(v_i) \forall v_i \in V, i \neq n \text{ and } g(v_n) = 0.$$

Then by the definition of  $f$  and  $g$  it is obvious that  $g < f$ .

$$\text{Now } g(v_n) = 0, \text{ but } \sum_{u \in N[v_n]} g(u) = g(v_{n-1}) + g(v_n) = 0 + 0 = 0 \neq 1.$$

Therefore  $g$  is not a undominating function.

Hence for all possibilities of defining a function  $g < f$ , it can be seen that  $g$  is not a undominating function.

Therefore  $f$  is a minimal undominating function.

$$\text{Now } \sum_{u \in V} f(u) = \underbrace{0 + 1 + 1 + 1 + 0}_{=3} + \dots + \underbrace{0 + 1 + 1 + 0}_{=2} + 0 + 1 = \frac{3(n-6)}{5} + 2 + 1 = \frac{3n-3}{5} = \left\lfloor \frac{3n}{5} \right\rfloor.$$

(We will take 5 vertices as one group so that their functional values sum is 3 and there are  $\frac{n-6}{5}$  such groups. The remaining vertices are 6 and their functional values sum is 2 + 1).

$$\text{Therefore } \Gamma_u(P_n) \geq \left\lfloor \frac{3n}{5} \right\rfloor \dots (1)$$

Let  $f$  be a minimal undominating function of  $P_n$ .

Suppose  $n = 6$ . Then the possible assignment of functional values to these six vertices is 1,0,0,1,1,0 or 0,1,1,0,0,1 or 0,1,0,0,1,0, so that  $f(V) \leq 3$  and

$$\Gamma_u(P_6) = 3 = \left\lfloor \frac{3n}{5} \right\rfloor = \left\lfloor \frac{18}{5} \right\rfloor.$$

Let  $n \geq 11$ .

As in Case 1 of this theorem we have  $\sum f(v_i) \leq 3$  for any five consecutive vertices.

$$\text{Therefore } \sum_{i=2}^n f(v_i) \leq \frac{3(n-1)}{5}.$$

Now we assign the functional value to  $v_1$  as follows.

Suppose  $f(v_1) = 0$ .

$$\text{Then } f(V) = f(v_1) + \sum_{i=2}^n f(v_i) \leq 0 + \frac{3(n-1)}{5} = \frac{3n-3}{5} = \left\lfloor \frac{3n}{5} \right\rfloor.$$

Suppose  $f(v_1) = 1$ .

In such case among the  $\frac{(n-1)}{5}$  sets of five consecutive vertices, there will be one set of five consecutive vertices whose functional values sum is 2. Otherwise the assignment of functional values makes  $f$  no more a minimal undominating function.

$$\begin{aligned} \text{Therefore } f(V) &= f(v_1) + \sum_{i=2}^{n-5} f(v_i) + \sum_{i=n-4}^n f(v_i) \\ &\leq 1 + \frac{3(n-6)}{5} + 2 = \frac{3n-3}{5} = \left\lfloor \frac{3n}{5} \right\rfloor. \end{aligned}$$

(Here this set need not be the last set of five consecutive vertices. It can be between the set of vertices  $v_2, v_3, \dots, v_{n-5}$ . For convenience we have taken the last set of five consecutive vertices).

Since  $f$  is arbitrary, it follows that  $\Gamma_u(P_n) \leq \left\lfloor \frac{3n}{5} \right\rfloor \dots (2)$

Therefore from the inequalities (1) and (2), we have  $\Gamma_u(P_n) = \left\lfloor \frac{3n}{5} \right\rfloor$ .

**Case 3:** Let  $n \equiv 2 \pmod{5}$ .

Define a function  $f: V \rightarrow \{0,1\}$  by

$$f(v_i) = \begin{cases} 1 & \text{for } i \equiv 2,3,4 \pmod{5}, \\ 0 & \text{for } i \equiv 0,1 \pmod{5}. \end{cases}$$

On similar lines to Case 1 we can show that  $f$  is a minimal undominating function.

$$\text{Now } f(V) = \sum_{u \in V} f(u) = \underbrace{0+1+1+1+0+\dots+0+1+1+1+0+0+1}_{3(n-2)} + 1 = \frac{3(n-2)}{5} + 1 = \frac{3n-1}{5} = \left\lfloor \frac{3n}{5} \right\rfloor.$$

By the definition of upper undomination number,

$$\Gamma_u(P_n) \geq \left\lfloor \frac{3n}{5} \right\rfloor \text{ --- (1)}$$

Let  $f$  be a minimal undominating function of  $P_n$ .

Suppose  $n = 2$ . Then the possibilities of assigning functional values to these two vertices is 1,0 or 0,1, so that

$$f(V) = 1 = \left\lfloor \frac{3n}{5} \right\rfloor = \left\lfloor \frac{6}{5} \right\rfloor.$$

Let  $n \geq 7$ .

Now  $n \equiv 2 \pmod{5} \Rightarrow n - 2 \equiv 0 \pmod{5}$ . So by Case 1 we have

$$\sum_{i=1}^{n-2} f(v_i) \leq \frac{3(n-2)}{5}.$$

Then for the vertices  $v_{n-1}$  and  $v_n$ , we have

$$f(v_n) = 0 \text{ or } 1 \text{ and } f(v_{n-1}) = 1 \text{ or } 0. \text{ So } f(v_{n-1}) + f(v_n) = 1.$$

$$\text{Therefore } \sum_{u \in V} f(u) = \sum_{i=1}^{n-2} f(v_i) + (f(v_{n-1}) + f(v_n)) \leq \frac{3(n-2)}{5} + 1 = \frac{3n-1}{5} = \left\lfloor \frac{3n}{5} \right\rfloor.$$

$$\text{Thus } \Gamma_u(P_n) \leq \left\lfloor \frac{3n}{5} \right\rfloor \text{ --- (2)}$$

Therefore from the inequalities (1) and (2), it follows that  $\Gamma_u(P_n) = \left\lfloor \frac{3n}{5} \right\rfloor$ .

**Case 4:** Let  $n \equiv 3 \pmod{5}$ .

Define a function  $f: V \rightarrow \{0,1\}$  by

$$f(v_i) = \begin{cases} 1 & \text{for } i \equiv 2,3,4 \pmod{5} \text{ and } i \neq n, \\ 0 & \text{for } i \equiv 0,1 \pmod{5}, \end{cases}$$

and  $f(v_n) = 0$ .

We can verify in similar lines as in Case 1 that  $f$  is a undominating function.

Now we check for the minimality of  $f$ .

Define a function  $g: V \rightarrow \{0,1\}$  by

$$g(v_i) = f(v_i) \forall v_i \in V, i \neq n-1 \text{ and } g(v_{n-1}) = 0.$$

Then by the definition of  $f$  and  $g$  it is obvious that  $g < f$ .

Now  $g(v_{n-1}) = 0$ , but

$$\sum_{u \in N[v_{n-1}]} g(u) = g(v_{n-2}) + g(v_{n-1}) + g(v_n) = 0 + 0 + 0 = 0 \neq 1.$$

Therefore  $g$  is not a undominating function.

It can be seen that for all possibilities of defining a function  $g < f$ ,  $g$  is not a undominating function.

Therefore  $f$  is a minimal undominating function.

$$\text{Now } \sum_{u \in V} f(u) = \underbrace{0+1+1+1+0+\dots+0+1+1+1+0+0+1+0}_{3(n-3)} + 1 = \frac{3(n-3)}{5} + 1 = \frac{3n-4}{5} = \left\lfloor \frac{3n}{5} \right\rfloor.$$

$$\text{Therefore } \Gamma_u(P_n) \geq \left\lfloor \frac{3n}{5} \right\rfloor \text{ --- (1)}$$

Let  $f$  be a minimal undominating function of  $P_n$ .

Suppose  $n = 3$ . Then the vertices  $v_1, v_2, v_3$  have functional values 0,1,0 and this is the only one possibility, so that

$$\Gamma_u(P_3) = f(V) = 1 = \left\lfloor \frac{3n}{5} \right\rfloor = \left\lfloor \frac{9}{5} \right\rfloor.$$

Let  $n \geq 8$ .

As in Case 1 of this theorem we have  $\sum f(v_i) \leq 3$  for any five consecutive vertices.

$$\text{Therefore } \sum_{i=2}^{n-2} f(v_i) \leq \frac{3(n-3)}{5}.$$

Similar to Case 3, for the vertices  $v_{n-1}$  and  $v_n$ , here also we have

$$f(v_n) = 0 \text{ or } 1 \text{ and } f(v_{n-1}) = 1 \text{ or } 0, \text{ so that } f(v_{n-1}) + f(v_n) = 1.$$

Now we assign the functional values to  $v_1$  as follows.

Suppose  $f(v_1) = 0$ .

$$\text{Then } f(V) = f(v_1) + \sum_{i=2}^{n-2} f(v_i) + (f(v_{n-1}) + f(v_n)) \leq 0 + \frac{3(n-3)}{5} + 1 = \frac{3n-4}{5} = \left\lfloor \frac{3n}{5} \right\rfloor.$$

Suppose  $f(v_1) = 1$ .

Then as in Case 2 we have

$$\sum_{i=2}^{n-2} f(v_i) = \sum_{i=2}^{n-7} f(v_i) + \sum_{i=n-6}^{n-2} f(v_i) \leq \frac{3(n-8)}{5} + 2.$$

Therefore  $f(V) = f(v_1) + \sum_{i=2}^{n-2} f(v_i) + (f(v_{n-1}) + f(v_n))$   
 $\leq 1 + \frac{3(n-8)}{5} + 2 + 1 = \frac{3n-4}{5} = \lfloor \frac{3n}{5} \rfloor.$

Since  $f$  is arbitrary, it follows that  $\Gamma_u(P_n) \leq \lfloor \frac{3n}{5} \rfloor$  --- (2)

Therefore from the inequalities (1) and (2), it follows that  $\Gamma_u(P_n) = \lfloor \frac{3n}{5} \rfloor.$

**Case 5:** Let  $n \equiv 4(mod 5).$

Define a function  $f:V \rightarrow \{0,1\}$  by

$$f(v_i) = \begin{cases} 1 & \text{for } i \equiv 2,3,4(mod 5), \text{ and } i \neq n \\ 0 & \text{otherwise.} \end{cases}$$

and  $f(v_n) = 0.$

Then on similar lines of Case 1 it can be shown that  $f$  is a minimal undominating function.

Further,

$$\sum_{u \in V} f(u) = \underbrace{0+1+1+1+0}_{i=2} + \dots + \underbrace{0+1+1+1+0}_{i=n-6} + \underbrace{0+1+1+0}_{i=n-4} = \frac{3(n-4)}{5} + 2 = \frac{3n-2}{5} = \lfloor \frac{3n}{5} \rfloor.$$

Therefore  $\Gamma_u(P_n) \geq \lfloor \frac{3n}{5} \rfloor$  --- (1)

Let  $f$  be a minimal undominating function.

Suppose  $n = 4.$  Then the possibilities of assigning functional values to these four vertices are 0,1,1,0 or 1,0,0,1, so that  $f(V) = 2 = \lfloor \frac{3n}{5} \rfloor = \lfloor \frac{12}{5} \rfloor.$

Let  $n \geq 9.$

If  $f$  is any minimal undominating function of  $P_n$  then the pendent vertices  $v_1$  and  $v_n$  must satisfy the following conditions,

$$f(v_1) + f(v_2) = 1 \text{ and } f(v_{n-1}) + f(v_n) = 1.$$

Now  $n \equiv 4(mod 5) \Rightarrow n - 4 \equiv 0(mod 5).$  Then as in Case 1,

$$\sum_{i=2}^{n-2} f(v_i) \leq \frac{3(n-4)}{5}.$$

Therefore  $f(V) = \sum_{u \in V} f(u) = f(v_1) + f(v_2) + \sum_{i=3}^{n-2} f(v_i) + (f(v_{n-1}) + f(v_n))$   
 $\leq 1 + \frac{3(n-4)}{5} + 1 = \frac{3n-4}{5} = \lfloor \frac{3n}{5} \rfloor.$

Since  $f$  is arbitrary, it follows that  $\Gamma_u(P_n) \leq \lfloor \frac{3n}{5} \rfloor$  --- (2)

Therefore from the inequalities (1) and (2),  $\Gamma_u(P_n) = \lfloor \frac{3n}{5} \rfloor.$

Thus for all possibilities of  $n,$  we have  $\Gamma_u(P_n) = \lfloor \frac{3n}{5} \rfloor.$  ■

**Theorem 3.2:** The number of minimal undominating functions of  $P_n$  with

$$\text{maximum weight is } \begin{cases} 1 & \text{when } n \equiv 0(mod 5), \\ \lfloor \frac{3n}{5} \rfloor & \text{when } n \equiv 1(mod 5), \\ 2 & \text{when } n \equiv 2(mod 5), \\ \lfloor \frac{n}{5} \rfloor + \frac{1}{2} \lfloor \frac{n}{5} \rfloor \lfloor \frac{n}{5} \rfloor + \lfloor \frac{n}{5} \rfloor & \text{when } n \equiv 3(mod 5), \\ \lfloor \frac{n}{5} \rfloor + 1 & \text{when } n \equiv 4(mod 5). \end{cases}$$

**Proof:** Let  $P_n$  be a path with vertex set  $V = \{v_1, v_2, \dots, v_n\}.$

Now we find the number of minimal undominating functions with maximum weight in the following five cases.

**Case 1:** Let  $n \equiv 0(mod 5).$

The function  $f$  defined in Case 1 of Theorem 3.1 is given by



The functional values of  $f$  are 0111001110 --- 01110.

Take  $a = 01110.$  Then the functional values of  $f$  are in the pattern of  $aaa \dots a$  (here there are  $\frac{n}{5}$   $a$ 's). These letters  $aaa \dots a$  can be arranged in one and only one way. Therefore there is one and only one minimal undominating function with maximum weight.

**Case 2:** Let  $n \equiv 1(mod 5).$

The function  $f$  defined in Case 2 of Theorem 3.1 is given by



The functional values of  $f$  are 0111001110 - - - 01110011001.

Taken  $a = 01110$ ,  $b = 0110$ . Then the functional values of  $f$  are in the pattern of

$aaa \dots ab01$  (here there are  $\frac{n-6}{5}$   $a$ 's). As there are  $\frac{n-6}{5}$   $a$ 's and one  $b$ , these letters can be arranged in  $\frac{\binom{\frac{n-6}{5}+1}{2}}{\binom{\frac{n-1}{5}}{2}} = \frac{n-1}{5}$

ways.

Therefore there are  $\frac{n-1}{5}$  minimal unidominating functions.

We further investigate some more minimal unidominating functions of  $P_n$  with maximum weight in the following way.

Define a function  $f_1: V \rightarrow \{0,1\}$  by

$$f_1(v_i) = \begin{cases} 1 & \text{for } i \equiv 0,1,4 \pmod{5}, i \neq n, \\ 0 & \text{for } i \equiv 2,3 \pmod{5}. \end{cases}$$

and  $f_1(v_n) = 0$ .

First we show that  $f_1$  is a unidominating function.

**Sub case 1:** Let  $i \equiv 0 \pmod{5}$  and  $i \neq n-1$ . Then  $f_1(v_i) = 1$ .

$$\text{Now } \sum_{u \in N(v_i)} f_1(u) = f_1(v_{i-1}) + f_1(v_i) + f_1(v_{i+1}) = 1 + 1 + 1 = 3 > 1.$$

$$\text{For } i = n-1, \sum_{u \in N(v_{n-1})} f_1(u) = f_1(v_{n-2}) + f_1(v_{n-1}) + f_1(v_n) = 1 + 1 + 0 = 2 > 1.$$

**Sub case 2:** Let  $i \equiv 1 \pmod{5}$  and  $i \neq 1, i \neq n$ . Then  $f_1(v_i) = 1$ .

$$\text{Now } \sum_{u \in N(v_i)} f_1(u) = f_1(v_{i-1}) + f_1(v_i) + f_1(v_{i+1}) = 1 + 1 + 0 = 2.$$

$$\text{For } i = 1, \sum_{u \in N(v_1)} f_1(u) = f_1(v_1) + f_1(v_2) = 1 + 0 = 1.$$

$$\text{For } i = n, \sum_{u \in N(v_n)} f_1(u) = f_1(v_{n-1}) + f_1(v_n) = 1 + 0 = 1.$$

**Sub case 3:** Let  $i \equiv 2 \pmod{5}$ . Then  $f_1(v_i) = 0$ .

$$\text{Now } \sum_{u \in N(v_i)} f_1(u) = f_1(v_{i-1}) + f_1(v_i) + f_1(v_{i+1}) = 1 + 0 + 0 = 1.$$

**Sub case 4:** Let  $i \equiv 3 \pmod{5}$ . Then  $f_1(v_i) = 0$ .

$$\text{Now } \sum_{u \in N(v_i)} f_1(u) = f_1(v_{i-1}) + f_1(v_i) + f_1(v_{i+1}) = 0 + 0 + 1 = 1.$$

**Sub case 5:** Let  $i \equiv 4 \pmod{5}$ . Then  $f_1(v_i) = 1$ .

$$\text{Now } \sum_{u \in N(v_i)} f_1(u) = f_1(v_{i-1}) + f_1(v_i) + f_1(v_{i+1}) = 0 + 1 + 1 = 2 > 1.$$

$$\text{Thus } \sum_{u \in N(v_i)} f_1(u) \geq 1 \text{ when } f_1(v_i) = 1 \text{ and } \sum_{u \in N(v_i)} f_1(u) = 1 \text{ when } f_1(v_i) = 0.$$

Then it follows that  $f_1$  is a unidominating function.

Now we check for the minimality of  $f_1$ .

Define a function  $g: V \rightarrow \{0,1\}$  by

$$g(v_i) = f_1(v_i) \text{ for } i = 1, 2, \dots, n, i \neq k \text{ for some } k \equiv 4 \pmod{5},$$

and  $g(v_k) = 0$ .

Obviously  $g < f_1$  and

$$\sum_{u \in N(v_{k-1})} g(u) = g(v_{k-2}) + g(v_{k-1}) + g(v_k) = 0 + 0 + 0 = 0 \neq 1.$$

$$\text{For } k = n-1, \sum_{u \in N(v_{n-1})} g(u) = g(v_{n-2}) + g(v_{n-1}) + g(v_n) = 0 + 0 + 0 = 0 \neq 1.$$

$$\text{That is } \sum_{u \in N(v_i)} g(u) \neq 1 \text{ for which } g(v_i) = 0.$$

This contradicts the definition of unidominating function.

Therefore  $g$  is not a unidominating function.

Similarly when  $k \equiv 0,1 \pmod{5}$  then also we can show that  $g$  is not a unidominating function.

Therefore for all possibilities of defining a function  $g < f_1$ , it can be seen that  $g$  is not a unidominating function.

Thus  $f_1$  is a minimal unidominating function.

Further,

$$\sum_{u \in V} f_1(u) = 1 + 0 + 0 + 1 + 1 + 1 + 0 + \dots + 0 + 1 + 1 + 1 + 0 + 0 + 1 + 1 + 0 = 1 + 3 \left( \frac{n-6}{5} \right) + 2 = \frac{3n-3}{5} = \left\lfloor \frac{3n}{5} \right\rfloor.$$

The functional values of  $f_1$  are given by



The functional values of  $f_1$  are 1001110 --- 011100110.

Take  $a = 01110$ ,  $b = 0110$ . Then the functional values of  $f_1$  are in the pattern of  $10aaa \dots ab$  (here there are  $\frac{n-6}{5}$   $a$ 's). In similar lines as above it can be proved that there exist  $\frac{n-1}{5}$  minimal undominating functions.

Thus there are  $\frac{n-1}{5} + \frac{n-1}{5} = \frac{2n-2}{5} = \lfloor \frac{2n}{5} \rfloor$  minimal undominating functions with maximum weight.

**Case 3:** Let  $n \equiv 2 \pmod{5}$ .

The function  $f$  defined in Case 3 of Theorem 3.1 is given by



The functional values of  $f$  are 01110 --- 0111001.

Take  $a = 01110$ . Then the functional values of  $f$  are in the pattern of  $aaa \dots a01$ .

As these letters  $aaa \dots a$  can be arranged in only one way, there exist one and only one minimal undominating function.

Now as in Case 2, we will get another minimal undominating function with the same weight.

Define a function  $f_1: V \rightarrow \{0,1\}$  by

$$f_1(v_i) = \begin{cases} 1 & \text{for } i \equiv 0,1,4 \pmod{5}, \\ 0 & \text{otherwise.} \end{cases}$$

On similar lines as in Case 2 of Theorem 3.2 we can show that  $f_1$  is a minimal undominating function.

Further,

$$\sum_{u \in V} f_1(u) = 1 + 0 + 0 + 1 + 1 + 1 + 0 + \dots + 0 + 1 + 1 + 1 + 0 = 1 + \frac{3(n-2)}{5} = \frac{3n-1}{5} = \lfloor \frac{3n}{5} \rfloor.$$

The functional values of  $f_1$  are given by



The functional values of  $f_1$  are 1001110 --- 01110.

Take  $a = 01110$ . Then the functional values of  $f_1$  are in the pattern of  $10aaa \dots a$ .

These letters  $aaa \dots a$  can be arranged in one and only one way. Therefore there exists only one function.

Thus there are two minimal undominating functions with maximum weight.

**Case 4:** Let  $n \equiv 3 \pmod{5}$ .

The function  $f$  defined in Case 4 of Theorem 3.1 is given by



The functional values of  $f$  are 0111001110 --- 01110010.

Take  $a = 01110$ ,  $c = 010$ . Then the functional values of  $f$  are in the pattern of  $aaa \dots ac$  (here there are  $\frac{n-2}{5}$   $a$ 's). Then as in similar lines of Case 2 it can be seen that there are

$$\frac{n-2}{5} + 1 = \frac{n+2}{5} = \lfloor \frac{n}{5} \rfloor$$

minimal undominating functions with maximum weight.

Now as in Case 2 and Case 3, we will get some other minimal undominating functions with maximum weight.

Define another function  $f_1: V \rightarrow \{0,1\}$  by

$$f_1(v_i) = f(v_i) \quad \forall v_i \in V, \text{ for } i \neq n-4, n-2,$$

$$\text{and } f_1(v_{n-4}) = 0 \text{ and } f_1(v_{n-2}) = 1, n \geq 8.$$

Then we can check easily the condition of undominating function in the closed neighbourhood of  $v_{n-4}$ ,  $v_{n-3}$ ,  $v_{n-2}$  and  $v_{n-1}$  and hence it follows that  $f_1$  is a undominating function and which is also minimal.

Then

$$\sum_{u \in V} f_1(u) = 0 + 1 + 1 + 1 + 0 + \dots + 0 + 1 + 1 + 1 + 0 + 0 + 1 + 1 + 0 + 0 + 1 + 1 + 0 = \frac{3(n-8)}{5} + 4 = \frac{3n-4}{5} = \lfloor \frac{3n}{5} \rfloor.$$

The function  $f_1$  is given by



The functional values of  $f_1$  are 01110 --- 0111001100110.

Take  $a = 01110$ ,  $b = 0110$ . Then the functional values of  $f_1$  are in the pattern of  $aa \dots abb$ . (here there are  $\frac{n-8}{5}$   $a$ 's).

As there are  $\frac{n-8}{5}$   $a$ 's and two  $b$ 's, these letters  $a$ 's and  $b$ 's can be arranged in

$$\frac{\binom{\frac{n-8}{5}+2}{2}}{\binom{\frac{n-8}{5}}{2}} = \frac{\binom{\frac{n+2}{5}}{2}}{\binom{\frac{n-8}{5}}{2}} = \frac{1}{2} \binom{\frac{n+2}{5}}{\frac{n-8}{5}} = \frac{1}{2} \lfloor \frac{n}{5} \rfloor \lfloor \frac{n}{5} \rfloor$$

ways. Therefore there exist  $\frac{1}{2} \lfloor \frac{n}{5} \rfloor \lfloor \frac{n}{5} \rfloor$  minimal undominating functions.

Define another function  $f_2: V \rightarrow \{0,1\}$  by

$$f_2(v_i) = \begin{cases} 1 & \text{for } i \equiv 0,1,4 \pmod{5}, i \neq n-2, \\ 0 & \text{for } i \equiv 2,3 \pmod{5}, i \neq n, \end{cases}$$

and  $f_2(v_{n-2}) = 0, f_2(v_n) = 1, n \geq 8$ .

Similar to earlier cases we can show that the function  $f_2$  is a minimal undominating function. Further,

$$\sum_{u \in V} f_2(u) = 1 + 0 + 0 + \underbrace{1 + 1 + 1 + 1 + 0}_{\dots} + \dots + \underbrace{0 + 1 + 1 + 1 + 1 + 0}_{\dots} + \underbrace{0 + 1 + 1 + 1 + 0}_{\dots} + 0 + 1 = 1 + \frac{3(n-8)}{5} + 2 + 1 = \frac{3n-4}{5}$$

$$= \left\lfloor \frac{3n}{5} \right\rfloor.$$

The function  $f_2$  is given by



The functional values of  $f_2$  are 1001110 --- 01110011001.

Take  $a = 01110, b = 0110$ . Then the functional values of  $f_2$  are in the pattern of  $10aaa \dots ab01$ . (here there are  $\frac{n-8}{5}$   $a$ 's.)

As there are  $\frac{n-8}{5}$   $a$ 's and one  $b$ , there exists  $\frac{n-8}{5} + 1 = \frac{n-3}{5} = \left\lfloor \frac{n}{5} \right\rfloor$  minimal undominating functions.

Thus there are  $\left\lfloor \frac{n}{5} \right\rfloor + 1 \cdot \left\lfloor \frac{n}{5} \right\rfloor + \left\lfloor \frac{n}{5} \right\rfloor$  minimal undominating functions with maximum weight.

**Case 5:** Let  $n \equiv 4 \pmod{5}$ .

The function  $f$  defined in Case 4 of Theorem 3.1 is given by



The functional values of  $f$  are 01110 --- 011100110.

Take  $a = 01110, b = 0110$ . Then the functional values of  $f$  are in the pattern of  $aa \dots ab$  (here there are  $\frac{n-4}{5}$   $a$ 's). On

similar lines to Case 4 we can see that there are

$\frac{n-4}{5} + 1 = \frac{n+1}{5} = \left\lfloor \frac{n}{5} \right\rfloor$  minimal undominating functions with maximum weight.

As in previous cases we investigate for another minimal undominating function with maximum weight.

Define another function  $f_1: V \rightarrow \{0,1\}$  by

$$f_1(v_i) = \begin{cases} 1 & \text{for } i \equiv 0,1,4 \pmod{5}, \\ 0 & \text{otherwise.} \end{cases}$$

Similar to earlier cases we can show that  $f_1$  is a minimal undominating function.

Now

$$\sum_{u \in V} f_1(u) = 1 + 0 + 0 + \underbrace{1 + 1 + 1 + 1 + 0}_{\dots} + \dots + \underbrace{0 + 1 + 1 + 1 + 0}_{\dots} + 0 + 1$$

$$= 1 + \frac{3(n-4)}{5} + 1 = \frac{3n-2}{5} = \left\lfloor \frac{3n}{5} \right\rfloor.$$

The function  $f_1$  is given by



The functional values of  $f_1$  are 1001110 --- 0111001.

Take  $a = 01110$ . Then the functional values of  $f_1$  are in the pattern of  $10aaa \dots a01$ .

As these letters  $aa \dots a$  can be arranged in only one way, there exists one and only one minimal undominating function.

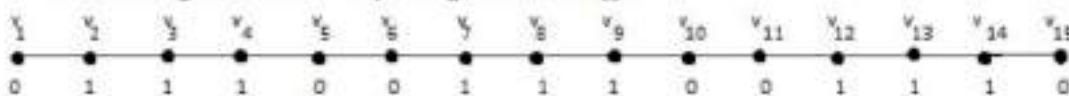
Thus there are  $\left\lfloor \frac{n}{5} \right\rfloor + 1$  minimal undominating functions with maximum weight. ■

#### IV. ILLUSTRATIONS

**Example 4.1:** Let  $n = 15$ .

Obviously  $15 \equiv 0 \pmod{5}$ .

The functional values of a minimal undominating function  $f$  defined in Case 1 of Theorem 3.1 are given at the corresponding vertices of  $P_{15}$ .



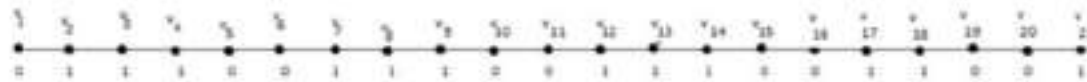
Upper undomination number of  $P_{15}$  is  $\Gamma_u(P_{15}) = \left\lfloor \frac{45}{5} \right\rfloor = 9$ .

There is only one minimal undominating function for  $P_{15}$  with maximum weight 9. ■

**Example 4.2:** Let  $n = 21$ .

Clearly  $21 \equiv 1 \pmod{5}$ .

The functional values of a minimal undominating function  $f$  defined in Case 2 of Theorem 3.1 are given at the corresponding vertices of  $P_{21}$ .



Upper undomination number of  $P_{21}$  is  $\Gamma_u(P_{21}) = \lfloor \frac{62}{5} \rfloor = 12$ .

There are 4 minimal undominating functions that exists from  $f$  with maximum weight 12. The functional values of another minimal undominating function  $f_1$  defined in Case 2 of Theorem 3.2 are given at the corresponding vertices of  $P_{21}$ .



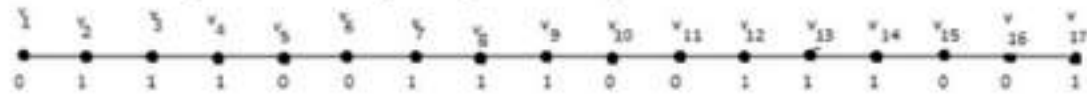
There are four such minimal undominating functions.

Thus there are  $\lfloor \frac{2x+21}{5} \rfloor = 8 = (4 + 4)$  minimal undominating functions with maximum weight 12. ■

**Example 4.3:** Let  $n = 17$ .

Clearly  $17 \equiv 2 \pmod{5}$ .

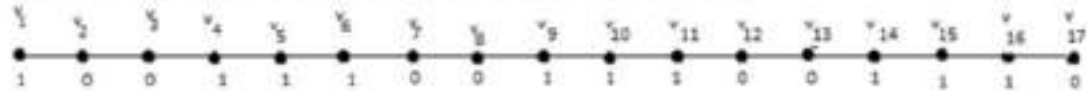
The functional values of a minimal undominating function  $f$  defined in Case 3 of Theorem 3.1 are given at the corresponding vertices of  $P_{17}$ .



Upper undomination number of  $P_{17}$  is  $\Gamma_u(P_{17}) = \lfloor \frac{51}{5} \rfloor = 10$ .

There exists only one minimal undominating function.

The functional values of another minimal undominating function  $f_1$  defined in Case 3 of Theorem 3.2 are given at the corresponding vertices of  $P_{17}$ .



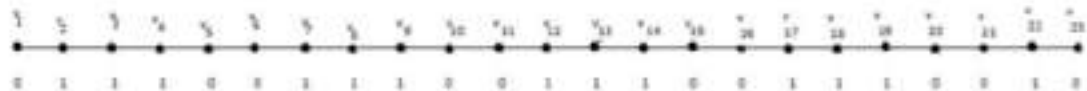
There exists only one minimal undominating function.

Thus there are two minimal undominating functions for  $P_{17}$  with maximum weight 10. ■

**Example 4.4:** Let  $n = 23$ .

We know that  $23 \equiv 3 \pmod{5}$ .

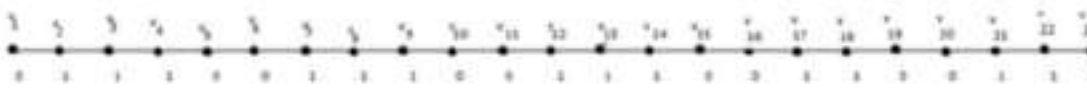
The functional values of a minimal undominating function  $f$  defined in Case 4 of Theorem 3.1 are given at the corresponding vertices of  $P_{23}$ .



Upper undomination number of  $P_{23}$  is  $\Gamma_u(P_{23}) = \lfloor \frac{69}{5} \rfloor = 13$ .

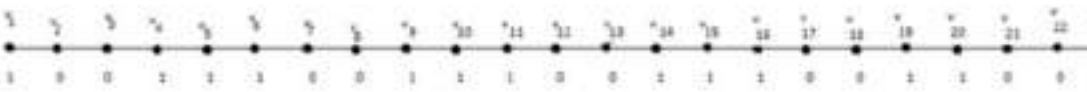
There are  $\lfloor \frac{22}{5} \rfloor = 5$  minimal undominating functions with maximum weight 13.

The functional values of another minimal undominating function  $f_1$  defined in Case 4 of Theorem 3.2 are given at the corresponding vertices of  $P_{23}$ .



There are  $\frac{1}{2} \lfloor \frac{22}{5} \rfloor \lfloor \frac{23}{5} \rfloor = 10$  minimal undominating functions with maximum weight 13.

The functional values of another minimal undominating function  $f_2$  defined in Case 4 of Theorem 3.2 are given at the corresponding vertices of  $P_{23}$ .



There are  $\lfloor \frac{22}{5} \rfloor = 4$  minimal undominating functions with maximum weight 13.

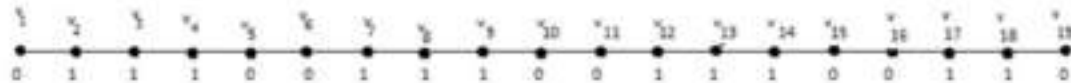
Thus there are  $5 + 10 + 4 = 19$  minimal undominating functions for  $P_{23}$  with maximum weight 13. ■

**Example 4.5:** Let  $n = 19$ .

Clearly  $19 \equiv 4 \pmod{5}$ .

The functional values of a minimal undominating function  $f$  defined in Case 5 of Theorem 3.1 are given at the corresponding vertices of  $P_{19}$ .

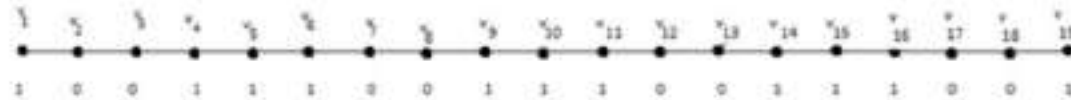




Upper undomination number of  $P_{19}$  is  $\Gamma_u(P_{19}) = \lfloor \frac{19}{2} \rfloor = 11$ .

There are  $\lfloor \frac{19}{2} \rfloor = 9$  minimal undominating functions with maximum weight 11.

The functional values of another minimal undominating function  $f_2$  defined in Case 5 of Theorem 3.2 are given at the corresponding vertices of  $P_{19}$ .



There is one and only one minimal undominating function with maximum weight 11.

Thus there are  $\lfloor \frac{19}{2} \rfloor + 1 = 10$  minimal undominating functions for  $P_{19}$  with maximum weight 11. ■

**V. CONCLUSION:** The upper undomination number of a path is proved in five cases basing on the number of vertices. This work gives a scope to find upper undomination number of a cycle and upper total undomination number of a path.

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### Baby Kamble's The Prisons We Broke: Breaking Free: Impacted

By Dr. B.R Ambedkar

#### Abstract

Out of poverty, superstitions, oppression and caste system in India, enlightened people like Baby Kamble have found a new way for self-assertion. Since the caste system denied education to shudras and panchamas, anti-caste writing was a way in which the oppressed were retaliating against the oppressors. It was a psychological literature. Baby Kamble's work is an autobiography. She portrays vividly the plight of her community, she also expresses her debt of gratitude to Dr. Ambedkar for his efforts in opening the eyes of the people. He makes them realize their oppression and enlightens the Mahar community. He shows them the path of liberty, equality and fraternity. He was the first Indian to break down the barriers in the way of advance of women in India. The present paper is an attempt to highlight how the Mahars were delivered from the strongholds of evil customs through the impact of the great and noble leaders like Ambedkar.

**Key Words:** Fraternity, Oppression, Superstitions.

#### Introduction:

In the words of Sharatchandra Muktibodh, "Dalit Literature is the literature produced by the Dalit consciousness. (Muktibodh, 270) It gave a voice to the exploited and unraveled the

caste politics in India. It very much differs from Main stream literature and this difference is made consciously by the writers. Mainstream literature is not objective and neutral. Mainstream literature includes voices of male upper class and upper caste only. Initially the Dalits felt very stifled with the way they were portrayed in upper cast works (While talking about Dalits they had a touch of sensibility e.g.: Mulk Raj Anand's Untouchables). Dalits were against such portrayals, when the upper castes wrote about the Dalits, they failed to talk about the realities of class oppression and used a sentimental tone, with no focus on a rebellion that could arise and this called for the creation of Dalit literature.

Major focus of Dalit literature was on day to day writing as opposed to mainstream writing (who wrote about spiritual reality, love and so on). Their writing focused on close portrayal of their social surrounding. The Dalits didn't write for entertainment or aesthetic values. They argue that mainstream writers were not visibly propagandist. They wanted to talk about the things that concern rather than talking about spiritual or philosophical things. They were against established literary traditions.

Subjectivity is a concept related to consciousness, personhood, reality, and truth. Subjectivity could mean an individual who possesses conscious experiences, viewpoints, feelings, beliefs, hopes. It could mean an entity that has agency. Subjectivity is the opposite of Objectivity and Subjectivity is shaped by culture and is return shaped by it. Just as how we all have two eyes but our view is different, subjectivity differs from people to people.

The 25<sup>th</sup> of December 1927 is an important day for the Mahars as the speech given by Dr.B.R.Ambedkar was followed by the burning of the Manusmiriti. It is celebrated today as Manavmukti din. This event is a symbolic event for the entire oppressed community. Many times, the oppressed castes are oppressed by the higher castes and they still want to imitate their oppressors. Take for instance the colonial hangover the people of India still reels under.

The oppressed people of the Mahar community found their voice through writing. Writing can be seen as a record of the suffering and the release from the pain. Buddhist literature, radical bhakti literature, writing of Phule awakened their sleeping conscience. Poet Raja Dhale wrote in an article in a Pune socialist magazine, Sadhana that people are fined more for 'dishonouring the national flag' than for raping a dalit woman. The era of literature written from 1960's to the mid 1980's can be called the 'golden age' of modern Marathi Dalit literature. Many works have been translated, incorporated in syllabus and have received recognition and fame.

In the book, *The Prisons We Broke*, the author articulates her sufferings in these words:

"Hindu philosophy had discarded us as dirt and thrown us into their garbage pits, on the outskirts of the village. We lived in the filthiest conditions possible. Yet Hindu rites and rituals were dearest to our hearts... We desperately tried to preserve whatever bits of Hindu culture we managed to lay our hands on. And yet no one tried to understand us. Our minds somehow kept hoping against hope-that we too would be able to live like the upper castes. The month of Ashad was kept in full honor and reverence, but they were looking for help to the same forces that oppressed them, they sought liberation and hope from the same forces that tied them down and robbed them of their hope." (Kamble, 18)

Superstitions like offering the eldest son to the deity as a vaghya, child marriages, illiteracy amongst women, eating the flesh of dead animals have been practiced ignorantly and ardently. They lived literally in dirt and filth.

The Impact of Dr. Ambedkar:

Mahatma Gandhi coined the term Harijan which meant children of God. This word is of Marathi origin and means 'held under check', 'suppressed', or 'crushed', or, in a looser sense 'oppressed' Maharashtra Dalit Activist and poet Namdeo Dhasal made it a symbol of pride to fight against social injustice." (Prasad, 8). The Hindus did not regard their own with honour; they felt more affinity towards the Muslims than with the people of the lower class.

"Dalit is the latest and currently most politically correct of many terms used for the caste. Offensive terms used mostly in the past include chura, bhangi, neech, kanjar and mirasi." (Prasad,8). The Dalits sought for identity in the Hindu religion and when they developed a crisis when they were not able to get a place where they belonged, they turned to Buddhism. According to French Marxist theorist, Louis Althusser, Ideology is not really a set of political ideas but it offers us coherent subject positions (roles).

Some of the roles of a Hindu is to read Hindu texts, visit temples and pray to the idols but these 'roles' were not available to be performed by the Untouchable and hence they were unable to fill this void that they felt gnawing in their hearts.

"A fourteen-year old girl from one of Jotirao's schools for untouchables wrote an essay in which she said, The Brahmins say that other castes should not read the Vedas; this leaves us without a scripture. Thus, are we without religion? Oh God, please tell us, what is our religion? [...]" (Joshi, 12)

"... Religion must have bribed you quite well to do this... you kept stealing our fates with your writings" (Kamble, 62)

The significance of the title comes in to play,

"Our lives were governed by various calamities. We were imprisoned in dark cells, our hands and feet bound by the chain of slavery. Our reason was gagged. But it is because of us

that the world stands... Shallow water makes a lot of noise, but still water runs deep! Like the ocean that covers mountains of sin under its huge expanse, we covered the sins of the high castes. That is why we, like the ocean, deserve the admiration of the whole world.”

From 1930 onwards, Buddhism started spreading its wings towards the Mahar community. People were greatly taken up by the works and talk of Dr. Babasaheb Ambedkar. Ambedkar was critical of the Hindu religion. He believed that education was a means of triumph from the life of poverty and ignorance. He wanted the caste system to be uprooted and wanted equality among all castes not sympathy or tolerance towards each other (promoted through practices of inter dining and other such customs). Many people from the lower castes were greatly taken up by him. He opened the eyes of the people to see their oppression and their plight in the hands of the upper caste

Mahatma Jotirao Phule was a social reformer, hailing from Maharashtra, belonging to the 19<sup>th</sup> century. He worked for the education of women and Dalits and for the downtrodden. He was radical in his thinking and was “one of the foremost exponents of modern humanitarian thought” (Joshi, 3) Jotirao realized that “though all Hindus followed one religion, Hinduism had not succeeded in creating a spiritual life based on unity.” (Joshi, 9)

Kabir was a bhakti who converted from Islam. He believed in the Nirguna form of worship. In his poem Padas he compares God the creator to a potter who has touched all human beings when he created them with his own hands, writes:

“... we eat by touching, we wash  
By touching, from a touch  
The world was born.  
So, who's untouched? Asks Kabir.  
Only she

Who's free from delusion?"(118)

Thus, with the influence of such radical minds the people of the Mahar community started to open their eyes and be aware of their subjugation. Ambedkar wanted to raise the educational standards of the untouchables so that they too can wield political power.

"Ambedkar planned his programmed to bring the Untouchable from a state of 'dehumanization' and 'slavery' into one of equality through the use of modern methods based on education and the exercise of legal and political rights." (Prasad, 2)

Ambedkar belonged to the Mahar community, he was educated and came back to lead his people from darkness to light, from bondage to freedom. He rejected Hinduism and embraced Buddhism.

"For Ambedkar, Buddhism represented the historical revolutionary experience in India, while Hinduism represented the counter-revolutionary experience seeking to bring back an orthodoxy founded upon the caste system." (Prasad, 1)

Ambedkar used concepts of Liberty, Equality and Fraternity from the French Revolution to justify eradication of untouchability.

The author's father was inspired by Dr. Babasaheb Ambedkar. He enrolled both his children into schools. In an education institution too, that was supposed to enlighten people and free them from superstition, caste practices were seen. The Mahar children had to sit in a corner from where the board was not visible. The author had a group of seven to eight girls and they felt united for the first time, they would deliberately gang up on the upper caste girls, touch them and beat them up. They no longer accepted their fates and no longer saw the higher



caste groups as “god’s own people”. They would insult Gandhi and the upper caste girls would try to insult Dr. Ambedkar.

Another wave of consciousness, the people decided not to celebrate Padva, the New Year of the Hindus, but to celebrate 14<sup>th</sup> April, the birthday of Dr. Ambedkar as the New Year keeping all the rituals and customs intact.

The two movies, Sant Tukarama and Satti Savitri greatly impacted their lives. Also, in the lives of the children of the author, they held family discussions on what to choose for their career. They didn’t just accept any lot that befell them, but were given the freedom of choice. While choosing a suitable groom for a girl, his educational qualification would come into play.

Seeking to exert their rights “as the sons of the soil”, the Mahars forcefully entered the Vitthal temple and the author and her friends too decide to do the same. And this incident made the author come to the conclusion that she would never think of those gods again.

Another event, the Mahar women were not given chair and Thakubai charged the Rani saying, “Your women are not allowing our women to sit on the chairs. Our Ambedkar has told us to demand our rights. I am going to forcefully remove your women from the chairs and seat my women there [...]” (Kamble, 133) and immediately chairs were arranged for them.

The author has educated all her children, the eldest son has done his M.Sc. in Agriculture, second son is a clerk and his wife is a teacher. Their third son is an officer and has married a teacher. Their daughter is a block development officer and her third daughter is married to a rich farmer. Their fourth daughter is married to a doctor.

The fighter’s spirit is seen in the author as she narrates incidents from her school days and also in her writing. She resolves to follow the path of Ambedkar. She shows the awakening of consciousness to a very large extent. The Hindu Code Bill helped the women break free

from the nets that entrapped them. She says that, "Veergaon has a lion's share in helping me perceive the truth." (Kamble, 102) She serves her community and uses her writing as a tool to start her struggle against the oppressive forces and to articulate her sufferings. "... it is because of him that my pen can scribble out some thoughts." (Kamble, 102)

## CONCLUSION

Dr. B.R. Ambedkar was against caste system and called it a many headed monster. He targeted caste system and said that it was just a different type of class system. According to him, caste is practiced, maintained and reproduced only through marriage. Caste determines who we will marry and that practice is known as endogamy (all the people within your caste you can marry). Exogamy on the other hand refers to all people in your caste you can't marry due to clan or gotra system. Ambedkar did not believe in inter caste dining as all these practices only reinforced the hierarchy, he believed that endogamy is the root of caste system and one should target it from its roots. Exogamy is sinful as incest is frowned upon by society. Endogamy has been created to promote caste system. There were four practices created to take care of surplus men and surplus women- Sati, forced widowhood, celibacy and child marriage. While Sati and forced widowhood were forced upon women, men could choose to become celibates or marry a young child in case he becomes a widower.

Dr. B.R. Ambedkar believed that Brahmins started the self-enclosed system and they did not allow inter caste marriage and one could convert to Brahmins, as Brahmins were created by being born into a Brahmin family only. Other caste groups wanted to imitate the Brahmins- Sanskritization means when you imitate the practices and rituals of the higher caste. Shudras do not practice forced widowhood and Sati because they are farthest from the high order. Kshatriyas practiced sati and forced widowhood was practiced by the Brahmins. Shudras

practiced neither sati nor forced widowhood. Vaishyas practiced forced widowhood, but at a small scale. Lower caste wanted to imitate the practices of higher caste.

Gandhi's understanding of caste was more humanistic. He called for a change of heart. He didn't say it on behalf of the rights of the Dalits, but to understand their religion. Ambedkar believed that it is the right of the Dalit, and that, they should have access to everything. He wanted it as a kind of civil right. He wanted legalistic jurisdiction to claim that caste system should not exist. Gandhi believed that Varna system was intentionally good but had been corrupted by men. Gandhi was not against caste-based occupation. Shudras have a class called Bhangi who deal with dead bodies and cleaned toilets. Gandhi said that there was nothing impure about cleaning toilets, he was asking for tolerance. Ambedkar said it is a very pitiful way of talking, he wanted to completely eradicate caste system and hence he was more practical.

"Both reformers (Gandhi and Ambedkar) had a vision of equality, but for Ambedkar equality meant not only equal status of the Varnas, but equal social, political, and economic opportunity for all." (Ambedkar and Indian Caste System, Singh, Ravindra Prasad, 2)

Gandhiji called them Harijans, but they rejected this word and wanted to use the word Dalits. For Ambedkar abolishing caste system was his first priority whereas pushing the colonial rule out of India was Gandhi's first priority. Nobody could address caste system in its entirety unlike Ambedkar. For him Hinduism was an immoral religion, and he was very critical of it. He used examples from Hinduism in his speeches to show its pitfalls and this sets him apart. He wanted a structural reorganization of the Hindu society. Ambedkar was more political and did not believe in the spiritual understanding of Hinduism. He did not believe in God given power. He understood it for what it really was- a system that kept people unequal.

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## TOTAL UNIDOMINATING FUNCTIONS AND TOTAL UNIDOMINATION NUMBER OF A 3-REGULARIZED WHEEL

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### Abstract

The theory of Domination in graphs is a rapidly growing area of research in Graph Theory. Domination in graphs has applications to several fields such as school bus routing, computer communication networks, Facility location problems, locating radar stations problem etc. Recently dominating functions in domination theory have received much attention. The concept of total unidominating function was introduced in [6]. The total unidominating functions of a cycle were studied in [7]. In this paper the authors define a graph named as 3-regularised wheel and study the total unidominating functions of this graph and determined its total unidomination number and the number of total unidominating functions with minimum weight.

**Key words:** Wheel, 3-regularized wheel, total unidominating function, total unidomination number.

### 1. INTRODUCTION

Graph theory has in numerous applications in different areas such as Physical Sciences, Biological Sciences and other branches of Mathematics etc. In addition, graph theory plays an important role in several areas of computer science such as switching theory, logical design etc.

Theory of domination is an important branch of graph theory that has applications in to several fields such as School bus routing, Computer communication networks, Facility location problems, Locating radar stations problem etc. Domination and its properties have

been extensively studied by T.W. Haynes et al [1, 2].

Recently dominating functions in domination theory have received much attention. Hedetniemi et al. [3] introduced the concept of dominating function. The concept of total dominating functions was introduced by Cockayne et al. [4]. Some inequalities relating to domination parameters in cubic graphs were studied in [5]. The concept of total unidominating function is introduced and studied the total unidominating functions of a path in [6], total unidominating functions of a cycle in [7].

In this paper we define a graph named as 3-regularised wheel and find the total unidomination number of a 3-regularised wheel, the number of total unidominating functions with minimum weight. Further the results obtained are illustrated.

3- Regularized wheel is defined as "A graph formed from  $W_{1,n}$  by replacing the center of  $W_{1,n}$  by a cycle  $C_n$  and each of the remaining  $n$  vertices in  $W_{1,n}$  are replaced by cycles  $C_3$ ".

### 2. TOTAL UNIDOMINATING FUNCTIONS AND TOTAL UNIDOMINATION NUMBER

In this section the concepts of total unidominating function and total unidomination number are introduced and defined as follows:

**Definition 2.1:** Let  $G(V, E)$  be a connected graph. A function  $f: V \rightarrow [0, 1]$  is said to be a **total unidominating function**, if

$$\sum_{u \in N(v)} f(u) \geq 1 \quad \forall v \in V \text{ and } f(v) = 1,$$

$$\sum_{u \in N(v)} f(u) = 1 \quad \forall v \in V \text{ and } f(v) = 0,$$

where  $N(v)$  is the open neighbourhood of the vertex  $v$ .

**Definition 2.2:** The total unidomination number of a connected graph  $G(V, E)$  is defined as

$\min\{f(V) / f \text{ is a total unidominating function}\}$ . It is denoted by  $\gamma_{tu}(G)$ .

Here  $f(V) = \sum_{u \in V} f(u)$  is called as the weight of the total unidominating function  $f$ .

### 3. TOTAL UNIDOMINATION NUMBER OF A 3-REGULARIZED WHEEL

**Theorem 3.1:** The total unidomination number of a 3-regularized wheel is  $\gamma_u(C_n) + n$ .

**Proof:** Let  $W_{1,n}$  be a wheel and  $C_n$  be the cycle replacing the center of  $W_{1,n}$  and  $C_3^1, C_3^2, \dots, C_3^n$  are the cycles replacing the  $n$  vertices in  $W_{1,n}$  respectively.

Let  $u_1, u_2, \dots, u_n$  be the vertices in  $C_n$  and  $v_1, v_2, \dots, v_n$  be the vertices in  $C_3^1, C_3^2, \dots, C_3^n$  respectively which are adjacent to  $u_1, u_2, \dots, u_n$  respectively. Let  $w_1, w_2, w_3, w_4, \dots, w_{2n-1}, w_{2n}$  be the remaining vertices in  $C_3^1, C_3^2, \dots, C_3^n$  respectively.

Here  $d(u_i) = d(v_1) = d(w_{2i}) = d(w_{2i-1}) = 3$  for  $i = 1, 2, \dots, n$ .

Let  $g$  be a unidominating function of  $C_n$  with minimum weight  $\gamma_u(C_n)$ , where  $\gamma_u(C_n)$  is the unidomination number of the cycle  $C_n$  obtained in [8].

Define a function  $f: V \rightarrow \{0, 1\}$  by

$$f(v) = \begin{cases} g(v) & \text{when } v = u_i, \quad i = 1, 2, \dots, n, \\ 1 & \text{when } v = v_i \text{ and } g(u_i) = 1, \\ 1 & \text{when } v = w_{2i}, w_{2i+1} \text{ and } g(u_i) = g(u_{i+1}) = 0, \\ 0 & \text{otherwise.} \end{cases}$$

Now we prove that  $f$  is a total unidominating function.

**Case 1:** Let  $g(u_i) = 1$  for some  $i = 1, 2, \dots, n$ . Then it follows that

$$f(u_i) = 1, f(v_i) = 1, f(w_{2i}) = 0, f(w_{2i+1}) = 0.$$

$$\text{Then } \sum_{u \in N(u_i)} f(u) = f(u_{i-1}) + f(u_{i+1}) + f(v_i) \geq f(v_i) = 1.$$

$$\sum_{u \in N(v_i)} f(u) = f(u_i) + f(w_{2i-1}) + f(w_{2i}) = 1 + 0 + 0 = 1.$$

$$\sum_{u \in N(w_{2i-1})} f(u) = f(v_i) + f(w_{2i-2}) + f(w_{2i}) = 1 + 0 + 0 = 1.$$

$$\sum_{u \in N(w_{2i})} f(u) = f(v_i) + f(w_{2i-1}) + f(w_{2i+1}) = 1 + 0 + 0 = 1.$$

**Case 2:** Let  $g(u_i) = 0$  and  $g(u_{i+1}) = 0$  for some  $i = 1, 2, \dots, n$ . Then it follows that

$$f(u_i) = 0, f(v_i) = 0, f(w_{2i-1}) = 0, f(w_{2i}) = 1, f(w_{2i+1}) = 1, f(w_{2i+2}) = 0.$$

$$\text{Then } \sum_{u \in N(u_i)} f(u) = f(u_{i-1}) + f(u_{i+1}) + f(v_i) = 1 + 0 + 0 = 1.$$

$$\sum_{u \in N(v_i)} f(u) = f(u_i) + f(w_{2i-1}) + f(w_{2i}) = 0 + 0 + 1 = 1.$$

$$\sum_{u \in N(w_{2i+1})} f(u) = f(u_{i+1}) + f(w_{2i+2}) + f(w_{2i+2}) = 0 + 1 + 0 = 1.$$

$$\sum_{u \in N(w_{2i+2})} f(u) = f(w_{2i}) + f(v_{i+1}) + f(w_{2i+2}) = 1 + 0 + 0 = 1.$$

$$\sum_{u \in N(w_{2i+2})} f(u) = f(w_{2i+1}) + f(v_{i+1}) + f(w_{2i+3}) = 1 + 0 + 0 = 1.$$

From Case 1 and Case 2 it follows that  $f$  is a total unidominating function.

From the definition of  $f$ , we have

$$\sum_{i=1}^n f(u_i) = \sum_{i=1}^n g(u_i) = \gamma_u(C_n), \quad \sum_{i=1}^n f(v_i) = \sum_{i=1}^n g(u_i) = \gamma_u(C_n),$$

$$\sum_{i=1}^n f(w_{2i-1}) + \sum_{i=1}^n f(w_{2i}) = \frac{1}{2} \left( n - \sum_{i=1}^n g(u_i) + n - \sum_{i=1}^n g(u_i) \right)$$

$$= \frac{1}{2} [2n - 2\gamma_u(C_n)] = n - \gamma_u(C_n).$$

$$\text{Therefore } \sum_{u \in V} f(u) = \sum_{i=1}^n f(u_i) + \sum_{i=1}^n f(v_i) + \sum_{i=1}^n f(w_{2i-1}) + \sum_{i=1}^n f(w_{2i})$$

$$= \gamma_u(C_n) + \gamma_u(C_n) + n - \gamma_u(C_n) = \gamma_u(C_n) + n.$$

By the definition of total unidomination number, it follows that

$$\gamma_{tu}(3\text{-regularised wheel}) \leq \gamma_u(C_n) + n \quad \dots (1)$$

Let  $f$  be a total undominating function. Then  $f$  has the following properties.

1. If  $f(u_i) = 0$  and  $f(u_{i-1}) = 1$  or  $f(u_{i+1}) = 1$  then  $f(v_i)$  must be 0 and  $f(w_{2i}) = 1$  or  $f(w_{2i+1}) = 1$  respectively. Otherwise if  $f(u_i) = 0$  and both of  $f(u_{i-1}), f(u_{i+1})$  are 0 then  $f(v_i), f(w_{2i-1}), f(w_{2i})$  must be 1.
2. If  $f(u_i) = 1$  and both of  $f(u_{i-1}), f(u_{i+1})$  are 0 then  $f(v_i)$  must be 1.

Let  $k_1$  be the number of  $u_i$ s such that  $f(u_i) = 0$  and any one of  $f(u_{i-1}), f(u_{i+1})$  is 1 then  $0 \leq k_1 \leq n - \gamma_u(C_n)$  and  $\sum_{k_1} f(v_i) + f(w_{2i-1}) + f(w_{2i}) = k_1$  for these  $k_1$  sets of vertices  $(v_i, w_{2i-1}, w_{2i})$  where  $i$  is such that  $f(u_i) = 0$  and  $f(u_{i-1}) = 1$  or  $f(u_{i+1}) = 1$ .

Let  $k_2$  be the number of  $u_i$ s such that  $f(u_i) = 0$  and  $f(u_{i-1}) = f(u_{i+1}) = 0$  then  $0 \leq k_2 \leq n$  and  $\sum_{k_2} f(v_i) + f(w_{2i-1}) + f(w_{2i}) = 3k_2$  for these  $k_2$  sets of vertices  $(v_i, w_{2i-1}, w_{2i})$ , where  $i$  is such that  $f(u_i) = 0$  and  $f(u_{i-1}) = f(u_{i+1}) = 0$ .

Then there are  $n - (k_1 + k_2)$   $u_i$ s such that  $f(u_i) = 1$  and  $\sum f(v_i) + f(w_{2i-1}) + f(w_{2i}) \geq n - (k_1 + k_2)$  for these  $n - (k_1 + k_2)$  sets of vertices  $(v_i, w_{2i-1}, w_{2i})$  where  $i$  is such that  $f(u_i) = 1$ .

Therefore  $f(V)$

$$\begin{aligned} &= \sum_{i=1}^n f(u_i) \\ &+ \sum_{k_1} f(v_i) + f(w_{2i-1}) + f(w_{2i}) \\ &+ \sum_{k_2} f(v_i) + f(w_{2i-1}) + f(w_{2i}) \\ &+ \sum_{n-(k_1+k_2)} f(v_i) + f(w_{2i-1}) + f(w_{2i}) \\ &\geq n - (k_1 + k_2) + k_1 + 3k_2 + n - (k_1 + k_2) \\ &= 2n - k_1 + k_2 \\ &\geq 2n - (n - \gamma_u(C_n)) + k_2 \geq n - \gamma_u(C_n). \end{aligned}$$

Since  $f$  is defined arbitrarily, it follows that  $\gamma_{tu}(3\text{-regularized wheel}) \geq \gamma_u(C_n) + n$  --- (2)

Therefore from the inequalities (1) and (2), we get  $\gamma_{tu}(3\text{-regularized wheel}) = \gamma_u(C_n) + n$ .

**Theorem 3.2:** The number of total undominating functions of a 3-regularized wheel with minimum weight  $\gamma_u(C_n) + n$  is the

number of undominating functions of  $C_n$  with minimum weigh  $\gamma_u(C_n)$ .

**Proof:** Consider the total undominating function  $f$  with minimum weight  $\gamma_u(C_n) + n$  given in Theorem 3.1. As the function  $f$  is given in terms of  $g$ , a undominating function of  $C_n$ , it is clear that the number of total undominating functions of 3-regularized graph with minimum weight is equal to the number of undominating functions of  $C_n$  with minimum weight.

Therefore the number of total undominating functions of a 3-regularized wheel are

$$\begin{cases} 3 & \text{when } n \equiv 0 \pmod{3}, \\ n & \text{when } n \equiv 1 \pmod{3}, \\ n \left(1 + \left\lfloor \frac{n}{6} \right\rfloor\right) & \text{when } n \equiv 2 \pmod{3}, n \neq 8, \\ 12 & \text{when } n = 8. \end{cases}$$

Now we verify that whether there is any other total undominating function with minimum weight.

Let  $f$  be a total undominating function. Then we have proved in Theorem 3.1 that

$$f(V) \geq 2n - k_1 + k_2.$$

If  $k_1 = k_2 = 0$  then  $f(V) \geq 2n > \gamma_u(C_n) + n$ .

If  $k_1 = 0, k_2 > 0$  then  $f(V) > 2n > \gamma_u(C_n) + n$ .

If  $k_1 < n - \gamma_u(C_n), k_2 > 0$  then  $f(V) \geq 2n - k_1 + k_2 > 2n - n + \gamma_u(C_n) = \gamma_u(C_n) + n$ .

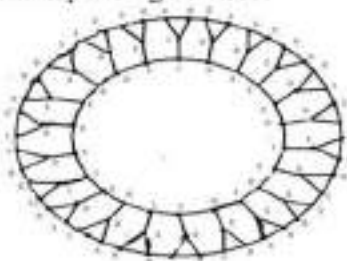
In the above three cases we have  $f(V) > \gamma_u(C_n) + n$ . Therefore  $f$  is not a function with minimum weight.

If  $k_1 = n - \gamma_u(C_n), k_2 = 0$  then  $f(V) = \gamma_u(C_n) + n$  and this function coincides with one of the above said functions.

Therefore there is no other total undominating function with minimum weight.

#### 4 ILLUSTRATIONS

**Example 4.1:** The functional values of a total undominating functions of a 3-regularized wheel formed from  $W_{1,22}$  are given at the corresponding vertices.



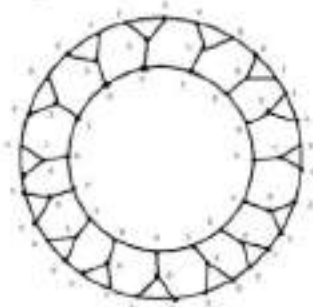
3-regularized wheel formed from  $W_{1,22}$

Total unidomination number of 3-regularized wheel formed from  $W_{1,22}$  is

$$\gamma_{tu}(3\text{-regularised wheel}) = \gamma_u(C_{22}) + 22 \\ = \left\lfloor \frac{22}{3} \right\rfloor + 22 = 8 + 22 = 30.$$

There are 22 total unidominating functions of the 3-regularized wheel formed from  $W_{1,22}$  having the minimum weight 30. ■

**Example 4.2:** The functional values of a total unidominating functions of a 3-regularised wheel formed from  $W_{1,15}$  are denoted at the corresponding vertices.



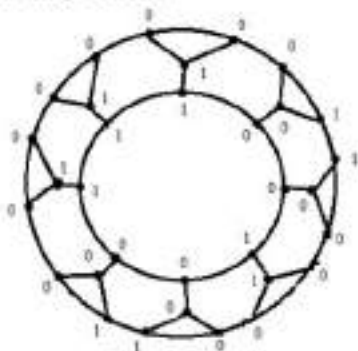
3-Regular wheel formed from  $W_{1,15}$

Total unidomination number of the regular wheel formed from  $W_{1,15}$  is

$$\gamma_{tu}(3\text{-regular wheel}) = \gamma_u(C_{15}) + 15 \\ = 20.$$

Number of total unidominating functions of the 3-regularized wheel formed from  $W_{1,15}$  having the minimum weight 20 are 3. ■

**Example 4.3:** The functional values of a total unidominating functions of a 3-regularized wheel formed from  $W_{1,8}$  are denoted at the corresponding vertices.



3-Regular wheel formed from  $W_{1,8}$

Total unidomination number of the regular wheel formed from  $W_{1,8}$  is

$$\gamma_{tu}(3\text{-regular wheel formed from } W_{1,8}) \\ = \gamma_u(C_8) + 8 = 12.$$

Number of total unidominating functions of the 3-regular wheel formed from  $W_{1,8}$  having the minimum weight 12 are 12.

## 5.CONCLUSION

This work gives a scope to find upper total unidomination number and the number of minimal total unidominating function with maximum weight of a 3-regularized wheel.

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## REPRODUCTIVE ECOLOGY OF SOME CARPET WEED SPECIES OF MOLLUGINACEAE

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### ABSTRACT

Reproductive ecology of five carpet weed species *Glinus lotoides* L., *G. oppositifolius* (L.) Aug. DC., *Mollugo cerviana* (L.) Ser., *M. nudicaulis* Lam. and *M. pentaphylla* L. (Molluginaceae) was studied. *Glinus* species are prostrate, spreading, annual herbs that carpet the ground in open sandy soils and agricultural lands. They have a perianth of five or rarely six tepals in quincuncial arrangement, functional stamens, petaloid staminodes and numerous ovules. The flowers are weakly protandrous, herkogamous and facultatively autogamous. Bees, thrips, ants and lycaenid butterflies pollinate the flowers. In both the species, the fruit is a dehiscent capsule and disperses scrotiform strophioled-seeds. Seed dispersal modes include anemochory, ombrohydrochory and hydrochory.

*Mollugo* species are annual herbs which usually grow throughout the year in open dry sandy and sandy and loamy soils but also occur in moist habitats, especially in cultivated lands. The flowers have a perianth consisting of five tepals in quincuncial aestivation, functional stamens and several ovules. The ovary has three carpels and three stigmas. In all, the flowers are facultative autogamous. *M. cerviana* is never visited by any insect species while *M. nudicaulis* and *M. pentaphylla* are pollinated by insects. Certain percentage of *in situ* pollen germination and the occurrence of pollen tubes on the stigma during the process of anthesis facilitates self-induced autogamy. Spontaneous autogamy occurs due to close proximity of dehiscent anthers of all five anthers to the stigmas in *M. cerviana* and due to close proximity of 1-3 dehiscent anthers to the stigmas in *M. nudicaulis* and *M. pentaphylla* during flower closure.

Seed dispersal modes include anemochory, ombrohydrochory and hydrochory.

**Keywords:** *Glinus lotoides*, *G. oppositifolius*, *Mollugo cerviana*, *M. nudicaulis*, *M. pentaphylla*, facultative autogamy, entomophily, polychory.

## INTRODUCTION

In general, weeds are mostly annuals or biennials and rarely perennials. Weeds are abundantly available, usually grow fast, and reproduce quickly and easily. Some species of weeds, called pioneer species, not only grow fast, but produce carbon quickly as well. This carbon, when they die, lasts a long time in the soil, helps build structure, and helps retain water. They have certain nutrients that they absorb from the soil, bring to the top, and release when they die. The weeds quickly colonize disturbed habitats created by man and also grow in areas far removed from their native ranges. They are genetically labile and phenotypically flexible, such characters enable them to pass through successfully the process of survival of the fittest under vagaries of nature such as adverse weather conditions, soils equipped with little water and nutrient content, presence of herbivores, predators, parasitoids, parasites and pathogens, and build up their populations very rapidly in many geographic regions (Mulligan and Kevan 1973; Andrewartha and Birch 1984). The weeds are excellent subjects for understanding mechanisms of breeding systems, life-history evolution and adaptation (Baker 1965; Stebbins 1989). The importance of understanding the biological requirements and peculiarities of herbs has been emphasized by many writers (Harlan 1965; King 1966; Chancellor 1968; Hammerton 1968).

Naidu (2012) explained the negative and positive effects of weeds. Weeds are undesired plants in agricultural systems as they deplete the nutrients, water and space allotted for the intended crop, and finally cause huge reduction in crop yield. Weeds in crop fields reduce input efficiency, interfere with agricultural operations, impair quality and act as alternate hosts for several insect pests and diseases. Some weeds release toxic substances which affect the crop growth. The obvious effect of these traits is the hike in cost of cultivation by several folds. Along with this, weeds affect and interfere in the management of all the terrestrial and aquatic resources. They endanger the native biodiversity by choking and deliberate takeover of the native plants. The animals which depend on this native biodiversity for their survival are also affected. As part of the primary producers within farming systems, weeds are also considered as important components of the agro-ecosystem. Reductions in abundances of weeds which act as hosts may affect associated insects and other taxa which are beneficial. Thus, weeds have a role within agro-ecosystems in supporting biodiversity more generally. Weed biology relates to the plant attributes such as morphology, seed dormancy and germination, physiology of growth, competitive ability and reproductive biology. Knowledge of weed biology is essential for the development of both economically and environmentally acceptable weed management systems. It is also essential to understand and predict how weed species, their populations and biotypes evolve in response to the selection pressures and play their role in soil conservation and eco-restoration. Vaidya *et al.* (1978) documented that weeds are generally classified based on their habitat. Weeds of cultivated crop fields (agricultural land) are called agrestals and those of non-agricultural land i.e. fallow land, harvested fields, along rail tracks, road-sides, hedges, waste places, on

old walls and roof tops etc. are called ruderals. There are some weeds which are not specific to any particular habitat i.e. they act as both agrestals and ruderals. Weeds show wide ecological amplitude by means of which they can resist the extreme conditions of environment.

The database of Plants For A Future (PFAF) indicates that there is limited information on wind-pollination and this information shows that wind-pollination is not a significant life history trait in flowering plants. Grasses are wind-pollinated, this bias may cause an underestimate of wind pollination in weeds. Baker (1965) predicted that wind-pollination is ideal for the reproductive success of weeds, but at the same he stated that generalized pollination is important over specialized pollination to enable weeds to spread and invade different niches. PFAF database does not distinguish between generalist insect pollination and specialist insect pollination. Weeds and non-weeds show significant differences in their pollination modes (Kartesz and Christopher 1999).

Invertebrate pollinators play important roles in the reproductive success of invasive indigenous and non-indigenous plant species, some or several of which threaten the native communities (Pascarella *et al.* 2001). Breeding system, flower morphology, pollinator traits, biomass allocated to vegetative versus reproductive tissues, all correlate with life history such as growth form, pattern of development, time to first breeding, number of reproduction events and offspring characteristics (Damgaard and Abbott 1995).

Molluginaceae is a small family in Caryophyllales order of flowering plants. It has 10 genera with 80 known species distributed in tropical, sub-tropical and even warm temperate regions of the world (Bogle 1970; Endress and Bittrich 1993). This family is coded as polymorphic and consists of taxa with uniseriate, undifferentiated perianth and taxa with differentiated perianth (Ronse De Craene 2008). Certain important characters in this family include quincuncial aestivation, dual role of perianth, petaloid staminodes, variation in stamen number, carpels and stigmas, closing of flowers and reniform seeds (Hofmann 1994). This family is of little economic value. Many species have a "weedy" nature and frequently invade disturbed places. Some species are important as forage plants while a few species are edible and used as potherbs (Kirk 1975). In *Glinus* and *Mollugo* genera, several species are weedy, and some species are used as vegetables and in traditional medicine (Bogle 1970; Endress and Bittrich 1993). The weedy *Glinus* and *Mollugo* species commonly known as carpet weeds are important in carpeting the soil in dry environments by their profuse prostrate growth and producing several batches of offspring in a year. But, there is no information on the reproductive ecology of these weedy species which is required to know how they are able to grow, thrive and carpet the soil.

The present study was contemplated to provide the details of reproductive ecology of five carpet weed species, *Glinus lotoides* L., *G. oppositifolius* (L.) Aug. DC., *Mollugo cerviana* (L.) Ser., *M. nudicaulis* Lam. and *M. pentaphylla* L. belonging to Molluginaceae family.

**The following objectives were investigated:** flowering phenology, floral structural and functional morphology, anthesis and anther dehiscence schedules, pollination mechanism, pollination, pollinators, sexual system, flower closing mechanism, fruiting ecology and seed dispersal. This information is important to understand the efficiency of sexual reproduction which enables them to be agrestals and ruderals as they occur both in natural and agricultural habitats, especially in dry environments. This study is useful to evaluate the abilities of these weeds to use local insect fauna as pollinators to achieve genetic variation through which they acquire adaptations to grow in different ecological niches and expand their distribution range, especially in open, dry soils which are devoid of any vegetative cover.

## REVIEW OF LITERATURE

The Caryophyllales is a group long recognized by its distinctive placentation and embryology, and is a major order of angiosperms representing about 5% of core eudicot diversity. This order exhibits wide variation in perianth structure and morphology (Takhtajan 1991). The perianth varies from an undifferentiated to differentiated structure with the concomitant evolution of petals from either bracts or stamens, in varying positions in the flower. More recently, molecular phylogenetics has improved the understanding of intra-ordinal relationships within the Caryophyllales (Cuenoud *et al.* 2002). In this order, the clade core eudicots characterized by tricolpate pollen grains included Aizoaceae as a large family in which the present **Molluginaceae** genera are included. In Aizoaceae, members of the early-diverging subfamilies Sesuvioideae and Aizoioideae possess a quincuncial uniseriate perianth; it is petaloid on the adaxial surface while it is sepaloid on the abaxial side. In the derived subfamilies Mesembryanthemoideae and Ruschioideae commonly referred to as mesembs, the petaloid staminodes are possibly stamen-derived as they possess a singular vascular trace and a narrow point of insertion. Both petals and stamens develop from primordia initiating in a centrifugal direction. Stamens and petals are linked by intermediates, as floral organs developing closest to the fertile stamens are increasingly filamentous while the outermost organs are increasingly petaloid (Hofmann 1994; Thiede *et al.* 2011). Concomitantly, the outer quincuncial uniseriate perianth loses all petaloid characters and resembles only a calyx (Brockington 2009).

Ronse De Craene (2012) reported that eudicots consist of a basal grade and a large (core eudicot) clade, which comprises the majority of species. Eudicots are a highly successful group of plants, occupying almost all habitats on earth. In core eudicots, the evolution of flowers is highly diverse and is driven by repeated diversifications of pollination mechanisms. Flowers of eudicots are mostly with parts in five and with differentiated perianth of sepals and petals. Specific pollination mechanisms have led to groups with specialized animal-pollinated zygomorphic flowers (Leguminosae and Lamiales) or wind-pollinated apetalous flowers (Fagales and Caryophyllales). Likewise, dispersal mechanisms of eudicots are also highly diverse.

Glover (2011) reported that flowering plants produce three classes of chemicals, the flavonoids, the betalains and the carotenoids. The flavonoids are the major floral pigments. They give rise to ivory and cream colours through flavonols and flavones, yellow and orange colours through aurones and chalcones and the red, purple and blue through anthocyanins. The betalains are not major floral pigments but when produced give red colour to flowers. The carotenoids are more widespread in plants but less significant as floral pigments than the flavonoids; they give yellow and orange colour to flowers. Rausher (2006) reported that anthocyanins color petals to attract pollinators while Gould (2004) noted that they provide protection to vegetative tissues against ultraviolet, herbivores, and pathogens. Hatlestad and Lloyd (2015) mentioned that plants also produce betalain pigments to attract pollinators to flowers. Stafford (1994) stated that a given plant species produces either anthocyanins or betalains but not both. Clement and Mabry (1996) stated that it is expensive for plants to produce both groups of pigments and perhaps the production of one group of pigments is cheaper under some environmental conditions or preferable if their metabolic precursors are limiting for some reason. Clement *et al.* (1994) and Stafford (1994) reported that in Caryophyllales order, anthocyanins are found only in two families, Caryophyllaceae and Molluginaceae while this pigmentation has been replaced by betalains in the rest of the Caryophyllales. Glover (2011) mentioned that the betalains are found exclusively in Caryophyllales, and nowhere else in the plant kingdom.

Floral evolution often modifies the androecium, resulting in either stamen loss or transformation of stamen function from pollen production and presentation to alternate functions. With the loss of their defining function as producers of viable male gametophytes, stamens become staminodes. Commonly implicated staminode roles include pollinator attraction through visual conspicuousness and/or provision of attractants and rewards, avoidance of self-pollination, and facilitation of pollen removal and receipt through various trigger-mechanisms. Direct evolution from stamen to functional staminode likely occurs when stamens initially serve purposes such as pollinator attraction in addition to pollen production and presentation. In this situation, functional constraints favor "division of labor," which converts some stamens into staminodes specialized for the ancillary function and allows specialization of the remaining stamens on their primary role. With indirect evolution, the nonfunctional phase preceding adoption of a new function allows staminodes to assume novel functions not expected of stamens. Therefore, the taxonomic distribution of staminodes reflect functional evolution and the variety of functions served by staminodes reveal the course of that evolution (Weberling 1989; Rouse De Craene and Smets 1993, 1995).

In animal-pollinated plants, reduction of entire stamen whorls usually involves actinomorphic flowers pollinated by diverse small insects with more than one whorl of fertile stamens (Stebbins 1974; Rouse De Craene and Smets 1993, 1995). Reallocation of resources to more, smaller flowers and/or adaptations that increase efficiency of pollen dispersal likely prompt reduced pollen production per flower through stamen loss. These adaptations include pollen packaging and pollen-dispensing mechanisms that limit pollen

removal by individual pollinators but maximize pollen dispersal, and more precise contact between pollinators and fertile anthers or pollinators and stigmas. Both adaptations increase the proportions of pollen grains delivered to stigmas (Harder and Thomson 1989).

Ehrendorfer (1976) proposed that ancestral taxa in Caryophyllales occupied "open, warm, dry and windy habitats with mineral soils". Because, many of the families in Caryophyllales currently inhabit xeric, marginal environments. In this open, pollinator-deprived environment, wind pollination may have prevailed, and anthocyanin pigmentation was lost as there was no need to attract pollinators. Subsequently, following the radiation of pollinator lineages and the colonization of less marginal habitats, reversion to zoophily engendered a return to pigmentation in the form of betalains rather than anthocyanins. Clement and Mabry (1996) also argued that anemophily was the ancestral condition in Caryophyllales because the ancestral species in this order have evolved in open, dry, marginal environments at a time when pollinators were scarce. Strauss and Whittall (2006) noted that it is unreasonable to explain the evolutionary changes in pigmentation as a result of the absence or presence of pollinators alone because anthocyanins and betalains accumulate and function in both vegetative and reproductive tissues. Friedman and Barrett (2008) reported that there is a strong correlation between the occurrence of open habitat and anemophily. These authors also noted that this correlation may not necessarily be due to pollinator scarcity but rather to the selective advantage of anemophily in an open environment.

Hutchinson (1926) recognized Molluginaceae as distinct from Aizoaceae. Molluginaceae genera previously included in the larger family Aizoaceae have been separated and treated them under Molluginaceae family in subsequent classifications of Angiosperm Phylogeny Group (APG) 1998, APG II of 2003, APG III of 2009 and APG IV of 2016. APG IV classification is the modern molecular-based system of plant taxonomy for flowering plants (angiosperms). In this classification, 10 genera and 80 known species have been assigned to Molluginaceae. The genera include *Adenogramma* Rehb., *Coelanthum* E. Mey. ex Fenzl, *Glinus* L., *Glischrothamnus* Pilg., *Hypertelis* E. Mey. ex Fenzl., *Mollugo* L., *Pharnaceum* L., *Polpoda* C. Presl., *Psammotropha* Eckl. & Zeyh, and *Suessenguthiella* Friedrich (Christenhusz and Byng 2016). The genus *Glinus* has been assigned 11 species, *G. bainesii* (Oliv.) Pax, *G. herniarioides* (Gagnep.) Tardieu, *G. lotoides* L., *G. microphyllus* Hauman, *G. oppositifolius* (L.) Aug. DC., *G. orygioides* F. Muell., *G. pauli-wilhelmi* Hochst., *G. radiatus* (Ruiz & Pav.) Rohrb., *G. runkewitzii* Tackh. & Boulos, *G. sessiliflorus* P.S. Short and *G. setiflorus* Forssk; all these have been accepted to species level in this genus except *G. pauli-wilhelmi* which is yet to be resolved. The genus *Mollugo* has been assigned 93 species out of which only 18 have been accepted while others have been considered to be either synonyms or un-assessed. The accepted species include *M. angustifolia* M.G. Gilbert & Thulin, *M. caespitosa* Scott-Elliot, *M. cerviana* (L.) Ser., *M. crockeri* Howell, *M. decandra* Scott-Elliot, *M. flavescens* Andersson, *M. floriana* (B.L. Rob.) Howell, *M. fragilis* Wawra, *M. namaquensis* Bolus, *M. nudicaulis* Lam., *M. pentaphylla* L., *M. pinosia* Urb., *M. pusilla* Adamson, *M. snodgrassii* B.L. Rob., *M.*

*stricta* L., *M. tenella* Bolus, *M. verticillata* L. and *M. walteri* Friedr. Cuenoud *et al.* (2002) reported that Molluginaceae is polyphyletic and hence have different lines of evolution.

Hofmann (1994) documented that in Caryophyllaceae, the terms sepaloid 'tepal' and petaloid 'tepal' are applied to the quincuncial perianth parts that are present in the core Caryophyllales while petaloid staminodes refer to perianth parts that are clearly androecium-derived. In Aizoaceae, androecial development proceeds centrifugally, and the basipetal members become progressively more sterile and petaloid with intermediates conceptually linking the outermost petals to the inner fertile stamens. This situation has been described in *Glinus* of Molluginaceae. Ronse De Craene (2008) coded Molluginaceae as polymorphic since this family exhibits taxa with uniseriate, undifferentiated perianth and taxa with differentiated perianth. Hofmann (1994) noted that *Glinus* possesses putatively staminodial petals while Ronse De Craene (2008) interpreted them as differentiated staminodial structures.

The genera *Glinus* and *Mollugo* are commonly called carpet weeds and their separation from each other is primarily based on seed characters, the former having appendaged seeds while the latter having non-appendaged seeds. Literature records concerning these weeds are limited to provide information on them. The genus *Glinus* is distributed in tropical and subtropical regions of the world. It originated from the Greek word "*glinos*" meaning a plant with sweet sap or juice. They are squat annual herbs with fuzzy to hairy green herbage. The fruit is a capsule containing many kidney-shaped seeds with a filiform appendaged aril and stellate hairs (Ronse De Craene 2013). *G. lotoides* is widely spread throughout the tropics and subtropics, especially in Africa, Asia, Australia and South Europe. It is native to Eurasia and Africa and has become widespread in tropical, subtropical, and warm-temperate areas worldwide. The species name "*lotoides*" means resembling the genus *Lotus*. It is a spreading annual herb distributed throughout India. The tender shoots and young leaves are used as green vegetable and in the indigenous system of medicine as antiseptic, anthelmintic, against diarrhoea, bilious attacks and as a purgative for curing boils, wounds and pains (Bhavani 2015; Hamed *et al.* 1996; Sastri 2002). Seeds are used for the treatment of tapeworm infections throughout Ethiopia, mainly among rural populations. In Ethiopia and Tanzania, it is currently given threatened status due to its regular harvest for medicinal purpose (Teshome and Feyissa 2015). *G. oppositifolius* is widely distributed in the Americas, tropical Asia, tropical Africa and Australia. The species name "*oppositifolius*" is derived from the Latin word "*oppositus*" meaning standing against or opposed and "*folium*" meaning a leaf, referring to the leaves arranged opposite each other (Huang and Wu 1998; Sahu *et al.* 2001). This species is widely used as a vegetable and in traditional medicine to treat skin diseases, joint pains, inflammation, diarrhoea, intestinal parasites, fever and malaria (Dutta *et al.* 2012).

*Glinus* species are generally characterized by herb life form with flowers in axillary whorls or fascicles, indumentum of stellate hairs, presence of staminodes and by the filiform-

appendaged aril on the seeds (Short 2002). Among different species of *Glinus*, the stamen number is unstable ranging from five to several series of stamens including outer staminodes (Hoffman 1994). In this genus, the sepals are five and display quincuncial aestivation (Ronse De Craene 2010). The stamen loss is variable in different species. The gynoecium and the sepals advancing centripetally act as two separate forces on the intervening androecium to cause stamen loss. The stamen loss is linked with a reduction of carpels from five to three or two. A strong correlation exists between the numbers of stamens in the upper tier and numbers of carpels. Upper stamens always alternate with carpels and an increase or decrease in the upper tier of stamens invariably affects the upper stamen whorl. In flowers with three carpels, the alternisepalous stamens tend to converge in pairs against the flanks of the carpels and are protected to an extent from pressure by the sepals but they leave less space for the initiation of the antesealous stamens. The outer stamens are replaced by petaloid structures and the petaloid number fluctuates enormously and these structures are either antesealous by replacing a fertile stamen or alternisepalous as an appendage of upper stamen (Brockington *et al.* 2013). In *Glinus*, a white aril of funicular origin develops into elongate, filiform strophiole (Bittrich 1990). In *G. lotoides*, the androecium is extremely variable and only the alternisepalous whorl is complete with staminodes and odd stamen is opposite to sepals (Ronse De Craene 2010). But, Sharma (1963) stated that this species shows rarely more than five stamens. Bittrich (1990) noted that *G. lotoides* fruit is a capsule and opens when moistened with the aid of expanding keels. Sharma (1963) reported that in *G. oppositifolius*, the stamens vary from 10 to 13 arranged in three whorls indicative of a tendency for reduction with the loss of the outer stamen whorl.

Erdtman (1986) stated that Molluginaceae is a stenopalynous family because the pollen morphology is uniform among species indicating that the pollen type is characteristic and constant. Perveen and Qaiser (2000) provided the details of pollen grains of this family. The pollen grains are radially symmetrical, isopolar, oblate-spheroidal to prolate-spheroidal or sub-prolate, tricolpate, and the colpal membrane is finely-coarsely granulate. Sexine is slightly thicker than nexine. Tectum is scabrate-spinulose. Ronse De Craene (2010) reported that in Molluginaceae, the ovary is isomerous with antesealous carpels or is reduced to three or two with ovules arranged on axile placentation with narrow partitions and styles are carinal. The fruit is a capsule with many seeds enclosed within persistent calyx. It is loculicidally dehiscent. Bittrich (1990) reported that in *M. verticillata*, the fruit capsule opens when moistened with the aid of expanding keels. Narayana (1962) and Hofmann (1973) noted that *Mollugo* species have a primordium-like swelling on the funiculus and it is considered to be a vestigial aril. Bittrich and Ihlenfeldt (1984) mentioned that *Mollugo* seeds germinate by means of an operculum.

The genus *Mollugo* is native to tropical to warm temperate parts of North and South America but it is distributed in Europe, Africa and Asia. The generic name is derived from the Latin word "*mollis*" meaning soft. *Mollugo* differs from other members of Molluginaceae by the presence of a combination of leaves in false whorls without stipules,



flowers borne in cymes or panicles and produce seeds lacking a caruncle or having a very small caruncle lacking an appendage (Short 2002). *M. verticillata* occasionally introduced in Europe, Africa and Asia. *M. disticha* is restricted to India and Sri Lanka. *M. brevipes*, *M. cubensis* and *M. pinosia* all are endemic to Cuba (Thulin *et al.* 2016). *M. cerviana* is native to India, Sri Lanka, Pakistan and Bangladesh. The species name is derived from the Latin word which means deer or fawn coloured. It is an ancient medicinal plant known as thread stem carpetweed. In India, it is widely used as a pot herb. It is also used in Ayurveda as an alternative treatment for various ailments such as skin diseases, rheumatism, piles, fever and snake bite (Parvathamma and Shanthamma 2000). Further, the extract of the plant has been reported to be a good inhibitor for the corrosion of mild steel (Arockiasamy *et al.* 2014). *M. pentaphylla* is distributed throughout India, Ceylon, Malacca, China, Japan and Fiji (Maharana *et al.* 2012). The species name refers to palmately compound leaf with five leaflets. It is used in traditional medicine as stomachic, antiseptic and to treat sore legs and promote menstrual discharge in women (Sahu *et al.* 2012). *M. nudicaulis* is distributed throughout tropical Africa and Asia (Burrows and Willis 2005). The species name is derived from the Latin word “*nudicaulis*” meaning naked or leafless stem. It is used in Indian phytotherapy for the treatment of inflammation, jaundice, urinary and kidney disorders, wounds, cold, cough, fever and body pain (Rajamanikandan *et al.* 2011).

Ronse De Craene (2010) reported that in *Mollugo*, the number of stamens ranges from five (*M. cerviana*) to three (*M. nudicaulis*). Perveen and Qaiser (2000) reported that *M. cerviana* and *M. pentaphylla* show a little variation in the exine pattern of pollen; the pollen grain is covered with a scabrate tectum in the former and with spinulose tectum in the latter.

Aizoaceae is closely associated with Molluginaceae in several leaf, flower and fruit characters. The flowers in these families are usually small or medium-sized and show adaptations to different categories of insects for pollination. In this connection, the literature available on pollination ecology of these two families has been reviewed and key aspects of pollination and pollinators have been presented.

Little information is available on the pollination biology or related aspects of Aizoaceae subfamilies Mesembryanthemoideae and Ruschoideae, commonly referred to as mesembs (Hartmann 1991; Chesselet *et al.* 1995; Juergens 2004; Peter *et al.* 2004; Thiede *et al.* 2011). The predominance of bright, showy petals and the presence of functional nectaries in the flowers of this family suggest insects as primary pollen vectors (Hartmann 1991; Ihlenfeldt 1994). In a detailed study, Chesselet *et al.* (1995) explained the importance of floral nectar as an attractant to insects in Aizoaceae species. Further, these authors also stated that the abundant pollen produced by many species is an important reward for many insects and insect pollination appears to be common in this family. On the contrary, Bittrich (1987) suggested that abundant pollen might be an adaptation for wind pollination. Ihlenfeldt (1994) noted that many flowers within the Aizoaceae attract a wide spectrum of floral

visitors and that most species are protandrous and self-sterile. Struck (1994) reported that the mesemb flowers are pollinated by masarid wasps in South Africa. Hartmann (1991) reported that the presence of prominent bright shiny petals and the open pollen presentation functional in diurnal flowering species of this family suggest insect pollination. He also stated that the flowers opening during the day are protandrous, with a very distinctive early male phase, followed by a later female phase. The flowers open repeatedly by basal growth of the androecial elements. In melittophilous flowers, the stigmas are at first shorter than the stamens. During the female phase, the stamens wither and collapse and the elongated stigmas become prominent in the middle of the flower. At this stage the stigmas spread and start to produce a copiously papillate surface, which is more intensively coloured than in the un-receptive stage. Such a sexual function in the flowers facilitate and promote cross-pollination by bees. Hammer (1995) reported that Aizoaceae members show synchrony in flowering time within populations. The period of flowering usually short and the repeated opening of flowers is usually restricted to a certain period of the day. Groen and Van Der Maesen (1999) reported that the populations of *Bergeranthus*, *Faucaria* and *Orthopterum* flower simultaneously. These authors considered this situation of flower synchrony in different species collectively promotes floral attraction to pollinator insects. Peter *et al.* (2004) speculated that *Bergeranthus* flowers stay closed at low ambient temperature and vapour pressure as a mechanism to protect the pollen from moisture because pollen fertility gets affected if it contacts with water. The flower closure mechanism probably evolved to protect pollen from water on cool humid days and from dew at night. Zietsman (2013) reported that *Stomatium bolusiae* flowers are hermaphroditic. In some flowers there is almost complete overlap of pollen presentation and stigma receptivity. In others, this time lapse is not more than a few minutes and in some others, there is complete overlap of male and female functions. The floral sexual function is indicative of facultative xenogamy.

In Molluginaceae, nectar secreting tissue is present in almost all species. In several genera, showy sepals or petals have evolved, both of which strongly suggest entomophily (Watson and Dallwitz 1992; Kubitzki *et al.* 1993). *Glinus lotoides* is an important source of pollen for honey bees in Radom area, South Darfur State, Sudan (Aldeen 2014). Hesperiid butterfly, *Carcharodus alceae* during its larval stage avoids *G. lotoides* if the latter is present in the habitat (Benyamini 2005). *Zizeeria karsandra* uses *G. lotoides* and *G. oppositifolius* as larval host plant in south Australia (Grund 1998). *Mollugo verticillata* is pollinated by syrphid fly, *Mesogramma marginata* (Robertson 1928). It is the larval host plant for the Pierid butterfly, *Nathalis iole* in Alabama, USA (Keener *et al.* 2017). The most widely spread, weedy species of *Mollugo verticillata*, *M. nudicaulis* and *M. cerviana* are self- and insect pollinated (Pax and Hoffmann 1934; Bogle 1970). In Taiwan, *M. pentaphylla* is a minor pollen source for *Apis mellifera* (Lin *et al.* 1993). In South India, honey bees use *Mollugo* species as pollen source and reciprocate the plants with pollination (Ponnuchamy *et al.* 2014). In South Africa, Andrenidae bees, *Meliturgula flavida* and *Meliturgula haematospila* use *Mollugo* species as pollen and nectar sources; the former

exhibits oligolecty by concentrating on *Mollugo* for forage collection while the latter exhibits polylecty by foraging on *Mollugo* and also on other forage plants growing simultaneously in the habitat.

*Glinus lotoides* and *G. oppositifolius* are  $C_3$  species (Rama Das and Raghavendra 1973; Christin *et al.* 2010). The genus *Mollugo* contains  $C_3$ ,  $C_4$  species and species with  $C_3$  and  $C_4$  intermediate characteristics (Edwards and Walker 1983). In *Mollugo*, *M. cerviana* is a  $C_4$  species, *M. nudicaulis*  $C_3$ - $C_4$  species and *M. pentaphylla*  $C_3$  species; the first species is distributed in hot arid regions from pantropics to temperate regions while the other two species are distributed from pantropical and subtropical regions (Christin *et al.* 2010). *M. nudicaulis* is a successful cosmopolitan weed of disturbed areas in warm climates (Vincent 2003). It produces some leaves with  $C_3$  characteristics and some other leaves with  $C_4$  characteristics according to their position on the stem. The leaves progress from  $C_3$  to  $C_4$  as they age. This photosynthetic variation in a single plant, correlated to the age of the leaf, indicates that  $C_4$  phenotype is controlled by more than just Mendelian genetics (Raghavendra *et al.* 1978). The  $C_3$ - $C_4$  photosynthesis is believed to be a relatively rare condition in plants, with only a few dozen identified species, many of which belong to *Flaveria* (Sage *et al.* 1999). Of all  $C_3$ - $C_4$  intermediates, *M. nudicaulis* and *M. verticillata* are the most widespread and abundant. Both are found in hot, ruderal habitats where competition is low and the potential for photorespiration is high. Their ability to survive on such sites is likely due to their  $C_3$ - $C_4$  pathway, which improves carbon gain in the reduced atmospheric  $CO_2$  levels. The ecological success of these  $C_3$ - $C_4$  *Mollugo* species demonstrates that  $C_3$ - $C_4$  intermediacy is a successful photosynthetic pathway in its own right and not merely a transitional phase to  $C_4$  photosynthesis (Vogan *et al.* 2007). *M. cerviana* is the only known  $C_4$  species in the Molluginaceae (Brockington *et al.* 2009).  $C_4$  plants possess a characteristic Kranz type of leaf anatomy which involves the occurrence of a chlorenchymatous bundle sheath in the leaves. These plants also consistently exhibit low carbon dioxide compensation point, while the  $C_3$  plants usually compensate at higher levels of carbon dioxide (Rama Das and Raghavendra 1973). The general pattern of abundance of  $C_4$  species in warm environments indicates that  $C_4$  pathway is physiologically advantageous for them to survive and broaden their niche (Lundgren *et al.* 2015).

Lundgren and Christin (2017) reported that  $C_3$ - $C_4$  taxa are remarkably widespread across geographical and environmental space, maintaining the ability to exist in both typical  $C_3$  and  $C_4$  niches. Their physiology does not strongly restrict the migration of species geographically or into new environments. These authors stated that  $C_3$ - $C_4$  lineages converged toward warm habitats, which may have facilitated the transition to  $C_4$  photosynthesis, effectively bridging the ecological gap between  $C_3$  and  $C_4$  plants. The intermediates retained some precipitation aspects of the habitats of their  $C_3$  ancestors, and likely transmitted them to their  $C_4$  descendants, contributing to the diversity among  $C_4$  lineages.

## MATERIALS AND METHODS

Five plant species, namely, *Glinus lotoides*, *G. oppositifolius*, *Mollugo cerviana*, *M. nudicaulis* and *M. pentaphylla* were selected for study during March 2014-May 2017. *Glinus* species were studied at Paravada area while *Mollugo* species were studied in the Andhra University Campus and also at Paravada area. These species are very small prostrate annuals and grow throughout the year if the soil has little moisture. They are called “carpet weeds” as they form huge populations carpeting the soil due to their very low prostrate habit. Of these plant species, *M. cerviana* with its wiry stems and linear-lanceolate leaves is not prominent and usually goes unnoticed or overlooked while all other plant species are prominent and can be easily noticed despite their low prostrate habit.

Field visits were made regularly to record the flowering season in the selected plant species. The inflorescence type, the number of flowers produced per inflorescence and per plant were noted. Twenty five fresh flowers were used for each plant species to record the floral details such as flower shape, colour, odour, sex, symmetry, floral mechanism, perianth, stamens, staminodes and style and stigma, etc. The floral configuration and floral rewards presentation aspects were examined in relation to the forage collection activity of insects and the attendant pollination effect.

Anthesis was initially recorded by observing marked mature buds in the field. Later, the observations were repeated 3 to 4 times on different days in order to record accurate anthesis schedule for each plant species. The same buds were followed for recording the time of anther dehiscence. The pollen presentation pattern was also investigated by recording how anthers dehisced, whether all anthers in a flower dehisce simultaneously or not and the same was confirmed by observing the anthers under a 10x hand lens.

In all five plant species, the flowers close back. The time of flower closure was recorded for each plant species. Field observations were also made to record whether the stamens and stigmas stay inside or not after the flower closure.

Nectar secretion was observed from mature bud stage to the time of flower closure. In all five plant species, the nectar traces appeared in mature bud stage itself and there is no further secretion during flower life. Since nectar is not produced in measurable quantity, it was not analyzed for its sugar concentration and chemical constituents such as sugar types, amino acids and proteins. As the nectar was secreted around the ovary base and enclosed by connate staminal filaments, it was considered to be producing sucrose-rich nectar because the nectar concealed in the flowers is usually sucrose-rich.

Pollen output was determined by taking 25 un-dehisced anthers from ten individuals for each plant species. The anthers collected from the sample of flowers were placed in a Petri dish. Later, each time a single anther was taken out and placed on a clean microscope slide (75 x 25 mm) and dabbed with a needle in a drop of lactophenol-aniline blue. The anther tissue was then observed under the microscope for pollen. The pollen mass was

drawn into a band, and the total number of pollen grains was counted under a compound microscope (40x objective, 10x eye piece). This procedure was followed for counting the number of pollen grains in each anther collected. Based on these counts, the mean number of pollen produced per anther was determined. The mean pollen output per anther was multiplied by the number of anthers in the flower for obtaining the mean number of pollen grains per flower. Another set of dehiscent anthers was collected in a Petri dish for each plant species and the pollen removed from these anthers was examined under microscope for recording the pollen grain features. The pollen-ovule ratio was determined by dividing the average number of pollen grains per flower by the average number of ovules per flower. The value thus obtained was taken as pollen-ovule ratio (Cruden 1977). The pollen-ovule ratios were calculated separately for 8 to 12-stamened flowers in *Glinus lotoides*, 4 to 6-stamened flowers in *G. oppositifolius*, 3 to 5-stamened flowers in *M. nudicaulis* and *M. pentaphylla*. The pollen-ovule ratio was constant in *M. cerviana* as it produces a fixed number of 5 stamens in all flowers. *In vitro* pollen germination was examined for the pollen of *G. oppositifolius* and *M. pentaphylla*. The pollen was collected from the anthers soon after anther dehiscence and transferred to Petri dish for storage. The pollen thus collected was placed in the cavity of slides, added modified Brewbaker and Kwack's medium and observed after one hour under microscope for germination. This was repeated at each hour from 1100 h to 1900 h to record the percentage of pollen germination in order to record the duration of pollen viability.

Ten flowers each from five individuals for each plant species were used to test stigma receptivity. It was tested with hydrogen peroxide from mature bud stage to flower closure and beyond as per Dafni *et al.* (2005). Hydrogen peroxide when applied to stigma does not stain but produces bubbles as a result of catalase (peroxidase) presence. This test is widely followed although it does not indicate the exact location of the receptive area. The period of release of bubbles from the surface of stigma following application of hydrogen peroxide was taken as the length of stigma receptivity period during flower life and also during closed state of flowers. Further, the receptivity was also observed visually whether the stigmas are shiny, wet or withering.

Based on the timings of maturation of anthers and receptivity of stigmas, the sexual system was defined and also elaborately explained its functionality to achieve spontaneous autogamy, geitonogamy and xenogamy. The positions of stamens and stigmas during and after anthesis were observed to evaluate as to how they facilitate spontaneous autogamy during anthesis and flower closure. Further, observations were also made to evaluate as to how these positions preclude self-pollination when flowers stay open.

After making preliminary observations on the foraging activities of insects on the plant species selected for study, a thorough knowledge of the local insect species was obtained by observing the representative species available with the Department of Environmental Sciences, Andhra University, Visakhapatnam. All butterflies were identified

to species level by consulting the books of Kunte (2007) and Gunathilagaraj *et al.* (1998) while other insects, some to species level while a few others to genus level only. The efforts to get the specimens identified to species level for the species which were identified up to genus level by Zoological Survey of India, Government of India were not successful during the study period. The insect species were observed with the naked eye and by using binoculars; the insect species that could not be identified on spot were captured and later identified with the help of the identified specimens available in the Department. The foraging activities of insects were recorded for 10 min at each hour during the open state of flowers on 4 occasions and the data was tabulated to use the same for further analysis, especially to understand the foraging activity rate. For each species, approximately fifty inflorescences were selected to record the foraging visits of insects. The data thus obtained was used to calculate the percentage of foraging visits made by each category of insects per day to evaluate their association and pollination role in the studied plant species. The insects feeding on nectar and/or pollen were carefully observed to assess their role in effecting pollination. They were observed on a number of occasions on each plant species for their foraging behaviour such as mode of approach, landing, probing behaviour, contact with essential organs to result in pollination, inter-plant foraging activity in terms of cross-pollination. Based on this data, the association between floral rewards of the studied plant species and insects was assessed.

All five plant species were used by thrips for breeding and feeding. They were collected from the flowers and identified using the key provided by Bhatti (1980) for Indian thrips. Field observations were made as to their mobility and foraging activity on flowers to assess their role in pollination. Further, their body washings were made to count the number of pollen grains in order to confirm whether they have any role in pollination or not.

Ten individuals of each insect species were captured while collecting pollen and/or nectar from the flowers of all the studied plant species; the collection was done during their peak foraging activity period. The captured specimens of insects were brought to the laboratory. They were washed first in ethyl alcohol and the contents stained with aniline-blue on a glass slide and observed under microscope to count the number of pollen grains present and evaluate their relative pollen carryover efficiency and pollination role.

Ten inflorescences each on ten individuals of each plant species were tagged prior to anthesis and followed for fruit and seed set for two weeks. The resulting fruit and seed output were pooled up for calculating fruit and seed set rates. Fruit and seed set rates were recorded separately for 8 to 12-stamened flowers in *Glinus lotoides*, 4 to 6-stamened flowers in *G. oppositifolius*, and 3 to 5-stamened flowers in *M. nudicaulis* and *M. pentaphylla*. Fruit and seed set rates were constant in *M. cerviana* as it produces a fixed number of stamens and ovules per flower.

Fruit and seed dispersal was carefully observed to draw practical inferences regarding their success as weeds. The role of wind and rain water in fruit and seed dispersal was

examined in the studied plant species. The fruit and seed morphological characteristics were observed in detail as to their adaptations for dispersal by different means in order to invade, colonize and establish populations in different areas. Seed is the only mode of propagation in all these plant species. Observations on seed germination were made in the field to know whether seeds germinate immediately after their dispersal or not, and if so, whether they form new plants or populations continuously or not.

## RESULTS

### *GLINUS LOTOIDES* L.

**Phenology:** It is a low-growing prostrate, spreading, annual herb that grows in open sandy soils, cultivated fields and open waste lands (Plate 2a,b). In soils with enough moisture, it produces well developed tap root and survives throughout the year producing flowers and fruits simultaneously or alternately. The stem is soft, succulent, pubescent and much-branched carpeting the soil with its foliage. Leaves are simple, basal ones borne in a rosette form while the upper ones in verticillate form or rarely arranged opposite, and densely stellate tomentose. The flowering is profuse when soil is very damp which occurs during July-October due to monsoonal rains. Flowers are borne on 1.5 mm long stalks in axillary cymes and each cyme consists of  $4.72 \pm 1.3$  flowers and each plant produces  $82.5 \pm 33.65$  flowers (Plate 2c,d).



Plate 1. Study area. a. Author (extreme left) surveying for the study species. b. Author examining study material under microscope.

**Flower morphology:** The flowers are small ( $6.1 \pm 1.28$  mm long,  $7.42 \pm 1.25$  mm wide), odourless, actinomorphic and bisexual. The calyx and corolla are represented by perianth with 5 or rarely 6 tepals. The tepals are succulent, free ( $6.02 \pm 0.19$  mm long,  $4.05 \pm 0.2$  mm wide), arranged in quincuncial aestivation, whitish green adaxially and green abaxially, ovate-oblong and covered with stellate hairs. The stamens are 8 to 12, free, white and arranged in two whorls. The stamens of inner whorl close to ovary base are long and form a short tube at the base around the ovary while those of the outer whorl are short. The stamens of both



Plate 2. *Grewia latifolia*: a. Mixed populations of *G. latifolia* (plants with silver colour leaves) and *Grewia oppositifolia* (plants with bright green leaves), b. Habitat with extensive population of *G. latifolia*, c. & d. Close-up view of flowering inflorescences.

whorls are usually anti-tepalous and occasionally alterni-tepalous. The flowers with 8-stamens constitute 14.28%, those with 9-stamens 21.42%, those with 10-stamens 42.85%, 11-stamens 19.04% and 12-stamens 2.38%. Anthers are H-shaped, white, dithecal and versatile. Staminodes are 5-7, petaloid, white, bifid and usually alterni-tepalous but occasionally in between short stamens; they extend beyond the height of long stamens (Plate 3c-e). The ovary ( $5 \pm 0.23$  mm long and  $2.03 \pm 0.17$  mm wide) is green, pentacarpellary and pentalocular syncarpous with variable number of ovules arranged in two rows in each locule on axile placentation. The ovule number varied from  $181.9 \pm 35.28$  to  $242.4 \pm 35.14$  in 8- and 12-stamened flowers (Plate 3g). The ovule production trend showed that the number of ovules produced gradually increased with a gradual increase in the number of stamens per anther and pollen out per flower but the variation is not significant. The style is absent and stigmas are five, greenish white, free, spreading, papillate, wet and shiny (Plate 3e,d,f).

**Floral biology:** Mature buds open during 1400-1500 h. Individual buds take 30 to 40 minutes from partial to full opening (Plate 3a,b, 4a-c). The anthers dehisce by longitudinal slits during anthesis (Table 1). The pollen output per anther varied from  $1,193.85 \pm 70.25$  to  $1,371.85 \pm 65.76$  and from  $10,974.85 \pm 526.08$  to  $14,326.28 \pm 843.03$  per flower in 8- to 12-stamened flowers (Table 2). The pollen production trend showed that the pollen output rate



gradually increased with a gradual decrease in the number of stamens produced per flower but the variation is not significant. The pollen-ovule ratio varied from 58: 1 to 61:1 in 8- to 12-stamened flowers. The pollen grains are white, spheroidal, tricolporate, tri-zonoaperturate, colpal membrane densely granulated,  $32.52 \pm 4.28 \mu\text{m}$  in size and tectum with scabrate ornamentation. The stigma attains receptivity after anthesis and continuous up to 2<sup>nd</sup> day noon time (Table 1). The nectar is secreted in traces during mature bud stage. The tepals together with the staminodes, stamens and stigma close back by 1700-1800 h on the same day and remain in place in fertilized flowers until fruit dispersal (Table 1). The un-fertilized flowers fall off after 7-10 days.

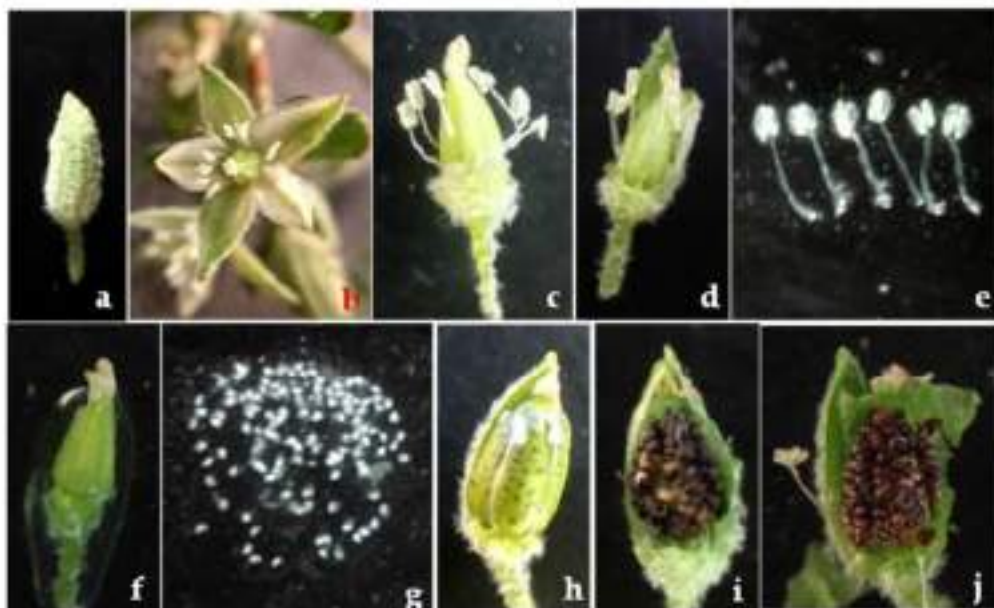


Plate 3. *Glinus lotoides*: a. Bud, b. Flower, c. & d. Relative positions of stamens and stigma, e. Stamens, f. Pistil, g. Ovules, h. Fruit capsule with stamens and stigma intact in withered state, i. & j. Fruit capsule with mature seeds.

Table 1. Anthesis schedules in the studied plant species

Plant species	Anthesis time (h)	Anther dehiscence	Period of stigma receptivity	Flower closing time (h)
<i>Glinus lotoides</i>	1400-1500	During anthesis	Starts after anthesis and continuous up to 2 <sup>nd</sup> day noon	1700-1800
<i>Glinus oppositifolius</i>	1200-1400	During anthesis	Starts after anthesis and continuous up to 2 <sup>nd</sup> day noon	1600-1800

<i>Mollugo cerviana</i>	0700-0800	During anthesis	Starts during anthesis and continuous up to 2 <sup>nd</sup> day noon	1000-1100
<i>Mollugo nudicaulis</i>	0700-0900	During anthesis	Starts during anthesis and continuous up to 2 <sup>nd</sup> day noon	1000-1200
<i>Mollugo pentaphylla</i>	0700-0800	During anthesis	Starts during anthesis and continuous up to 2 <sup>nd</sup> day noon	1000-1100

Table 2. Pollen aspects in *Glinus lotoides*

Flower type	Percentage of occurrence	Mean pollen output/anther	Mean pollen output/flower	Mean no. of ovules/flower	Pollen: ovule ratio
8-stamened	14.20	1371.85 ± 65.76	10974.85 ± 526.08	181.9 ± 35.28	60 : 1
9-stamened	21.42	1331.85 ± 91.46	11986.71 ± 823.21	196.7 ± 35.78	61 : 1
10-stamened	42.85	1304.57 ± 62.65	13045.71 ± 626.57	221.8 ± 40.67	59 : 1
11-stamened	19.04	1221.85 ± 61.36	13440.42 ± 674.99	230.2 ± 34.23	58 : 1
12-stamened	2.38	1193.85 ± 70.25	14326.28 ± 843.03	242.4 ± 35.14	59 : 1

**Pollination mechanism:** The fully open flowers show different positions of the stamens and the stigmas. The stigmas are situated beyond the height of short stamens but below the height of long stamens. Further, both the sex organs are spatially separated and precludes spontaneous autogamy during open state of the flower. However, during the closure of the flower, the closely spaced dehiscent anthers of long stamens contact the stigmas facilitating spontaneous autogamy but its occurrence is dependent on the availability of pollen in the anthers (Plate 4d).

**Thrips breeding, feeding and pollination:** Thrips species, *Haplothrips* sp. (Thysanoptera: Thripidae) ovi-positing during early stage of floral bud (Plate 4e). The larvae emerged from the eggs in synchrony with anthesis and nectar production in flowers. The larvae and adults foraged for pollen and nectar. Individual thrips were dusted with pollen during their movements within the flowers. The pollen morphological characters facilitated the thrips to carry 428 to 635 pollen grains on their body setae, wings and legs. The thrips dispersed the pollen on free spreading papillate stigmas due to their active movement, rubbing the abdomen against the stigmatic surface, cleansing of their body parts with their hind legs and also by their wing combing mechanism. The near homogamous nature of the flowers facilitate self-pollination within the same flower or different flowers of the same plant by thrips. As the plant occurs as small or large populations, thrips could fly to migrate to the flowers of other closely spaced plants and effect cross-pollination by feeding on the forage.



Plate 4. *Glans luteoides*: a. Initiation of anthesis by mature bud, b. Half-open flower, c. Fully open flower, d. Flower closure at 1800 h, e. *Haplodipris* sp. feeding on nectar, f. *Apis cerana* collecting pollen, g. *Apis cerana* collecting nectar, h. *Camponotus* sp. collecting nectar, i. Lycaenid butterfly, *Zizeeria karsandra* collecting nectar.

**Insect visitors and Pollination:** The flowers were foraged by bees for pollen and nectar, ants and butterflies for only nectar during 1400-1700 h with concentrated foraging activity during 1500-1600 h (Figure 1,2). The bees were *Apis cerana* (Plate 4f), *A. florea* (Plate 4g), *Trigona iridipennis* and *Ceratina* sp. The ants included *Camponotus* sp. (Plate 4h) and *Crematogaster* sp. The butterflies included only lycaenids, namely, *Zizula hylax*, *Zizeeria karsandra* (Plate 4i), *Zizina otis*, *Freyeria trochylus*, *Chilades laius* and *Chilades pandava* (Table 3). All these insects approached the flowers in upright position, landed on the tepals and then probed for forage collection. Bees first accessed anthers to collect pollen and then moved to the flower base to collect nectar, if available in the same and/or different visits. Ants were resident foragers and continuously crawled all over the plant and accessed the floral base to collect nectar. Butterflies stretched their proboscis and inserted into the flower base to collect nectar. All insect species collected forage from several flowers of different cymes of the same or different plants to collect the forage. The bees during pollen collection brushed against the stigmas with their ventral surface effecting sternotribic pollination. Further, the bees and also ants during nectar collection brushed against anthers and stigmas with their dorsal surface effecting nototribic pollination. The butterflies during nectar collection contacted the stamens and stigmas with their proboscis and front side of head and ventral surface of thorax and abdomen effecting sternotribic pollination. Their wings never contacted the stamens and stigma during nectar collection as they kept them in vertical position. Bees made 47%, ants 9% and lycaenids 44% of total foraging visits (Figure 3). The body washings of insects collected from the flowers during

peak foraging period revealed that all insects carry pollen but bees carry the highest number of pollen grains. Further, the mean number of pollen grains varied with each insect species (Table 4). The nectar secretion in traces and its depletion by thrips during and after anthesis appeared to be driving the insects to visit as many flowering cymes as possible to quench their thirst for nectar. Such a foraging behavior was considered to be facilitating the promotion of cross-pollination.

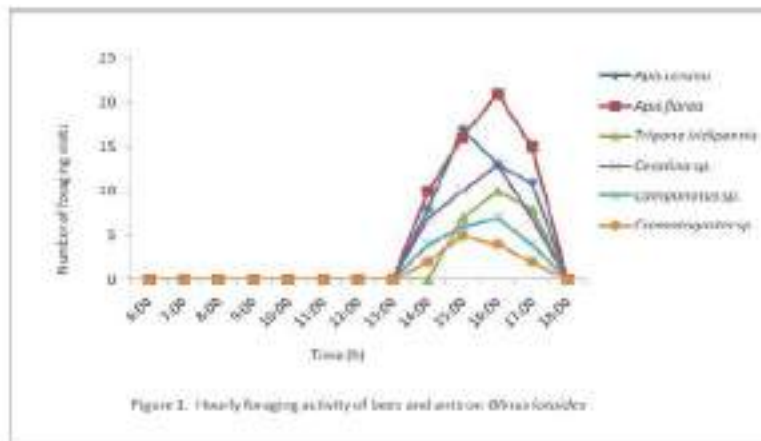


Figure 2. Hourly foraging activity of bees and ants on *Glinus lotoides*.

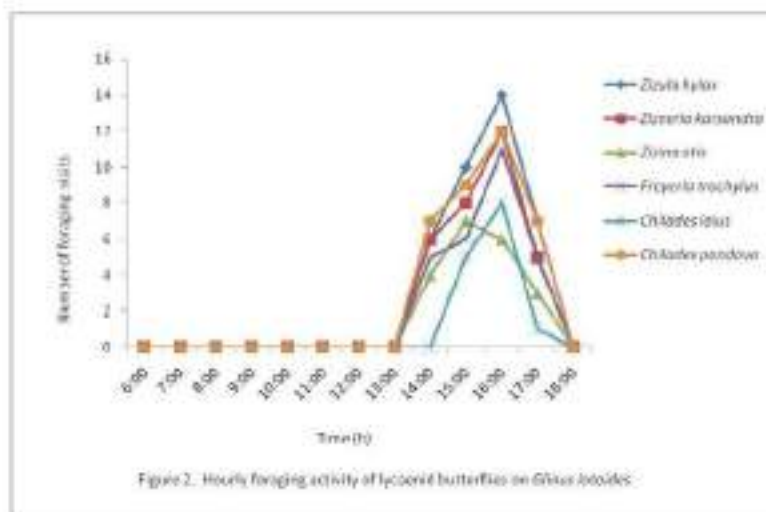


Figure 2. Hourly foraging activity of lycaenid butterflies on *Glinus lotoides*.

Table 3. List of insect foragers on *Glinus lotoides*

Order	Family	Genus	Species	Common name	Forage sought
Hymenoptera	Apidae	<i>Apis</i>	<i>cerana</i> E.	Indian Honey Bee	Pollen + Nectar
		<i>Apis</i>	<i>florea</i> E.	Dwarf Honey Bee	Pollen + Nectar

		<i>Trigona</i>	<i>iridipennis</i> Smith	Stingless Honey Bee	Pollen + Nectar
		<i>Ceratina</i>	sp.	Small Carpenter Bee	Pollen + Nectar
	Formicidae	<i>Camponotus</i>	sp.	Carpenter Ant	Nectar
		<i>Crematogaster</i>	sp.	Cocktail Ant	Nectar
Lepidoptera	Lycaenidae	<i>Zizula</i>	<i>hylax</i> F.	Tiny Grass Blue	Nectar
		<i>Zizeeria</i>	<i>karsandra</i> Moore	Dark Grass Blue	Nectar
		<i>Zizina</i>	<i>otis</i> F.	Lesser Grass Blue	Nectar
		<i>Freyeria</i>	<i>trochylus</i> Freyer	Grass Jewel	Nectar
		<i>Chilades</i>	<i>latus</i> Stoll	Lime Blue	Nectar
		<i>Chilades</i>	<i>pandava</i> Horsfield	Plains Cupid	Nectar

Table 4. Pollen recorded in the body washings of insects on *Glinus lotoides*

Insect species	Sample size(N)	Number of pollen grains		
		Range	Mean	S.D
<i>Apis cerana</i>	10	92-307	208.2	55.45
<i>Apis florea</i>	10	78-252	167.1	56.83
<i>Trigona iridipennis</i>	10	43-214	129.2	46.26
<i>Ceratina</i> sp.	10	35-94	64.1	17.44
<i>Camponotus</i> sp.	10	27-58	39.4	8.59
<i>Crematogaster</i> sp.	10	23-46	34.5	6.43
<i>Zizula hylax</i>	10	8-31	21.4	6.29
<i>Zizeeria karsandra</i>	10	15-40	27.8	7.40
<i>Zizina otis</i>	10	11-29	20.8	5.24
<i>Freyeria trochylus</i>	10	10-38	25.4	7.4
<i>Chilades latus</i>	10	12-34	23.3	6.37
<i>Chilades pandava</i>	10	10-42	28.4	8.27

**Fruiting ecology and seed dispersal:** The pollinated and fertilized flowers grow continually and produce fruits within 8-12 days. The stamens and stigmas are persistent and remain inside due to the closure of the flower (Plate 3h). The tepals bulge gradually and protect the bulging ovary in which the seeds form and mature (Plate 3i,j). Natural fruit set rate varied from 88% to 92% while seed set rate varied from 85% to 93% in 8- to 12-stamened flowers

(Table 5). Fruit is a loculicidal 5-valved capsule, stalked, membranous, densely pubescent,  $6.05 \pm 0.75$  mm long and  $4.1 \pm 0.64$  mm wide. The seeds are small, reniform, smooth, 0.7 mm long and 0.5 mm wide, initially red and finally dark brown and have a white aril of funicular origin developed into elongate filiform strophiole. They are arranged in two rows in each locule. Dry capsules break open when tepals are wet and expose the seeds. But the seeds remain so and gradually separate and fall to the ground on their own due to their smooth and slippery nature on clear sunny days. In dry season, when the capsules are ripe, the plant dies, dries out and becomes brittle. In this state, the base of the stem breaks off and it is more so when high winds prevail. Then, the plant parts roll readily and fruit and seeds disperse to other areas. On rainy days, drops of water falling on the distal opening after the locules are filled with rain water result in an explosive expulsion of water droplets and seeds. Further, water acts as an efficient dispersal agent for the dispersal of seeds fallen on the soil during rainy season. Therefore, seed dispersal is characteristically anemochorous, ombrohydrochory and hydrochory.

Table 5. Natural fruit and seed set rate in *Gliricidia sepium*

Flower type	Number of flowers sampled	Number of flowers set fruit	Fruit set (%)	Seed set (%)
8-stamened	50	45	90	85
9-stamened	60	53	88	87
10-stamened	110	98	89	90
11-stamened	45	41	91	92
12-stamened	25	23	92	93

#### **GLINUS OPPOSITIFOLIUS (L.) AUG. DC.**

**Phenology:** It is a low-growing prostrate, spreading, annual herb that grows in open sandy soils, cultivated fields and open waste lands (Plate 5a). In soils with enough moisture, it produces well developed tap root and survives throughout the year producing flowers and fruits simultaneously or alternately (Plate 5b-d). The stem is soft, succulent, sub-glabrous and much-branched covers the soil with its foliage. Leaves are petiolate, simple, arranged in pseudo-whorls of 3-6 or opposite to each other. Leaf blade is spatulate-oblongate. Its margins are covered with sparse teeth. The flowering is profuse when soil is very damp which occurs during July-October due to monsoonal rains. Flowers are borne on 3-7 mm long pedicels in axillary fascicles and each fascicle consists of  $7.5 \pm 1.5$  flowers and each plant produces  $64.52 \pm 41.28$  flowers.

**Flower morphology:** The flowers are small ( $3.51 \pm 0.5$  mm long,  $8.57 \pm 0.7$  mm wide), odourless, actinomorphic and bisexual. The calyx and corolla are represented by a perianth with 5 or rarely 6 tepals. The tepals are succulent, free ( $4.01 \pm 0.2$  mm long,  $2.03 \pm 0.2$  mm

wide), arranged in quincuncial aestivation, creamy white adaxially and brownish orange abaxially, ovate-oblong and pubescent. The stamens are 4 to 6, free but connate at the base, and alterni-tepalous. The flowers with 4-stamens constitute 5%, those with 5-stamens 80% and those with 6-stamens 15%. Anthers are H-shaped, white, dithecal and versatile. Staminodes are 5 or 6, petaloid, white, bifid and anti-tepalous. The ovary ( $4.07 \pm 0.16$  mm long and 2 mm wide) is green, tri-carpellary and tri-locular syncarpous with variable number of ovules arranged in two rows in each locule on axile placentation. The ovule number varied from  $115.5 \pm 7.1$  to  $137.7 \pm 9.9$  in 4- and 6-stamened flowers. The ovule production trend showed that the number of ovules produced gradually increased with a gradual increase in the number of stamens per anther and pollen out per flower but the variation is not significant. The style is absent and stigmas are three, creamy white, free, spreading, papillate, wet and shiny (Plate 6d).

**Floral biology:** Mature buds open during 1200-1400 h (Plate 6a). Individual buds take 10 to 15 minutes from partial to full opening. The anthers dehisce by longitudinal slits during anthesis (Table 1, Plate 6b). The pollen output per anther varied from  $1,151 \pm 67.22$  to  $957.33 \pm 49.1$  and from  $4,604 \pm 268.9$  to  $5,744 \pm 295.1$  per flower in 4- to 6-stamened flowers (Table 6). The pollen production trend showed that the pollen output rate gradually increased with a gradual decrease in the number of stamens produced per flower but the variation is not significant. The pollen-ovule ratio is constant despite variation in the number of stamens and ovules; it is 40:1. The pollen grains are white, spheroidal, tricolporate, tri-zonoperturate, colpal membrane densely granulated,  $29.34 \pm 4.26$   $\mu\text{m}$  in size and tectum with scabrate ornamentation (Plate 6c). *In vitro* pollen viability test indicated that the pollen grains are viable during the open state of flowers only (Table 7). The pollen is available from anthesis onwards and remain so until the closure of the flower; it is the highest immediately after anthesis and gradually decreases towards the time of closing of the flower. The stigma attains receptivity after anthesis and continuous up to 2<sup>nd</sup> day noon time (Table 1). The nectar is secreted in traces during mature bud stage. The tepals together with the staminodes, stamens and stigma close back by 1600-1800 h on the same day and remain in place until fruit dispersal in fertilized flowers (Table 1). The un-fertilized flowers fall off after 6-8 days.

**Pollination mechanism:** The fully open flowers show different positions of the stamens and the stigmas. The stamens, staminodes and stigmas are situated at the same height. But, both the sex organs are spatially separated and precludes spontaneous autogamy during open state of the flower. However, during the closure of the flower, the anthers contact the stigmas facilitating spontaneous autogamy but its occurrence is dependent on the availability of pollen in the anthers.

**Thrips breeding, feeding and pollination:** Thrips species, *Haplothrips sp.* (Thysanoptera: Thripidae) ovi-positing during early stage of floral bud. The larval emergence from the eggs was in synchrony with anthesis and nectar production in flowers. Both larvae

and adults foraged for pollen and nectar. Individual thrips were dusted with pollen during their movements within the flowers. The pollen morphology facilitated the thrips to carry 157 to 253 pollen grains on their body setae, wings and legs. The thrips dispersed the pollen on free papillate spreading stigmas due to their active movement, rubbing of the abdomen against the stigmatic surface, cleansing of their body parts with their hind legs and also by their wing combing mechanism. The near homogamous nature of the flowers facilitate self-pollination within the same flower or different flowers of the same plant by thrips. As the plant occurs as small or large populations, thrips could fly to migrate to the flowers of other closely spaced plants and effect cross-pollination by feeding on the forage.



Plate 5. *Glintseppinèssin* a. Habit, b. & c. Bud stage, d. Flowering phase.

**Insect visitors and Pollination:** The flowers were foraged by bees for pollen and nectar, ants and butterflies for only nectar during 1300-1700 h with concentrated foraging activity during 1500-1600 h (Figure 4,5). The bees were *Apis cerana*, *A. florea* (Plate 6g,h), *Ceratina* sp. (Plate 6i,j), *Ceratina smaragdula* (Plate 6k), *Halictus* sp. and *Megachile* sp. (Plate 6l). The ants included *Camponotus* sp. and *Crematogaster* sp. The butterflies included only lycaenids, namely, *Zizula hylax* (Plate 6m), *Zizeeria karsandra* (Plate 6n,o), *Zizina otis* (Plate 6p), *Freyeria trochylus*, *Chilades laius* and *Chilades pandava* (Table 8). All these insects approached the flowers in upright position, landed on the tepals and then probed for forage collection. Bees first accessed anthers to collect pollen and then moved to the flower base to collect nectar, if available in the same and/or different visits. Ants were resident foragers and continuously crawled all over the plant and accessed the floral base to collect nectar. Butterflies stretched out their proboscis and inserted into the flower



base to collect nectar. All insect species collected forage from several flowers of different fascicles of the same or different plants to collect the forage. The bees during pollen collection brushed against the stigmas with their ventral surface effecting sternotribic pollination. Further, the bees and also ants during nectar collection brushed against anthers and stigmas with their dorsal surface effecting nototribic pollination. The butterflies during nectar collection contacted the stamens and stigmas with their proboscis and front side of head and ventral surface of thorax and abdomen effecting sternotribic pollination. Their wings never contacted the stamens and stigma during nectar collection as they kept them in vertical position. Bees made 60%, ants 5% and lycaenids 35% of total foraging visits (Figure 6). The body washings of insects collected from the flowers during peak foraging period revealed that all insects carry pollen but bees carry the highest number of pollen grains. Further, the mean number of pollen grains varied with each insect species (Table 9). The nectar secretion in traces and its depletion by thrips during and after anthesis appeared to be driving the insects to visit as many flowering cymes as possible to quench their thirst for nectar. Such a foraging behavior was considered to be facilitating the promotion of cross-pollination.

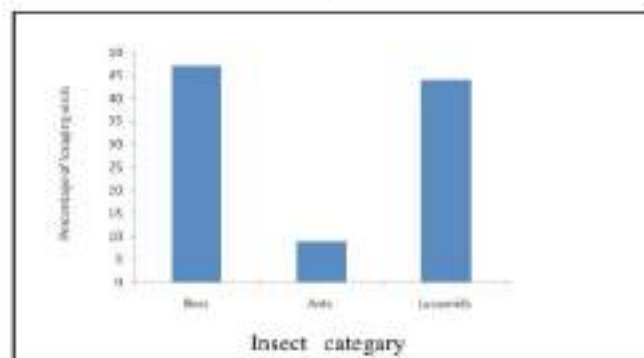


Figure 3. Percentage of foraging visits of bees, ants and lycaenid butterflies of *Glinus lotoides*.

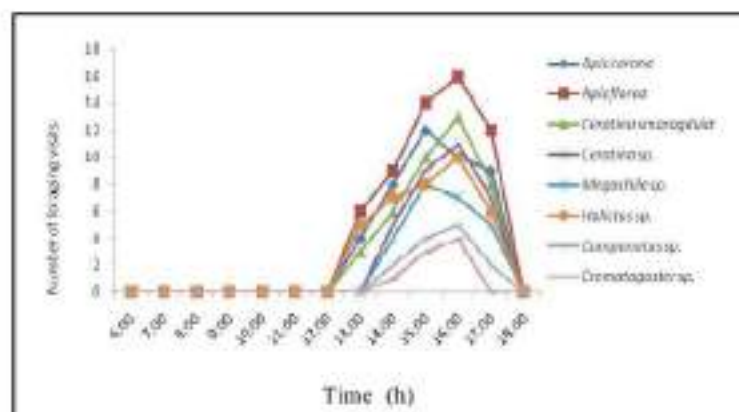


Figure 4. Hourly foraging activity of bees and ants on *Glinus oppositifolius*.

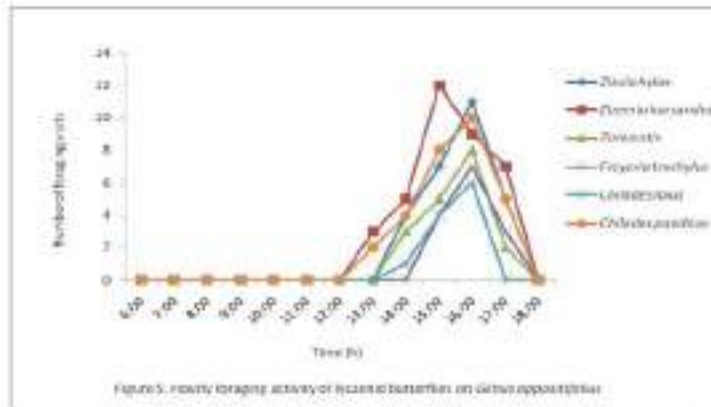


Plate 6. *Glinus oppositifolius*: a. Flower, b. Dehiscing stamens, c. Pollen grain, d. Ovary with three stigmas, e. & f. Seeds, g-l. Pollen and nectar foragers - g. *Apis florea* collecting pollen, h. *Apis florea* collecting nectar, i. *Corithia* sp. collecting pollen, j. *Corithia* sp. collecting nectar, k. *Corithia aureogibba* collecting nectar, l. *Megachile* sp. collecting nectar, m-p. Nectar foragers (Lycaenid butterflies) - m. *Zizula hylei*, n. *Zizania leucandra*, o. *Zizania leucandra* in mating state, p. *Zizania* etc.

Table 6. Pollen aspects in *Glinus oppositifolius*

Flower type	Percentage of occurrence	Mean pollen output/anther	Mean pollen output/flower	Mean no. of ovules/flower	Pollen: ovule ratio
4-stamened	5	1151 ± 67.2	4604 ± 268.9	115.5 ± 7.1	40 : 1
5-stamened	80	1027 ± 55.5	5133 ± 277.5	128.6 ± 7.7	40 : 1
6-stamened	15	957 ± 49.1	5744 ± 295.1	137.7 ± 9.9	40 : 1

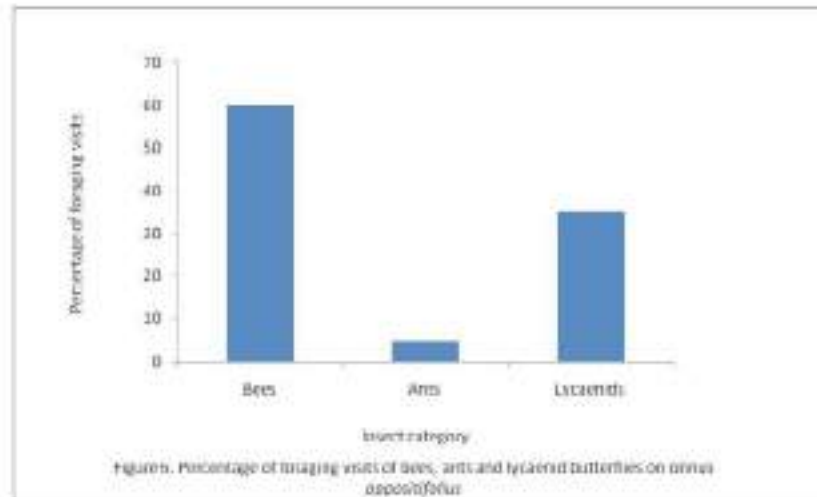


Table 7. *In vitro* pollen germination in *Glinus oppositifolius*

Time (h)	Pollen sample	No. of germinated pollen grains	Germination (%)
1100	—	—	—
1200	224	210	94
1300	367	307	84
1400	22	16	73
1500	233	118	51
1600	90	42	47
1700	45	8	18
1800	34	3	9
1900	—	—	—

Modified Brewbaker and Kwack's medium

Table 8. List of insect foragers on *Glinus oppositifolius*

Order	Family	Genus	Species	Common Name	Forage Sought
Hymenoptera	Apidae	<i>Apis</i>	<i>cerana</i> F.	Indian Honey Bee	Pollen + Nectar
		<i>Apis</i>	<i>florea</i> F.	Dwarf Honey Bee	Pollen + Nectar
		<i>Ceratina</i>	<i>smaragdula</i> F.	Small Carpenter Bee	Pollen + Nectar
		<i>Ceratina</i>	sp.	Small Carpenter Bee	Pollen + Nectar

	Haliictidae	<i>Halicus</i>	sp.	Sweat Bee	Pollen + Nectar
	Megachilidae	<i>Megachile</i>	sp.	Leafcutter Bee	Pollen + Nectar
	Formicidae	<i>Camponotus</i>	sp.	Carpenter Ant	Nectar
		<i>Crematogaster</i>	sp.	Cocktail Ant	Nectar
Lepidoptera	Lycaenidae	<i>Zizula</i>	<i>hylax</i> F.	Tiny Grass Blue	Nectar
		<i>Zizeeria</i>	<i>karsandra</i> Moore	Dark Grass Blue	Nectar
		<i>Zizina</i>	<i>otis</i> F.	Lesser Grass Blue	Nectar
		<i>Freyeria</i>	<i>trochylus</i> Freyer	Grass jewel	Nectar
		<i>Chilades</i>	<i>latus</i> Stoll	Lime Blue	Nectar
		<i>Chilades</i>	<i>pandava</i> Horsfield	Plains Cupid	Nectar

Table 9. Pollen recorded in the body washings of insects on *Glinus oppositifolius*

Insect species	Sample size(N)	Number of pollen grains		
		Range	Mean	S.D
<i>Apis cerana</i>	10	87-236	161.5	46.6
<i>Apis florea</i>	10	66-214	140.5	42.9
<i>Ceratina smaragdula</i>	10	68-164	115.9	24.8
<i>Ceratina</i> sp.	10	41-117	84.2	21.6
<i>Megachile</i> sp.	10	43-102	74.8	16.1
<i>Halicus</i> sp.	10	27-63	44.1	9.1
<i>Camponotus</i> sp.	10	22-45	31.3	7.4
<i>Crematogaster</i> sp.	10	13-34	25.1	6.4
<i>Zizula hylax</i>	10	11-38	26.8	7.2
<i>Zizeeria karsandra</i>	10	9-46	28.8	9.2
<i>Zizina otis</i>	10	14-30	23.1	5.1
<i>Freyeria trochylus</i>	10	7-29	19.3	6.1
<i>Chilades latus</i>	10	10-35	24.8	7.0
<i>Chilades pandava</i>	10	12-41	28.1	7.2

**Fruiting ecology and seed dispersal:** The pollinated and fertilized flowers grow continually and produce fruits within 7-10 days. The stamens and stigmas are persistent and remain inside due to the closure of the flower. The tepals bulge gradually and protect the bulging ovary in which the seeds form and mature. Natural fruit set rate varied from 88% to 92% while seed set rate varied from 88% to 91% in 4- to 6-stamened flowers (Table 10).

Fruit is an ellipsoid loculicidal 3-valved capsule, short-stalked, membranous, densely pubescent,  $5.5 \pm 0.5$  mm long and  $2.1 \pm 0.2$  mm wide. The seeds are small, sub-reniform, granulose, 0.9 mm long and 0.7 mm wide, reddish-brown and have a white aril of funicular origin developed into extensively curved scrotiform strophiole (Plate 6e,f). They are arranged in two rows in each locule. Dry capsules break open when tepals are wet and expose the seeds. But the seeds remain so and gradually separate and fall to the ground on their own on clear sunny days. In dry season, when the capsules are ripe, the plant dies, dries out and becomes brittle. In this state, the base of the stem breaks off and it is more so when high winds prevail. Then, the plant parts roll readily and fruit and seeds disperse to other areas. On rainy days, drops of water falling on the distal opening after the locules are filled with rain water result in an explosive expulsion of water droplets and strophiolate seeds. Further, water acts as an efficient dispersal agent for the dispersal of seeds fallen on the soil during rainy season. Therefore, seed dispersal is characteristically anemochorous, ombrohydrochory and hydrochory.

Table 10. Natural fruit and seed set rate in *Glinus oppositifolius*.

Flower type	Number of flowers sampled	Number of flowers set fruit	Fruit set (%)	Seed set (%)
4-stamened	61	54	88	88
5-stamened	225	206	91	90
6-stamened	105	97	92	91

#### **MOLLUGO CERVIANA (L.) SER.**

**Phenology:** It is small, glabrous, slender annual herb. It is common in open dry sandy and semi-dry soils along roadsides, waste places, bare ground and dry river beds (Plate 7a). Its presence is easily overlooked due to its very low ground habit, wiry reddish orange stems and thin linear leaves. The stems are numerous, upright, thin and stiff. Leaves are sessile, grey green and linear with acute apex; they arise in whorls on the stem but some are in a rosette at the base. The plant appears simultaneously in vegetative, flowering and fruiting phases in different populations growing in different habitats throughout the year (Plate 7b). An individual plant, however, has a short life cycle of 3 months from seed germination to seed dispersal. Although it appears throughout the year, it shows robust vegetative growth and profuse flowering and fruiting during July-October when soil is damp due to occurrence of rains. The flowers are borne on 7-8 mm long pedicels in dichotomous and trichotomous umbellate cymes produced terminally or in leaf axils.

**Flower morphology:** The flowers are small ( $2.52 \pm 0.4$  mm long,  $1.51 \pm 0.5$  mm wide), whitish green on adaxial side and green on abaxial side, odourless, actinomorphic and bisexual. The sepals and petals are represented by a monochlamydeous perianth of 5 herbaceous scarious, elliptic to oblong,  $2.45 \pm 0.4$  mm long,  $1.13 \pm 0.2$  mm wide long free tepals with

white margins. The stamens are 5, anti-tepalous, free but connate at base, white,  $1.22 \pm 0.3$  mm long with dorsifixed, golden yellow, less than 1 mm long and dithecous anthers. The ovary is light green, tri-carpellary, tri-locular syncarpous with  $58.2 \pm 8.16$  D-shaped ovules arranged on axile placentation (Plate 8g,h). The style is absent but the ovary is terminated with 3 free stigmas (Plate 8f). The stigmas are minutely denticulate with membranous flaps.



Plate 7. *Mollepis coronata*: a. Habitat with *Mollepis coronata* and *M. sudlowii*. b. *Mollepis coronata* in flowering phase.

**Floral biology:** Mature buds open during 0700-0800 h. Individual buds take 5 to 10 minutes from partial to full opening (Plate 8a,b). The flowers are homogamous as the anthers and stigmas attain maturity at the same time during anthesis; the former dehisce by longitudinal slits (Plate 8d) and the latter continue receptivity until the noon of the 2<sup>nd</sup> day (Table 1). The pollen output is  $159.7 \pm 14.5$  per anther and  $798.5 \pm 69.5$  per flower. The pollen-ovule ratio is 14:1. The pollen grains are pale yellow, spheroidal, tri-colpate, tri-zonoperturate, granulated, tectum scabrate,  $21.9 \pm 4.12$   $\mu\text{m}$  (Plate 8c). The nectar is secreted in traces during mature bud stage. The tepals together with the stamens and stigmas close back by 1000-1100 h (Table 1).

**Pollination mechanism and Pollinators:** In dehisced anthers collected during anthesis, 20-35% of pollen grains were found with pollen tubes indicating *in situ* germination. Further, the pollen tubes were also found on the stigma. The pollen germination and formation of tubes both within the dehisced anthers and on the stigma indicate that the presence of self-induced autogamy. During and after anthesis, the dehisced anthers and receptive stigmas contact each other due to their close proximity and their position at the same height (Plate 8c). With this situation, the anthers brush against the stigmas causing autogamy. Further, the stamens and stigmas contact each other very closely during the closing of the flower assuring autogamy if it did not occur during open state of the flower.

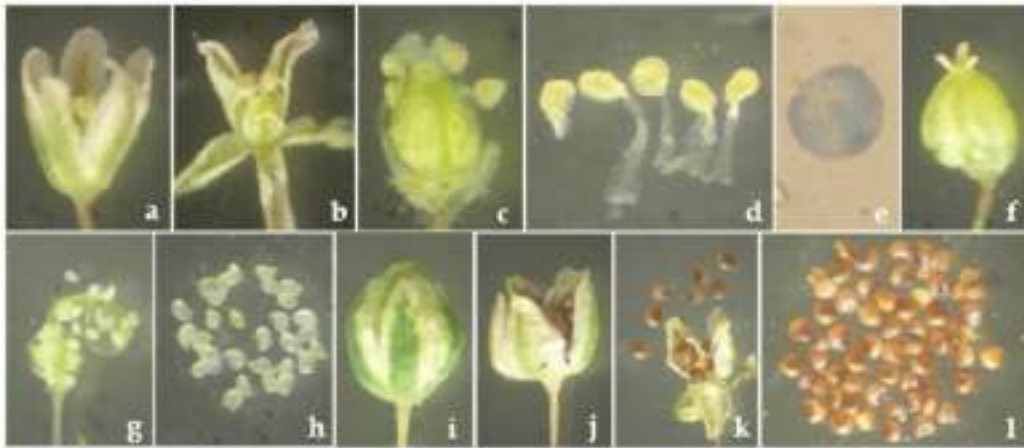


Plate 8. *Mollego cerviana*: a. & b. Flowering-opening phase, c. Position of stigmatic lobes and anthers at the same height contacting each other at anthesis, d. Dehiscent anthers, e. Pollen grain, f. Ovary with three stigmas, g. & h. Multi-ovuled ovary, i. Maturing fruit, j. & k. Dehiscent fruit capsule, l. Seeds.

The floral characters such as radial symmetry, whitish green adaxial surface and minute amount of nectar are considered as adaptations for insect-pollination. But, insects never visited the flowers during the study period. In the habitats of *M. cerviana*, *M. nudicaulis* and *M. pentaphylla* also grow as pockets or as large carpet populations; here the insects visited the last two species for forage. In open habitats where *M. cerviana* alone occurred, insects were not found and hence this plant is obligately autogamous. However, thrips feeding activity contributed to self- and cross-pollination.

**Thrips breeding, feeding and pollination:** The thrips species, *Haplothrips* sp. (Thysanoptera: Thripidae) used flower buds for breeding and flowers for feeding. The larvae emerged from the eggs in synchrony with anthesis and nectar production in flowers. The larvae and adults foraged for pollen and nectar. Individual thrips were dusted with pollen during their movements within the flowers. They carried 87 to 176 pollen grains on their body setae, wings and legs. The thrips dispersed the pollen on free denticulate and membranous stigmas due to their active movement, rubbing of abdomen against the stigmatic surface, cleansing of their body parts with their hind legs and also by their wing combing mechanism. The homogamous flowers were found to facilitate self-pollination in the same or different flowers of the same plant. As the plant occurs as small or large populations, thrips could fly to migrate to the flowers of other closely spaced plants and effect cross-pollination by feeding on the forage.

**Fruiting ecology and seed dispersal:** The pollinated and fertilized flowers grow continually and produce fruits within 8-10 days. The stamens and stigmas are persistent and remain inside due to the closure of the flower. The tepals bulge gradually and protect the

bulging ovary in which the seeds form and mature (Plate 8i). Natural fruit set is 91.27% and seed set is 61.94%. Fruit is a loculicidal 3-valved broadly-ellipsoid capsule, stalked, membranous, densely pubescent,  $2.35 \pm 0.36$  mm and  $1.85 \pm 0.23$  mm wide. The seeds are arranged in two rows in each locule. They are tiny, brown, shiny, D-shaped and faintly striate dorsally (Plate 8i). The seed coat is studded with minute granular excrescences with reticulate ornamentation. Dry capsules break open when fruit pericarp and tepals are dry and expose the seeds (Plate 8j,k). But the seeds remain so and gradually separate and fall to the ground on their own on clear sunny days. On rainy days, the water droplets falling on the dehiscent capsules washout seeds to the ground. Further, water acts as an efficient dispersal agent for the dispersal of seeds fallen on the soil during rainy season. Seeds do not have adaptations for wind dispersal. But, wind disperses the dry cymes together with dry dehiscent capsules to short distances and subsequently the seeds fall to the ground from capsules. Therefore, seed dispersal modes include ombrohydrochory, hydrochory and anemochory. The seeds produced from plants growing in cultivated lands have the potential to be dispersed as a cereal grain contaminant and in effect agricultural produce movement contributes to seed dispersal and expansion of its distribution.



Plate 9. *Mollugo nudicaulis*: a. Habat - flowering phase, b. Individual plant in flowering, c. & d. New plants.

***MOLLUGO NUDICAULIS* LAM.**

**Phenology:** It is a small acaulescent annual herb with a rosette of prostrate leaves. It is



common in open dry sandy and moist soils along roadsides, waste places, bare ground and cultivated lands (Plate 9a,b). Leaves are sessile, succulent, glabrous, obovate to spatulate, margin entire and apex rounded. The plant appears simultaneously in vegetative, flowering and fruiting phases in different populations growing in different habitats throughout the year. An individual plant, however, has a short life cycle of 3 months from seed germination to seed dispersal. Although it appears throughout the year, it shows robust vegetative growth and profuse flowering and fruiting during July-October when soil is damp due to occurrence of rains. The inflorescence is a polychasial cyme which arises from the rosette of basal leaves. The dichasial or trichasial cymes are common during dry season while polychasial cymes are common during wet season. It is spreading, pedunculate (7-8 mm long) and produces pedicellate (4 mm long) flowers. The peduncle and pedicel are wiry and stiff. A polychasial cyme produces  $7.5 \pm 1.5$  flowers.

**Flower morphology:** The flowers are small ( $3.51 \pm 0.4$  mm long,  $4.03 \pm 0.3$  mm wide), creamy white on adaxial side and light green on abaxial side, odourless, actinomorphic and bisexual. The sepals and petals are represented by a monochlamydeous perianth of 5 tepals. The tepals are free but connate at base, elliptic to oblong,  $3.28 \pm 0.41$  mm long,  $1.82 \pm 0.33$  mm wide and hooded. The stamens are 3 to 6, free, but connate at base, creamy white,  $2.27 \pm 0.17$  mm long with dorsifixed, light yellow, dithecal anthers. The flowers with 3-stamens constituted 60%, those with 4-stamens 33% and those with 5-stamens 7%. All the three types of flowers were found on the same plant. The flowers with 6-stamens are very rare. A single plant all with 5-stamened flowers was encountered during the study period and these flowers are prominently larger than other types of flowers. In 3-stamened flowers, one stamen is alterni-tepalous while the other two are anti-tepalous (Plate 10b). In 4-stamened flowers, three stamens are alterni-tepalous while the other one is anti-tepalous. In 5-stamened flowers, two stamens are alterni-tepalous while the other three are anti-tepalous (Plate 10c). In 6-stamened flowers, three stamens are alterni-tepalous while three others are anti-tepalous (Plate 10d). The ovary is light green, oblong, tri-carpellary, tri-locular syncarpous with D-shaped ovules arranged on axile placentation. The ovule number varied with change in stamen number (Plate 10h). It is  $17.45 \pm 3.51$  in 3-stamened flowers,  $19.9 \pm 2.88$  in 4-stamened flowers and  $23.1 \pm 3.70$  in 5-stamened flowers. The style is absent but the ovary is terminated with 3 free densely papillose, shiny, spreading stigmas (Plate 10g).

**Floral biology:** Mature buds open during 0700-0900 h (Plate 10a). Individual buds take 5 to 10 minutes from partial to full opening. The flowers are homogamous as the anthers and stigmas attain maturity at the same time during anthesis; the former dehisce by longitudinal slits (Plate 10e) and the latter continue receptivity until the noon of the 2<sup>nd</sup> day (Table 1). The pollen output is varied with change in stamen number. It varied from  $209.6 \pm 17.12$  to  $171.4 \pm 13.44$  per anther and from  $628.8 \pm 51.36$  to  $857 \pm 67.2$  per flower in 3- to 5-stamened flowers (Table 11). The pollen production trend showed that pollen output rate gradually increased with a gradual decrease in the number of stamens produced per flower but the variation is slight significant. The pollen-ovule ratio is 36:1 in 3-stamened flowers

while it is 37:1 in 4- and 5-stamened flowers. The pollen grains are pale yellow, spheroidal, tri-colpate, tri-zonoaperturate, granulated, tectum scabrate,  $25.3 \pm 0.5 \mu\text{m}$  on polar axis. The nectar is secreted in traces during mature bud stage (Plate 10f). The tepals together with the stamens and stigmas close back by 1000-1200 h (Table 1).

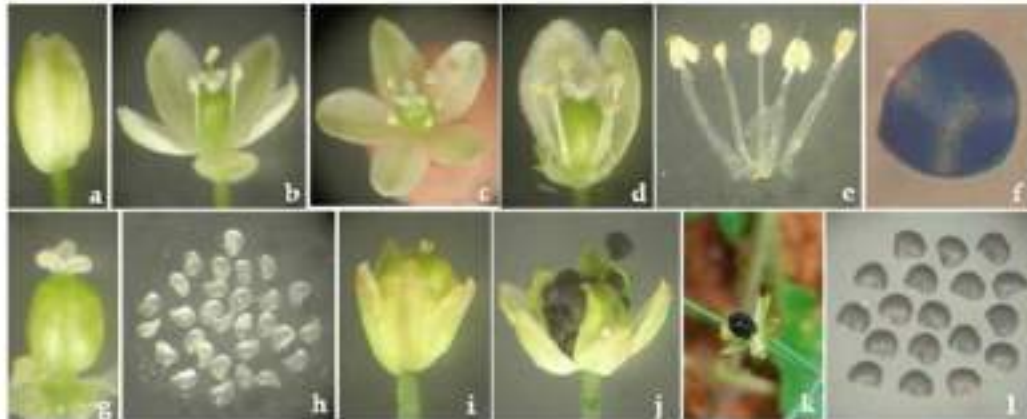


Plate 10. *Mollugo nudicaulis*: a. Bud, b. 3-stamened flower, c. 5-stamened flower, d. 4-stamened flower, e. Dehiscid anthers, f. Pollen grain, g. Ovary with three styles, h. Ovules, i. Maturing fruit, j. Dehiscid fruit capsule, k. Dehiscid fruit capsule with seeds intact, l. Seeds.

Table 11. Pollen aspects in *Mollugo nudicaulis*

Flower type	Percentage of occurrence	Mean pollen output/anther	Mean pollen output/flower	Mean no. of ovules/flower	Pollen: ovule ratio
3-stamened	60	$209.6 \pm 17.12$	$628.8 \pm 51.36$	$17.45 \pm 3.51$	36 : 1
4-stamened	33	$184.4 \pm 13.12$	$737.6 \pm 52.48$	$19.90 \pm 2.88$	37 : 1
5-stamened	7	$171.4 \pm 13.44$	$857.0 \pm 67.20$	$23.10 \pm 3.70$	37 : 1

**Pollination mechanism:** In dehiscid anthers collected during anthesis, 11-21% of pollen grains were found with pollen tubes indicating *in situ* germination. Further, the pollen tubes were also found on the stigma. The pollen germination and formation of tubes both within the dehiscid anthers and on the stigma indicate that the presence of self-induced autogamy. During anthesis, one anther in 3-stamened flowers and 2-3 anthers in 4- and 5-stamened flowers contact the stigmas due to their close proximity and their position at the same height. With this situation, the anthers brush against the stigmas causing autogamy. After anthesis, all anthers move away from the stigmas but both the sex organs are situated at the same height facilitating vector-mediated self- or cross-pollination. Further, the stamens and stigmas contact each other very closely during the closing of the flower assuring autogamy if it did not occur during open state of the flower.

**Thrips breeding, feeding and pollination:** The thrips species, *Haplothrips* sp.

(Thysanoptera: Thripidae) used flower buds for breeding and flowers for feeding. The larvae emerged from the eggs in synchrony with anthesis and nectar production in flowers. The larvae and adults foraged for pollen and nectar. Individual thrips were dusted with pollen during their movements within the flowers. They carried 69 to 158 pollen grains on their body setae, wings and legs. The thrips dispersed the pollen on free densely papillose spreading stigmas due to their active movement, rubbing of abdomen against the stigmatic surface, cleansing of their body parts with their hind legs and also by their wing combing mechanism. The homogamous flowers were found to facilitate self-pollination in the same or different flowers of the same plant. As the plant occurs as small or large populations, thrips could fly to migrate to the flowers of other closely spaced plants and effect cross-pollination by feeding on the forage.

**Insect visitors and Pollination:** The flowers were foraged by bees and flies for pollen and nectar, and butterflies for only nectar during noon time from 0800-1100 h with concentrated foraging activity during 0900-1000 h (Figure 7,8). The bees were *Apis cerana* (Plate 11a), *A. florea* (Plate 11b), *Trigona iridipennis* (Plate 11c), *Ceratina* sp. (Plate 11d) and *Halictus* sp (Plate 11e). The flies included *Eristalinus* sp. (Plate 11f) and *Musca* sp. (Plate 11g). The butterflies included only lycaenids, namely, *Zizula hylax*, *Zizeeria karsandra* (Plate 11h), *Zizina otis* (Plate 11i), *Freyeria trochylus* and *Chilades pandava* (Plate 11j) (Table 12). All these insects approached the flowers in upright position, landed on the tepals and then probed for forage collection. Bees first accessed anthers to collect pollen and then moved to the flower base to collect nectar, if available in the same and/or different visits. Flies and butterflies stretched out their proboscis and inserted into the flower base to collect nectar. All insect species collected forage from several flowers of different cymes of the same or different plants to collect the forage. The bees during pollen collection brushed against the stigmas with their ventral surface effecting sternotribic pollination. Further, these insects during nectar collection brushed against anthers and stigmas with their dorsal surface effecting nototribic pollination. The flies and butterflies during nectar collection contacted the stamens and stigmas with their proboscis and occasionally front side of head and ventral surface of thorax and abdomen effecting sternotribic pollination. Their wings never contacted the stamens and stigma during nectar collection as they kept them in vertical position. Bees made 49%, flies 11% and lycaenids 40% of total foraging visits (Figure 9). The body washings of insects collected from the flowers during peak foraging period revealed that all insects carry pollen but bees carry the highest number of pollen grains. Further, the mean number of pollen grains varied with each insect species (Table 13). The nectar secretion in traces and its depletion by thrips during and after anthesis appeared to be driving the insects to visit as many flowering cymes as possible to quench their thirst for nectar. Such a foraging behavior was considered to be facilitating the promotion of cross-pollination.

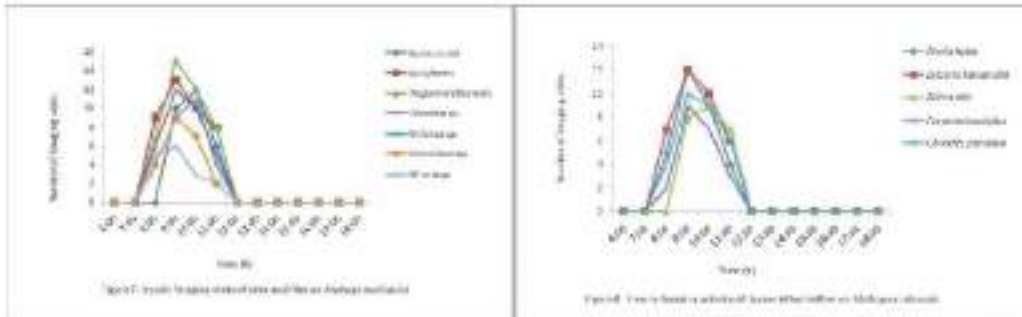


Plate 11. *Moraga mucknallii*: a. *Apis cerana*, b. *Apis florea*, c. *Trigona indigena*, d. *Crematogaster* sp., e. *Helictes* sp., f. *Eristalis* sp., g. *Megachile* sp., h-j. Lycaenids - h. *Zizania leucosticta*, i. *Zizania* sp., j. *Chloris pectorata*.

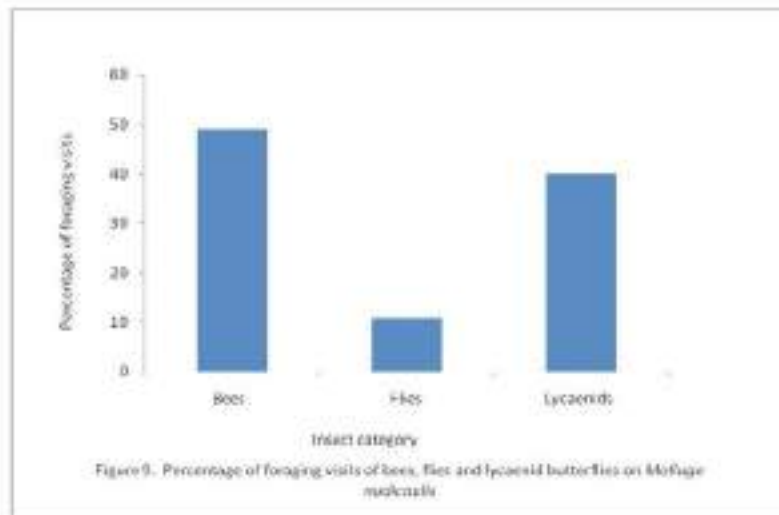


Table 12. List of insect foragers on *Mollugo nudicaulis*

Order	Family	Genus	Species	Common Name	Forage Sought
Hymenoptera	Apidae	<i>Apis</i>	<i>cerana</i> F.	Indian Honey Bee	Pollen + Nectar
		<i>Apis</i>	<i>florea</i> F.	Dwarf Honey Bee	Pollen + Nectar
		<i>Trigona</i>	<i>iridipennis</i> Smith	Stingless honey Bee	Pollen + Nectar
		<i>Ceratina</i>	sp.	Small Carpenter Bee	Pollen + Nectar
	Halictidae	<i>Halictus</i>	sp.	Sweat Bee	Pollen + Nectar
	Syrphidae	<i>Eristalmus</i>	Sp	Hover fly	Nectar
	Muscidae	<i>Musca</i>	sp.	House Fly	Nectar
Lepidoptera	Lycacnidae	<i>Zizula</i>	<i>hylax</i> F.	Tiny Grass Blue	Nectar
		<i>Zizeeria</i>	<i>karsandra</i> Moore	Dark Grass Blue	Nectar
		<i>Zizina</i>	<i>otis</i> F.	Lesser Grass Blue	Nectar
		<i>Freyeria</i>	<i>trochylus</i> Freyer	Grass jewel	Nectar
		<i>Chilades</i>	<i>pandava</i> Horsfield	Plains Cupid	Nectar

Table 13. Pollen recorded in the body washings of insects on *Mollugo nudicaulis*

Insect species	Sample size(N)	Number of pollen grains		
		Range	Mean	S.D
<i>Apis cerana</i>	10	73-204	133.5	37.5
<i>Apis florea</i>	10	61-183	126.1	33.31
<i>Trigona iridipennis</i>	10	37-95	63.4	14.4
<i>Ceratina</i> sp.	10	34-62	47.8	8.27
<i>Halictus</i> sp.	10	41-87	69.8	12.2
<i>Eristalmus</i> sp.	10	26-50	38.2	7.26
<i>Musca</i> sp.	10	11-38	27.9	7.5
<i>Zizula hylax</i>	10	9-28	21.3	5.47
<i>Zizeeria karsandra</i>	10	13-32	23.8	5.57
<i>Zizina otis</i>	10	16-40	28.1	6.48
<i>Freyeria trochylus</i>	10	8-31	24.4	7.19
<i>Chilades pandava</i>	10	15-36	28.5	6.27

**Fruiting ecology and seed dispersal:** The pollinated and fertilized flowers grow continually and produce fruits within 8-10 days. The stamens and stigmas are persistent and remain inside due to the closure of the flower. The tepals bulge gradually and protect the bulging ovary in which the seeds form and mature. Natural fruit set varied from 86 to 89% while seed set varied from 88 to 92% in 3-, 4- and 5-stamened flowers (Table 14). Fruit is a loculicidal 3-valved broadly-ellipsoid capsule, stalked, membranous, densely pubescent,  $3.4 \pm 0.4$  mm long and  $2.33 \pm 0.39$ mm wide. The seeds are arranged in two rows in each locule. They are tiny, black, slightly shiny, reniform and concentrically ridged. The seed coat is closely packed with uniformly distributed, pebble-like, lyrate and chipped areoles. Dry capsules break open when fruit pericarp and tepals are dry and expose the seeds (Plate 10j,k). But the seeds remain so and gradually separate and fall to the ground on their own on clear sunny days. On rainy days, the water droplets falling on the dehisced capsules washout seeds to the ground. Further, water acts as an efficient dispersal agent for the dispersal of seeds fallen on the soil during rainy season. Seeds do not have adaptations for wind dispersal. But, wind disperses the dry cymes together with dry dehisced capsules to short distances and subsequently the seeds fall to the ground from capsules. Therefore, seed dispersal modes include ombrohydrochory, hydrochory and anemochory. The seeds produced from plants growing in cultivated lands have the potential to be dispersed as a produce contaminant and in effect agricultural produce movement contributes to seed dispersal and expansion of its distribution (Plate 9c,d; Plate 10l).

Table 14. Natural fruit and seed set rate in *Mollugo nudicaulis*

Flower type	Number of flowers sampled	Number of flowers set fruit	Fruit set (%)	Seed set (%)
3-stamened	320	286	89	88
4-stamened	85	73	86	91
5-stamened	40	35	88	92

#### **MOLLUGO PENTAPHYLLA L.**

**Phenology:** It is a small much-branched annual herb with a thin tap root. It is common in open dry and moist sandy and sandy loamy soils along roadsides, waste places and cultivated lands (Plate 12a). The stem is thin, angular, glabrous and tinged with brownish red when old. Leaves are petiolate, unequal, succulent, glabrous, obovate to spatulate, margin entire and apex mucronate. The basal leaves are 5 or more in rosette form while those upwards vary from 4 to 1. The plant appears simultaneously in vegetative, flowering and fruiting phases in different populations growing in different habitats throughout the year (Plate 12b). An individual plant, however, has a short life cycle of 3 months from seed germination to seed dispersal. Although it appears throughout the year, it shows robust vegetative growth and profuse flowering and fruiting during July-October when soil is damp

due to occurrence of rains. The inflorescence is a polychasial cyme which arises from leaf axils and terminally during wet season while it is usually di- or tri-chasial cyme during dry season. It is spreading, pedunculate (5-8 mm long) and produces pedicellate (2-4 mm long) flowers. The peduncle and pedicel are wiry and stiff. An polychasial produces  $13.83 \pm 4.9$  flowers.

**Flower morphology:** The flowers are small ( $2.75 \pm 0.4$  mm long,  $1.8 \pm 0.4$  mm wide), white on adaxial and abaxial side, odourless, actinomorphic and bisexual. The sepals and petals are represented by a monochlamydeous perianth of 5 tepals. The tepals are free, 2-3 mm long, but connate at base, elliptic to oblong and hooded. The stamens are 3 to 5, free, but connate at base, white,  $1.8 \pm 0.17$  mm long with broadly flat filaments but dilated at base and dorsifixed, white, ditheous anthers. The flowers with 3-stamens constituted 91%, those with 4-stamens 7% and those with 5-stamens 2%. All the three types of flowers were found on the same plant. In 3-stamened flowers, one stamen is alterni-tepalous while the other two are anti-tepalous. In 4-stamened flowers, three stamens are alterni-tepalous while the other one is anti-tepalous. In 5-stamened flowers, two stamens are alterni-tepalous while the other three are anti-tepalous. The ovary is white, ovoid,  $1.16 \pm 0.24$  mm long, 1.5 mm broad, tri-carpellary, tri-locular syncarpous with reniform ovules arranged on axile placentation. The ovule number varied with change in stamen number. It is  $16.02 \pm 4.0$  in 3-stamened flowers,  $18.44 \pm 2.0$  in 4-stamened flowers and  $20.11 \pm 2.6$  in 5-stamened flowers (Plate 12i). The style is absent but the ovary is terminated with 3 free densely papillose, shiny, spreading stigmas (Plate 12h).



Plate 12. *Mollige jostaphylla*: a. Habit, b. Flowering phase, c-e. Different stages of anthesis, f. Position of anthers and stigmas at the same height, g. Pollen grain, h. Ovary with three stigmas, i. Ovules.

**Floral biology:** Mature buds open during 0700-0800 h. Individual buds take 5 to 10 minutes from partial to full opening (Plate 12c-e). The flowers are homogamous as the anthers and stigmas attain maturity at the same time during anthesis; the former dehisce by longitudinal slits and the latter continue receptivity until the noon of the 2<sup>nd</sup> day (Table 1). The pollen output varied with change in stamen number. It varied from  $277.2 \pm 13.4$  to  $213.4 \pm 12.9$  per anther and from  $831.6 \pm 40.2$  to  $1067 \pm 64.5$  per flower in 3- to 5-stamened flowers (Table 15). The pollen production trend showed that pollen output rate gradually slightly increased with a gradual decrease in the number of stamens produced per flower. The pollen-ovule ratio is 52:1 in 3-stamened flowers while it is 53:1 in 4- and 5-stamened flowers. The pollen grains are pale yellow, spheroidal, tri-colpate, tri-zonoaperturate, granulated, tectum scabrate,  $26.4 \pm 0.5 \mu\text{m}$  (Plate 12g). *In vitro* pollen viability test indicated that the pollen grains are viable from anthesis onwards and remain so even after the closure of the flower (Table 16). The nectar is secreted in traces during mature bud stage. The tepals together with the stamens and stigmas close back by 1000-1100 h (Table 1).

Table 15. Pollen aspects in *Mollugo pentaphylla*

Flower type	Percentage of occurrence	Mean pollen output/anther	Mean pollen output/flower	Mean no. of ovules/flower	Pollen: ovule ratio
3-stamened	91	$277.2 \pm 13.4$	$831.6 \pm 40.2$	$16.02 \pm 4.0$	52 : 1
4-stamened	7	$242.6 \pm 19.6$	$970.4 \pm 78.4$	$18.44 \pm 2.0$	53 : 1
5-stamened	2	$213.4 \pm 12.9$	$1067 \pm 64.5$	$20.11 \pm 2.6$	53 : 1

Table 16. *In vitro* pollen germination in *Mollugo pentaphylla*

Time (h)	Pollen sample	No. of germinated pollen grains	Germination (%)
0600	-	-	-
0700	50	26	52
0800	34	23	68
0900	39	28	72
1000	23	14	61
1100	15	9	60
1200	46	18	39
1300	15	4	27
1400	35	3	9
1500	-	-	-
1600	-	-	-

Modified Brewbaker and Kwack's medium



**Pollination mechanism:** In dehisced anthers collected during anthesis, 18-26% of pollen grains were found with pollen tubes indicating *in situ* germination. Further, the pollen tubes were also found on the stigma. The pollen germination and formation of tubes both within the dehisced anthers and on the stigma indicate that the presence of self-induced autogamy. During anthesis, one anther in 3-stamened flowers and 2-3 anthers in 4- and 5-stamened flowers contact the stigmas due to their close proximity and their position at the same height (Plate 12f). With this situation, the anthers brush against the stigmas causing autogamy. After anthesis, all anthers move away from the stigmas but both the sex organs are situated at the same height facilitating vector-mediated self- or cross-pollination. Further, the stamens and stigmas contact each other very closely during the closing of the flower assuring autogamy if it did not occur during open state of the flower.

**Thrips breeding, feeding and pollination:** The thrips species, *Haplothrips* sp. (Thysanoptera: Thripidae) used flower buds for breeding and flowers for feeding. The larvae emerged from the eggs in synchrony with anthesis and nectar production in flowers. The larvae and adults foraged for pollen and nectar. Individual thrips were dusted with pollen during their movements within the flowers. They carried 89 to 217 pollen grains on their body setae, wings and legs. The thrips dispersed the pollen on free densely papillose spreading stigmas due to their active movement, rubbing of abdomen against the stigmatic surface, cleansing of their body parts with their hind legs and also by their wing combing mechanism. The homogamous flowers were found to facilitate self-pollination in the same or different flowers of the same plant. As the plant occurs as small or large populations, thrips could fly to migrate to the flowers of other closely spaced plants and effect cross-pollination by feeding on the forage.

**Insect visitors and Pollination:** The flowers were foraged by bees for pollen and nectar while butterflies for only nectar during noon time from 0800-1100 h with concentrated foraging activity during 0900-1000 h (Figure 10,11). The bees were *Apis cerana* (Plate 13a), *A. florea* (Plate 13b), *Trigona iridipennis* (Plate 13c), *Ceratina* sp. and *Thyreus histrio* (Plate 13e). The butterflies included only lycaenids, namely, *Castalius rosimon* (Plate 13f), *Zizula hylax* (Plate 13g), *Zizeeria karsandra* (Plate 13h), *Zizina otis* and *Chilades pandava* (Table 17). All these insects approached the flowers in upright position, landed on the tepals and then probed for forage collection. Bees first accessed anthers to collect pollen and then moved to the flower base to collect nectar, if available in the same and/or different visits. Butterflies stretched out their proboscis and inserted into the flower base to collect nectar. All insect species collected forage from several flowers of different cymes of the same or different plants to collect the forage. The bees during pollen collection brushed against the stigmas with their ventral surface effecting sternotribic pollination. Further, these insects during nectar collection brushed against anthers and stigmas with their dorsal surface effecting nototribic pollination. The butterflies during nectar collection contacted the stamens and stigmas with their proboscis and occasionally front side of head and ventral surface of thorax and abdomen effecting sternotribic pollination. Their wings

never contacted the stamens and stigma during nectar collection as they kept them in vertical position. Bees made 56% and lycaenids 44% of total foraging visits (Figure 12). The body washings of insects collected from the flowers during peak foraging period revealed that all insects carry pollen but bees carry the highest number of pollen grains. Further, the mean number of pollen grains varied with each insect species (Table 18). The nectar secretion in traces and its depletion by thrips during and after anthesis appeared to be driving the insects to visit as many flowering cymes as possible to quench their thirst for nectar. Such a foraging behavior was considered to be facilitating the promotion of cross-pollination.

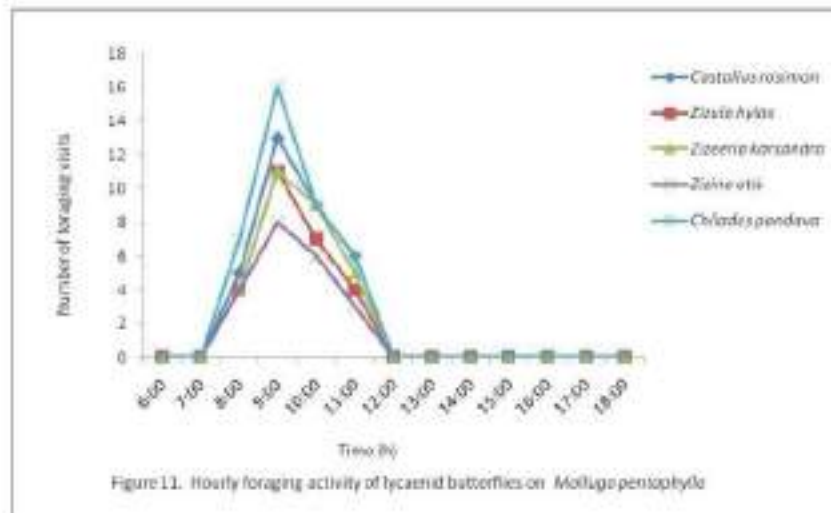
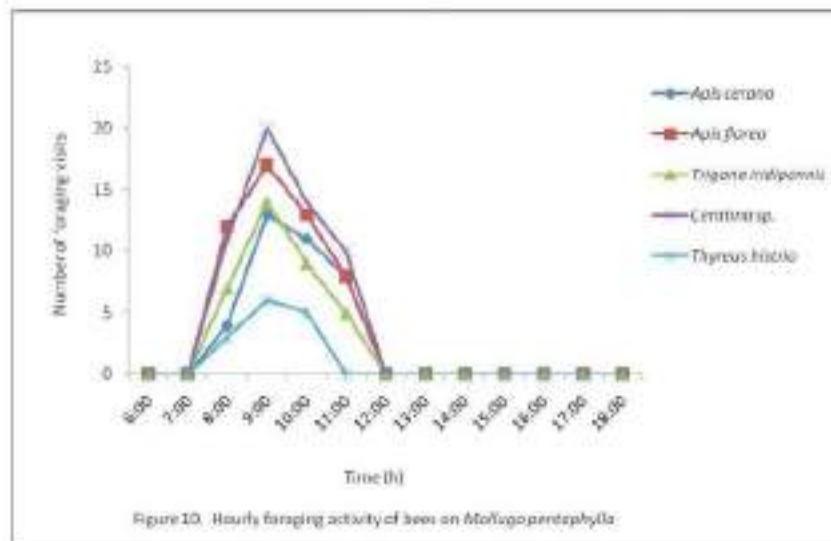




Plate 13. *Molugo pentaphylla*: Foragers - a. *Apis cerana*, b. *Apis florea*, c. *Trigona iridipennis*, d. *Ceratina* sp., e. *Thyreus histria*, f. *Castalius rosimon*, g. *Zizula hylax*, h. *Zizeeria karsandra*.

Table 17. List of insect foragers on *Molugo pentaphylla*

Order	Family	Genus	Species	Common name	Forage sought
Hymenoptera	Apidae	<i>Apis</i>	<i>cerana</i> F.	Indian Honey Bee	Pollen + Nectar
		<i>Apis</i>	<i>florea</i> F.	Dwarf Honey Bee	Pollen + Nectar
		<i>Trigona</i>	<i>iridipennis</i> Smith	Stingless Honey Bee	Pollen + Nectar
		<i>Ceratina</i>	sp.	Small Carpenter Bee	Pollen + Nectar
		<i>Thyreus</i>	<i>histria</i> F.	Bee-fly	Pollen + Nectar
Lepidoptera	Lycaenidae	<i>Castalius</i>	<i>rosimon</i> F.	Common Pierrot	Nectar
		<i>Zizula</i>	<i>hylax</i> F.	Tiny Grass Blue	Nectar
		<i>Zizeeria</i>	<i>karsandra</i> Moore	Dark Grass Blue	Nectar
		<i>Zizina</i>	<i>otis</i> F.	Lesser Grass Blue	Nectar
		<i>Chilades</i>	<i>pandava</i> Horsfield	Plains Cupid	Nectar

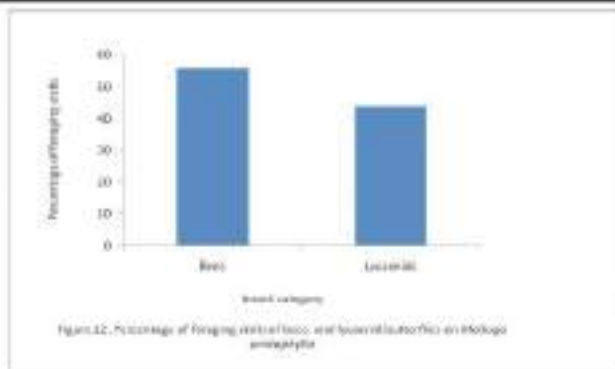


Table 18. Pollen recorded in the body washings of insects on *Mollugo pentaphylla*

Insect species	Sample size (N)	Number of pollen grains		
		Range	Mean	S.D
<i>Apis cerana</i>	10	82-246	159.2	51.8
<i>Apis florea</i>	10	68-217	145.1	43.56
<i>Trigona iridipennis</i>	10	31-86	62.1	13.5
<i>Ceratina</i> sp.	10	24-51	38.2	9.07
<i>Thyreus histrio</i>	10	19-43	30.6	7.60
<i>Castalix rosomon</i>	10	15-41	29.5	6.38
<i>Zizula hylax</i>	10	9-30	20.6	5.27
<i>Zizeeria karsandra</i>	10	13-45	27.8	9.49
<i>Zizina otis</i>	10	10-36	23.7	6.63
<i>Chilades pandava</i>	10	8-43	30.7	8.8

**Fruiting ecology and seed dispersal:** The pollinated and fertilized flowers grow continually and produce fruits within 8-12 days (Plate 14a). The stamens and stigmas are persistent and remain inside due to the closure of the flower. The tepals bulge gradually and protect the bulging ovary in which the seeds form and mature (Plate 14b). Natural fruit set varied from 83 to 88% while seed set varied from 83 to 86% in 3-, 4- and 5-stamened flowers (Table 19). Fruit is a loculicidal 3-valved broadly-ellipsoid capsule, stalked, membranous, glabrous,  $2.67 \pm 0.4$  mm long and  $1.97 \pm 1.4$  mm wide. The seeds are arranged in two rows in each locule. They are tiny, black, slightly shiny, reniform and concentrically ridged (Plate 14e). The seed coat is closely packed with uniformly distributed, pebble-like, lyrate and chipped areoles. Dry capsules break open when fruit pericarp and tepals are dry and expose the seeds (Plate 14c). But the seeds remain so (Plate 14d) and gradually separate and fall to the ground on their own on clear sunny days. On rainy days, the water droplets falling on the dehiscent capsules washout seeds to the ground. Further, water acts as an efficient dispersal agent for the dispersal of seeds fallen on the soil during rainy season. Seeds do not have adaptations for wind dispersal. But, wind disperses the dry cymes together with dry dehiscent capsules to short distances and subsequently the seeds fall to the ground from capsules. Therefore, seed dispersal modes include ombrohydrochory, hydrochory and anemochory. The seeds produced from plants growing in cultivated lands have the potential to be dispersed as a produce contaminant and in effect agricultural produce movement contributes to seed dispersal and expansion of its distribution (Plate 14f,g).

The study shows that all five plant species studied display certain common characters such as homogamy, spontaneous autogamy, brief period of open state of flowers, flower closure and facultative autogamy. These species serve as breeding and feeding host plants

for *Haplothrips* species which in turn reciprocate pollination service. *M. cerviana* is never foraged by any insect species. The other four plant species are pollinated by bees and lycaenid butterflies. *G. oppositifolius* is also pollinated by ants while *M. nudicaulis* is also pollinated by flies (Table 20).



Plate 14. *Mollugo pentaphylla*: a. Fruiting phase, b. Maturing fruits, c. Dehiscing fruit capsule, d. Dehiscing fruit capsule with seeds intact, e. Seeds, f. & g. New plants.

Table 19. Natural fruit and seed set rate in *Mollugo pentaphylla*

Flower type	Number of flowers sampled	Number of flowers set fruit	Fruit set (%)	Seed set (%)
3-stamened	250	220	88	83
4-stamened	150	130	87	84
5-stamened	75	62	83	86

Table 20. Consolidated list of insect foragers on the studied plant species

Order/Family	Insect species	<i>Glinus</i>	<i>Glinus oppositifolius</i>	<i>Mollugo nudicaulis</i>	<i>Mollugo pentaphylla</i>	Forage sought P: Pollen, N: Nectar
<b>Hymenoptera</b>						
Apidae	<i>Apis cerana</i> F.	+	+	+	+	P + N
	<i>Apis florea</i> F.	+	+	+	+	P + N

	<i>Trigona iridipennis</i> Smith	+	-	+	+	P + N
	<i>Ceratina smaragdula</i> F.	+	+	-	-	P + N
	<i>Ceratina</i> sp.	+	+	+	+	P + N
	<i>Thyreus histrio</i>	-	-	-	+	P + N
Halictidae	<i>Halictus</i> sp.	-	+	+	-	P + N
Megachilidae	<i>Megachile</i> sp.	-	+	-	-	P + N
Formicidae	<i>Camponotus</i> sp.	+	+	-	-	N
	<i>Crematogaster</i> sp.	+	+	-	-	N
<b>Diptera</b>						
Syrphidae	<i>Eristalinus</i> sp.	-	-	+	-	N
Muscidae	<i>Musca</i> sp.	-	-	+	-	N
<b>Lepidoptera</b>						
Lycanidae	<i>Castalius rosimon</i> F.	-	-	-	+	N
	<i>Zizula hylax</i> F.	+	+	+	+	N
	<i>Zizeeria karsandra</i> Moore	+	+	+	+	N
	<i>Zizina otis</i> F.	+	+	+	+	N
	<i>Freyeria trochylus</i> Freyer	+	+	+	-	N
	<i>Chilades lains</i> Stoll	+	+	-	-	N
	<i>Chilades pandava</i> Horsfield	+	+	+	+	N

## DISCUSSION

### Pollination ecology of *Glinus lotoides* and *G. oppositifolius*

*Glinus lotoides* and *G. oppositifolius* are prostrate, spreading, annual herbs that carpet the ground in open sandy soils and agricultural lands. They occur throughout the year and show vegetative, flowering and fruiting phases in different areas. But, their robust growth, profuse flowering and fruiting is confined to wet season. The flowers borne in axillary cymes in *G. lotoides* and axillary fascicles in *G. oppositifolius* stand erect above the foliage and display their prominence. In both the species, the stems produce many branches and each branch produces several cymes or fascicles. Since the plants usually grow as green carpets, the simultaneous display of several flowers from individual plants

and from the entire population(s) enhances their attraction to insect pollinators.

Several anonymous authors provided the floral descriptions of *Glinus* species. Ronse De Craene (2010) stated that *Glinus* species have five sepals in quincuncial aestivation. In the present study, *G. lotoides* and *G. oppositifolius* have been found to have five tepals as common and six tepals as rare. The study also indicates that the word "tepal" is the appropriate word since it acts as petal adaxially and sepal abaxially. This is further substantiated by two different colours displayed on adaxial and abaxial surface. The tepals are whitish green on adaxial surface and green abaxial surface in *G. lotoides* and creamy white on adaxial surface and brownish orange on abaxial surface in *G. oppositifolius*.

Hoffman (1994) stated that the stamen number is unstable among different species of *Glinus* and the number varies from five to several series which includes outer staminodes. Brockington *et al.* (2013) used the word "petaloid" for "staminode" in *Glinus*. These authors reported that in this genus, petaloid number can fluctuate enormously and can either be antisepalous replacing a fertile stamen or alternisepalous as an appendage of upper stamen. They also stated that the outer stamens are replaced by petaloid structures. Weberling (1989) noted that the stamens that lost their function as producers of viable pollen have become staminodes or petaloids in this genus. Ronse De Craene (2010) reported that the androecium of *G. lotoides* is extremely variable. Further, he reported that the flower material he examined showed that the alternisepalous whorl is complete with staminodes and odd stamen opposite to petals. Sharma (1963) mentioned that *G. lotoides* flowers produce rarely more than five stamens while *G. oppositifolius* produce 10-13 stamens in three whorls. He also suggested a tendency for reduction with the loss of the outer stamen whorl in androecium. In the present study, *G. lotoides* flowers have 8-12 functional stamens arranged in two whorls and they are usually antitepalous. The flowers also have 5-7 staminodes arranged in outer whorl and they are usually alternitepalous. In *G. oppositifolius*, the flowers have 4-6 functional stamens arranged in one whorl and they are alternitepalous; the staminodes are 5-6 arranged in outer whorl and they are antitepalous. In both the species, the staminodes are petaloids indicating the fusion of two adjacent stamens that lost the function of producing viable pollen in course of the evolution of flowers. These staminodes are integral features of floral morphology and appear to have evolved to serve as attractants to pollinators, reduce self-pollination rate and optimize the available nutrients for enhanced reproductive output in water and nutrient deficient habitats. Further, the production of staminodes appears to be an adaptation to reduce pollen production per flower, increase efficiency of pollen dispersal by limiting pollen removal by individual pollinators and enable precise contact between pollinators and pollen presenters or pollinators and stigmas. Therefore, the petaloid staminodes are evolved to perform different roles in the flowers and are unique for *Glinus* within Molluginaceae (Stebbins 1974; Ronse De Craene and Smets 1993, 1995; Ronse De Craene 2013).

Hammer (1995) reported that different populations of Aizoaceae growing in the

same habitat exhibit synchrony in flowering time. The period of flowering is usually short and the flowers show repeated opening but this phenomenon is restricted to a certain period of the day. Groen and Van Der Maesen (1999) observed that the mixed populations of Aizoaceae genera, *Bergeranthus*, *Faucaria* and *Orthopterum* flower simultaneously. These authors suggested that such a synchrony in flowering in these genera in the same habitat collectively enable them to enhance their floral attraction to pollinators. In the present study, it is found that *Glinus* species form mixed and distinct populations in the same and different habitats depending on soil moisture and nutrient conditions. These species exhibit synchrony in flowering by opening flowers in the afternoon. Further, the flowers are too small, lack corolla, tepals not vividly coloured and stay open for a brief period, for three hours in *G. lotoides* and four hours in *G. oppositifolius* for visitation by insects. Therefore, the synchrony in anthesis schedule and massive floral display appear to be imperative for them to attract pollinators during the brief period of open state of flowers.

Peter *et al.* (2004) reported that the temperature and relative humidity are probably important cues determining flower opening in the afternoon. The specific timing of anthesis in the late afternoon is a likely mechanism to filter out generalist pollinators most active at midday, rather targeting specific group of insects, primarily bees, still active in the late afternoon. The present study indicates that afternoon anthesis in *Glinus* species is probably evolved in course of time to avoid competition for pollinators in pollinator-deprived environment, especially in habitats where other herbaceous plant species flower simultaneously, show anthesis during forenoon period and attract insect pollinators with their vivid floral colours. *Glinus* species provide sufficient forage for insect pollinators in the afternoon period and accordingly bees, ants and lycaenid butterflies collect forage and pollinate flowers. Bees and butterflies are generalists which visit a wide range of flowers and hence are polylectic. Since they are active throughout day, they soon switch to fresh forage available in the habitat. *Glinus* species with afternoon anthesis readily provide forage, and bees and butterflies begin to shift to these floral sources and concentrate on forage collection from them. Therefore, afternoon anthesis in *Glinus* species ensures insect pollination and reciprocate the insect pollinators with pollen and/or nectar.

Watson and Dallwitz (1992) stated that Molluginaceae members are entomophilous. These authors considered nectar secreting tissue and showy tepals in several species as adaptations for entomophily. In *Glinus* species, the floral characters such as the erect position of flowers above foliage, adaxial surface of the tepals, petaloid staminodes and nectar secreting tissue between the ovary base, connate part of staminal filaments and scabrate ornamentation of pollen grains appear to be adaptations for insect pollination. The bees while collecting pollen and butterflies while collecting nectar effect sternotribic pollination. Further, the bees and ants while collecting nectar effect nototribic pollination. In both *G. lotoides* and *G. oppositifolius*, the pollen output per anther varies with the number of functional stamens present in the flowers; it increases with a decrease in the stamen number. The pollen output per flower in *G. lotoides* is more than double the amount produced per



flower in *G. oppositifolius*. The variation in pollen production in these plant species is partly attributable to the number of stamens produced. The varied amount of pollen output in the flowers of the same and different inflorescences on the same plant drives the pollen collecting bees to visit the flowers across population(s) in search of more pollen collection and such a foraging activity contributes to both self- and cross-pollination. The nectar secreted in traces in both the species and nectar removal by thrips species, *Haplothrips* also drives the nectar collecting bees, ants and lycaenid butterflies to visit flowers across population(s) due to which both self- and cross-pollinations occur. *Glinus* species appear to be important sources of pollen for bees, especially for honey bees. Aldeen (2014) also noted that *G. lotoides* is an important pollen source for honey bees in Radom area, South Darfur State, Sudan. Further, these plant species in the study area are important nectar sources for ants and lycaenid butterflies. Among butterflies, lycaenids are the smallest, low-flying and appropriate pollinators for prostrate herbs such as *Glinus* species. Grund (1998) reported that the lycaenid butterfly, *Zizeeria karsandra* uses both *G. lotoides* and *G. oppositifolius* as larval host plants in South Australia. This report suggests that this butterfly uses *Glinus* species as both larval and nectar host plants. Studies on the life cycle of other lycaenid butterflies visiting *Glinus* species may throw more light on the relationships between *Glinus* species and lycaenid butterflies.

In both *Glinus* species, the flowers are weakly protandrous because there is a brief gap between anther dehiscence and commencement of stigma receptivity. Since both male and female sexes mature almost at the same time, the flowers in these plant species can be stated as homogamous. Further, the stamens and stigmas are spatially separated in both the species; such a situation suggests that the flowers are also herkogamous. Herkogamy does not facilitate the occurrence of spontaneous autogamy despite the flowers being homogamous. However, the thrips emerging from the floral buds during anthesis and their movements in the flowers after anthesis for pollen and nectar collection result in autogamy. They also bring about geitonogamy due to their migration to different inflorescences on the same plant for forage collection and xenogamy due to their migration to other conspecific plants for forage collection. Further, the movement of tepals together with stamens towards the pistil during flower closure facilitates contact between the sex organs and effects spontaneous autogamy if pollen is still available in the dehiscent stamens. *In vitro* pollen germination test for *G. oppositifolius* indicated that pollen is viable for a brief period only from the time of anther dehiscence to the time of flower closure suggesting that there is no possibility for the occurrence of spontaneous autogamy after flower closure. However, the tiny thrips have the possibility to carry pollen from other flowers, enter the closed flowers from the apical portion and laterally, and deposit the same on the stigmas effecting either geitonogamy or xenogamy. *In vitro* pollen germination test for *G. lotoides* was not done. Since it is an allied species of *G. oppositifolius*, it is possible that its pollen also displays the same duration of viability and thrips may effect pollination in the closed flowers. Therefore, *G. lotoides* and *G. oppositifolius* while keeping the options open for spontaneous or vector-mediated

selfing exhibit polyphily involving bees, ants, butterflies and thrips as pollinators.

In the present study, *Glinus* species show variation in the number of carpels and ovules per flower. The flowers of *G. lotoides* produce five carpels while those of *G. oppositifolius* produce three carpels. Likewise, the number of ovules also varies depending on the number of stamens and pollen output per flower; the ovules are more in *G. lotoides* than in *G. oppositifolius*. This ovule production trend indicates that the pollen output increases with an increase in ovule number in order to provide sufficient pollen to fertilize as many ovules as possible through spontaneous autogamy or vector-mediated pollination. This situation is reflected in the natural fruit and seed set rates in both the plant species. The highest fruit and seed set rates and the lowest pollen-ovule ratios recorded in *G. lotoides* and *G. oppositifolius* indicate that they are facultatively autogamous.

Bittrich (1990) reported that there is only one genus, *Adenogramma* which has one-seeded nutlet in Molluginaceae. All other genera produce capsules with many seeds and the capsules dehisce loculicidally to expose seeds. In *Glinus lotoides*, the capsules open when moistened with the aid of expanding keels. In the present study, in *G. lotoides* and *G. oppositifolius*, the fertilized flowers produce fruits within a week or two. The fruit is a capsule but it is 5-valved in *G. lotoides* and 3-valved in *G. oppositifolius*. In dry season, plants with ripe and dry capsules break off which are then dispersed by wind. Dry capsules break open loculicidally when tepals become wet and then expose the seeds. However, the seeds remain attached to the base of the perianth. In both the species, the seeds exposed from the capsules fall to the ground on clear sunny days. On rainy days, water drops find their way into the fruit through the distal opening and the fruit filled with water expels both water and seeds explosively. Further, seeds fallen on the ground disperse through surface water runoff during rain fall. Therefore, *G. lotoides* and *G. oppositifolius* exhibit anemochory, ombrohydrochory and hydrochory.

Narayana (1962) and Hofmann (1973) noted that *Glinus* species produce seeds with a white aril of funicular origin which develops into elongate, filiform strophiole. Ronse De Craene (2013) reported that *Glinus* genus is well characterized morphologically by its seeds with a filiform appendaged aril and indumentum of often stellate hairs. In the present study, it is found that *G. lotoides* produces small reniform, smooth and dark brown seeds with a white aril of funicular origin developed into elongate filiform strophiole. In *G. oppositifolius*, the seeds are small, sub-reniform and reddish-brown with a white aril of funicular origin formed into extensively curved scrotiform strophiole. The presence of filiform or scrotiform strophiole in *Glinus* species appears to be an adaptation for seed dispersal by ants. But, ants have not been found to use the strophiole as food and carry seeds of these species. However, further studies if taken up on this aspect may throw more light either to confirm or refute this observation.

Balcha (2009) reported that *Glinus lotoides* has short seed viability period. Teshome and Feyissa (2015) also reported that this species propagates by seed but short period of

seed viability and poor seed germination percentage are the limiting factors for its invasiveness. The present study showed that both *G. lotoides* and *G. oppositifolius* produce several batches of populations in a year and their seeds germinate as soon as they are dispersed but their germination is related to soil moisture which plays an important role in breaking the seed coat. Therefore, *Glinus* species appear to have short period of seed viability and also the viability may also be attributable to the extent of genetic variation achieved through vector-mediated pollination.

#### **Pollination ecology of *Mollugo cerviana*, *M. nudicaulis* and *M. pentaphylla***

*Mollugo* species are annual herbs which usually grow in open dry sandy and sandy and loamy soils but also occur in moist habitats, especially in cultivated lands. In this study, it is found that *M. cerviana*, *M. nudicaulis* and *M. pentaphylla* with their low ground habit populate the soil and for this reason, they are often called as carpet weeds. Of these, *M. cerviana* does not cover the soil extensively due to its wiry stems and thin, linear leaves. *M. nudicaulis* without any stem covers the soil with a rosette of prostrate leaves. *M. pentaphylla* with branched stems carpets the soil with its basal rosette form of leaves and upper spatulate leaves. All the three plant species grow throughout the year displaying vegetative, flowering and fruiting phases in different populations. However, their robust growth, profuse flowering and fruiting is confined to wet season. Individual plants complete their life cycle within three months from seed germination to seed dispersal. Similarly, Owens and Lund (2009) reported that *M. cerviana* is a herbaceous ephemeral species and completes its life cycle in a very short time. In the present study, it is found that the inflorescence is a dichotomous or trichotomous umbellate cyme in *M. cerviana* while it is di- or tri- or poly-chasial cyme in *M. nudicaulis* and *M. pentaphylla*. In the last two species, di-/tri-chasial cymes are common during dry season while poly-chasial cymes are common during wet season, suggesting that the branching of inflorescences and the production rate of flowers is regulated by the soil moisture and nutrient environment. *M. cerviana* and *M. pentaphylla* produce inflorescences in leaf axils and terminally while *M. nudicaulis* produces inflorescences from the axils of rosette of leaves due to lack of stems. Since all the three plant species usually grow as green carpets, the simultaneous display of several flowers from individual plants and from the entire population(s) enhances their attraction to insect pollinators.

The floral descriptions of *Mollugo* species provided by different authors are not accurate. The present study provides details of the floral descriptions, especially of perianth, androecium and gynoecium in *M. cerviana*, *M. nudicaulis* and *M. pentaphylla* as these are important from the pollination of point view. In these species, perianth typically consists of five tepals which serve the function of calyx (sepals) and corolla (petals). In *M. cerviana* and *M. nudicaulis*, the abaxial surface of the perianth serves the role of calyx while the adaxial surface of the perianth serves the role of corolla due to display two different colours on each surface. But, in *M. pentaphylla*, the perianth is white on both abaxial and adaxial

surface. The study shows that *M. cerviana* with perianth acting as both calyx and corolla is unable to attract any insect pollinators in pollinator-deprived habitat or pollinator-available habitat. Such a situation explains that *M. cerviana* is not dependent on insect foragers for pollination. *M. nudicaulis* with perianth displaying light green on its abaxial surface and creamy white on its adaxial surface, and *M. pentaphylla* with perianth displaying white colour on both adaxial and abaxial surface attract insect foragers. Eckardt (1974) and Stannard (1988) reported that the sister genera of *Mollugo*, *Corbichonia* and *Lophiocarpus* have only four stamens of which three alternate with sepals and one is opposite a sepal. Batenburg and Moeliono (1982) reported that the presence of one stamen opposite a sepal is unusual in these genera and indicate that this stamen is derived from an original condition with five alternisepalous stamens by a fusion of two stamens under the influence of a reduced tetramerous ovary which is similar to a process occurring in *Mollugo*. Ronse De Craene (2010) reported that in Molluginaceae including *Mollugo*, the androecium consists of generally of five stamens alternating with the sepals. In *Mollugo*, the number of stamens ranges from five in *M. cerviana* to three in *M. nudicaulis*. The present study shows that *M. cerviana* flowers produce a fixed number of 5 stamens and all are opposite to tepals suggesting that there is no process evolving to produce flowers with 3 or 4 stamens. *M. nudicaulis* produces flowers with 3-6 stamens while *M. pentaphylla* produces flowers with 3-5 stamens on the same plant. In these species, 3-stamened flowers have one stamen between two tepals and two stamens opposite to tepals, the 4-stamened flowers have three stamens alternate to tepals and one stamen opposite to a tepal, and the 5-stamened flowers have two stamens alternate to tepals and three stamens opposite to tepals. In *M. nudicaulis*, the 6-stamened flowers have three stamens alternate to tepals and three stamens opposite to tepals. The study indicates that all the three plant species produce trimerous ovary with three stigmas irrespective of the number of stamens produced in the flowers. In *M. nudicaulis* and *M. pentaphylla*, the production of 5-stamened flowers appears to be a residual trait still functional because these flowers are occasionally or rarely produced. In *M. nudicaulis*, the rarity of 6-stamened flowers may be a trait of polyploidy. In *M. nudicaulis* and *M. pentaphylla*, the two stamens opposite to tepals in 3-stamened flowers appear to be derived from the pair-wise fusion of four stamens (Ronse De Craene 2010) and the stamen opposite a tepal in 4-stamened flowers appear to be derived from the fusion of two stamens as in *Corbichonia* and *Lophiocarpus*, *sensu* Batenburg and Moeliono (1982). In *M. nudicaulis* and *M. pentaphylla*, the variation in pollen output levels due to variation in stamen number in 3-5(6) stamened flowers make bees and/or flies to fly from flower to flower in quick succession to collect pollen from the same or different conspecific plants and effect both self- and cross-pollination.

Hammer (1995) reported that different populations of Aizoaceae growing in the same habitat exhibit synchrony in flowering time. The period of flowering is usually short and the flowers show repeated opening but this phenomenon is restricted to a certain period of the day. Groen and Van Der Maesen (1999) observed that the mixed populations of

Aizoaceae genera, *Bergeranthus*, *Faucaria* and *Orthopterum* flower simultaneously. These authors suggested that such a synchrony in flowering in these genera in the same habitat collectively enable them to enhance their floral attraction to pollinators. In the present study, it is found that *Mollugo* species form mixed and distinct populations in the same and different habitats depending on soil moisture and nutrient conditions. These species exhibit synchrony in flowering by opening flowers during morning time. Further, the flowers are too small, lack corolla, tepals not vividly coloured and stay open for a brief period of three hours for visitation by insects. Therefore, the synchrony in anthesis schedule and massive floral display appear to be imperative for them to attract pollinators during the brief period of open state of flowers.

Peter *et al.* (2004) reported that the temperature and relative humidity are probably important cues determining flower opening in the afternoon. In the present study, the anthesis during morning time in *Mollugo* species is attributable to their predominance in open, dry habitats where herbaceous flora usually do not grow. With synchrony in anthesis schedule, these species provide sufficient forage but insect foragers collect forage only from *M. nudicaulis* and *M. pentaphylla*. Bees and lycaenid butterflies visit and pollinate both the plant species while flies additionally visit and pollinate *M. nudicaulis*. Bees and butterflies are generalists which visit a wide range of flowers and hence are polylectic. Since *Mollugo* species keep the flowers open only for a brief period, the polylectic foragers soon switch over to other plant species which provide forage in the nearby habitats. The morning anthesis in *Mollugo* species ensures insect pollination and reciprocate the insect pollinators with pollen and/or nectar. The total absence of insect foraging activity on *M. cerviana* could be attributable to its common occurrence in pollinator-excluded or deprived habitats and production of tiny flowers which can be overlooked or unnoticed by foragers.

Watson and Dallwitz (1992) stated that Molluginaceae members are entomophilous. These authors considered nectar secreting tissue and showy tepals in several species as adaptations for entomophily. Robertson (1928) reported that *Mollugo verticillata* is pollinated by syrphid fly, *Mesogramma marginata*. Pax and Hoffmann (1934) and Bogle (1970) stated that the showy sepals or petals evolved in several genera of Molluginaceae suggest entomophily. *Mollugo verticillata*, *M. cerviana* and *M. nudicaulis* are the most widely spread, weedy species and adapted for self- and insect-pollination. In the present study, it is found that in *Mollugo* species, the floral characters such as the erect position of flowers above foliage, adaxial surface of the tepals and nectar secreting tissue between the ovary base and connate part of staminal filaments appear to be adaptations for insect pollination. In *M. nudicaulis*, the bees while collecting pollen, and flies and butterflies while collecting nectar effect sternotribic pollination. Further, the bees while collecting nectar effect nototribic pollination. In *M. pentaphylla*, the bees while collecting pollen effect nototribic pollination. The bees and also butterflies while collecting nectar effect sternotribic pollination. In *M. nudicaulis* and *M. pentaphylla*, the pollen output per anther varies with the number of stamens present in the flowers; it increases with a decrease in the stamen number. The

pollen output per flower in *M. pentaphylla* is more than in *M. nudicaulis*. The variation in pollen production in these plant species is partly attributable to the number of stamens produced. The varying amount of pollen output in the flowers of the same and different inflorescences on the same plant drives the pollen collecting bees to visit the flowers across population(s) in search of more pollen and such a foraging activity contributes to both self- and cross-pollination. The nectar secreted in traces in both the species and nectar removal by thrips species, *Haplothrips* also drives the nectar collecting bees, flies and lycaenid butterflies to visit flowers across population(s) due to which both self- and cross-pollinations occur. *M. nudicaulis* and *M. pentaphylla* appear to be important sources of pollen for bees, especially for honey bees. Further, these plant species in the study area are important nectar sources for lycaenid butterflies. Among butterflies, lycaenids are the smallest, low-flying and appropriate pollinators for prostrate herbs, *M. nudicaulis* and *M. pentaphylla*.

Dipterans mostly take nectar with a preponderance of sucrose (Goldblatt *et al.* 1997). The nectar used by most flies is characteristically hexose-rich and of relatively high sugar concentration (Kevan and Baker 1999). The sugars in nectar may crystallize, but many generalist flies are able to re-liquify the nectar with saliva and then imbibe it (Willmer 2011). Both sexes of most flies use the carbohydrates in nectar for short-term energy needs (Downes and Smith 1969), especially during periods of peak activity such as swarming, mating and oviposition, dispersal, and migration (Willmer 2011). Several authors have indicated that nectar can contain amino acids and they have a nutritional role for flies (Rathman *et al.* 1990; Gardener and Gillman 2002; Vrzal *et al.* 2010). In the present study, nectar analysis was not done for *Mollugo* species but it is most likely that their flowers produce sucrose-rich nectar with high sugar concentration and the plants grow in arid areas. Since flies in general have the ability to re-liquify the nectar with their saliva and then imbibe it, they use the traces of nectar present in *M. nudicaulis* without any difficulty to meet their short-term energy needs. Therefore, the interaction between *M. nudicaulis* and flies is mutualistic, the former achieves pollination while the latter obtains the food.

Bhargava (1934) and Kshirsagar (1960) reported *in situ* pollen germination in *M. nudicaulis* and *M. pentaphylla*. Johri *et al.* (1992) noted that self-pollination seems to occur in these species as pollen tubes reached the ovules of ovaries in un-opened flowers and pollen grains with pollen tubes occur both inside the anther and on the stigma of the same flowers. But, these authors did not mention the time of the occurrence of these events in unopened flowers. In the present study, all the three *Mollugo* species show certain percentage of pollen germination only in the dehisced anthers and also the pollen tube formation on the stigma during anthesis process which occurs in individual flowers over a period of five to ten minutes. Such *in situ* pollen germination and the occurrence pollen tubes on the stigma during the process of anthesis facilitates self-induced autogamy to some extent. In *M. cerviana*, the close proximity of dehisced anthers of all five anthers to the stigmas facilitate the occurrence of spontaneous autogamy. In *M. nudicaulis* and *M. pentaphylla*, the close proximity of one dehisced anther in 3-stamened flowers and 2-3

dehisced anthers in 4- and 5-stamened flowers facilitate the occurrence of spontaneous autogamy. The minutely denticulate stigmas with membranous flaps in *M. cerviana* and densely papillose spreading stigmas in *M. nudicaulis* and *M. pentaphylla* capture pollen easily from the dehisced anthers to result in pollination. Further, in all the three *Mollugo* species, the thrips emerging from the floral buds during anthesis and their movements in the flowers after anthesis for pollen and nectar collection result in autogamy. They also bring about geitonogamy due to their migration to different inflorescences on the same plant for forage collection and xenogamy due to their migration to other conspecific plants for forage collection. In these plant species, the movement of tepals together with stamens towards the pistil during the flower closure facilitates contact between the sex organs and effects spontaneous autogamy if pollen is still available in the dehisced stamens. *In vitro* pollen germination test for *M. pentaphylla* indicated that the pollen is viable from the time of anther dehiscence and until three hours after flower closure suggesting that there is a possibility for the occurrence of spontaneous autogamy even after flower closure. Further, the tiny thrips have the possibility to carry pollen from other flowers, enter the closed flowers from the apical portion and laterally, and deposit the same on the stigmas effecting either geitonogamy or xenogamy. Therefore, all the three *Mollugo* species have specialized floral structural and functional behaviours for self-induced and spontaneous pollination while keeping the options open for vector-mediated pollination during the open state of flowers.

In the present study, all the three *Mollugo* species have three carpels with variation in ovule number per flower which is highest in *M. cerviana* and lowest in the other two *Mollugo* species. In *M. nudicaulis* and *M. pentaphylla*, the ovule number also varies depending on the number of stamens and pollen output per flower. This ovule production trend indicates that the pollen output increases with an increase in ovule number in order to provide sufficient pollen to fertilize as many ovules as possible. This situation is reflected in the natural fruit and seed set rates in both the plant species. The highest fruit and seed set rates and also the lowest pollen-ovule ratios recorded in *Mollugo* species now studied indicate that they are facultatively autogamous.

Bittrich (1990) reported that in Molluginaceae, *Adenogramma* is the only genus which produces one-seeded nutlets. All other genera produce capsules with many seeds which become exposed by loculicidal dehiscence. Soerjani *et al.* (1987) reported that *Mollugo pentaphylla* is hydrochorous. In the present study, the *Mollugo* species produce fruits within a week or slightly more than a week from the fertilized flowers. The fruit is a 3-valved broadly ellipsoid capsule which breaks open and exposes the seeds on clear sunny days; the seeds subsequently fall to the ground. On rainy days, water drops find their way into the fruits which are then filled with water. In effect, the fruits expel both water and seeds explosively. Further, wind disperses the dry cymes together with dry dehisced capsules to short distances and subsequently the seeds fall to the ground from the capsules. The seeds that reach the ground through these modes are further disseminated through surface water runoff during rain fall. Therefore, *Mollugo* species now studied exhibit anemochory.

ombrohydrochory and hydrochory.

Narayana (1962) and Hofmann (1973) noted that *Mollugo* species produce seeds with a primordium-like swelling on the funiculus and this structure is considered to be a vestigial aril. In the present study, it is found that *M. cerviana* produces tiny, brown, shiny, D-shaped seeds with faintly striate dorsal surface. The seed coat is studded with minute granular excrescences with reticulate ornamentation. *M. nudicaulis* and *M. pentaphylla* produce tiny, black, slightly shiny, reniform and concentrically ridged seeds. The seed coat is closely packed with uniformly distributed, pebble-like, lyrate and chipped areoles. Since the seeds of these plant species lack any aril or strophiole-like structure that usually serves as food for ants, the possibility for myrmecochory is ruled out. Wagner *et al.* (1999) noted that *Mollugo* species produce fruit capsules and inside seeds that lack means of external attachment for dispersal by animals. The present study is also in agreement with this report as all the three *Mollugo* species now studied do not have external structures that aid in the dispersal of seeds by animals. Therefore, seed dispersal by animals is totally ruled out.

Bittrich and Ihlenfeldt (1984) reported that *Mollugo* seeds germinate by means of an operculum. *M. cerviana* and *M. pentaphylla* propagate by seeds and reseed themselves, often forming colonies. The present study showed that *Mollugo* species produce several batches of populations in a year and their seeds germinate as soon as they are dispersed but their germination is related to soil moisture which plays an important role in breaking the seed coat. As therophytes, these species are best adapted to survive in open dry habitats as they take advantage of any sign of temporary humidity that allows them to complete their life cycle quickly. Jurado *et al.* (1991) reported that *M. cerviana* does not form dense cover that inhibits other vegetation and compete well in crowded conditions. The present study also indicates that all the three *Mollugo* species do not grow in shaded habitats or form dense populations that inhibit other vegetation but *M. nudicaulis* and *M. pentaphylla* share insect pollinators along with other simultaneously flowering herbaceous taxa in certain habitats.

Rama Das and Raghavendra (1973) noted that *Glinus lotoides* and *G. oppositifolius* are  $C_3$  species. Brockington *et al.* (2009) reported that *Mollugo cerviana* is the only known  $C_2$  species in the family Molluginaceae. Edwards and Walker (1983) noted that the genus *Mollugo* contains  $C_3$ ,  $C_4$  and  $C_3$ - $C_4$  species. Christin *et al.* (2010) reported that *M. cerviana* being a  $C_4$  species is distributed in hot arid regions of tropical and temperate latitudes. *M. nudicaulis* is a  $C_3$ - $C_4$  species while *M. pentaphylla* is a  $C_3$  species but both are distributed in tropical and subtropical regions of the world. Raghavendra *et al.* (1978) reported that *M. nudicaulis* produces some leaves with  $C_3$  characteristics and some other leaves with  $C_4$  characteristics according to their position on the stem. Sage *et al.* (1999) documented that  $C_3$ - $C_4$  photosynthesis is believed to be a relatively rare condition in plants and only a few dozen species have been identified so far, many of which belong to *Flaveria* (Asteraceae). The present study shows that *Glinus* species and *M. pentaphylla*



with  $C_3$  photosynthesis usually occur in dry habitats displaying the sparse growth of a few other prostrate or erect herbs and the presence of insect pollinators although they grow in cultivated lands that enable herbaceous flora, especially weeds and insect pollinators thrive well. Their occurrence in habitats with scanty or robust vegetation indicates that  $C_3$  photosynthesis does not facilitate them to grow in habitats without any vegetation or insect pollinators. On the contrary, *M. cerviana* with  $C_4$  photosynthesis grows only in dry habitats which are almost devoid of other vegetation and also devoid of pollinator fauna. This finding is in line with the statement by Lundgren *et al.* (2015) that  $C_4$  species are usually abundant in warm but not cool environments and this photosynthetic pathway is physiologically advantageous for their niche broadening in warm environments. Williams *et al.* (2013) explained the advantages of  $C_4$  photosynthesis.  $C_4$  plants grow faster than  $C_3$  plants, and they require less water. In a hot climate, however, a plant can lose a lot of water through the pores in its leaves: closing these pores allows the plant to retain water, but this also reduces the supply of carbon dioxide. Under these circumstances this causes problems because RuBisCO uses oxygen to break down RuBP, instead of creating sugars, when carbon dioxide is not readily available.  $C_4$  photosynthesis prevents wastage of a lot of energy and resources but it is more complex than  $C_3$  photosynthesis. In  $C_4$  plants, the mesophyll which is the region that is associated with the capture of carbon dioxide by RuBisCO in  $C_3$  plants, contains high levels of an alternative enzyme called PEPC that converts carbon dioxide molecules into an acid that contains four carbon atoms. To avoid carbon dioxide being captured by both enzymes,  $C_4$  plants evolved to relocate RuBisCO from the mesophyll to a second set of cells in an airtight structure known as the bundle sheath. The four-carbon acids produced by PEPC diffuse to the cells in the bundle sheath, where they are broken down into carbon dioxide molecules, and photosynthesis then proceeds as normal. This process allows photosynthesis to continue when the level of carbon dioxide in the leaves is low due to closure of pores to retain water in the plant. This specialized photosynthetic pathway allows *M. cerviana* to survive and build up its populations in warm and dry habitats or environments. *M. nudicaulis* with  $C_3$ - $C_4$  photosynthesis is versatile to flourish well both in dry habitats and cultivated areas with scanty and robust vegetation comprising of herbaceous flora that support insect pollinators. The  $C_3$ - $C_4$  photosynthetic pathway enables this species to grow in warm and cool habitats which in turn enables it to be widespread and abundant. Vogan *et al.* (2007) reported that of all  $C_3$ - $C_4$  intermediates, *M. nudicaulis* and *M. verticillata* are the most widespread and also abundant. These species are found in hot, ruderal habitats where competition is low and the potential for photorespiration is high. Their ability to survive in such habitats is likely due to their  $C_3$ - $C_4$  pathway. Their ecological success demonstrates that  $C_3$ - $C_4$  intermediacy is a successful photosynthetic pathway in its own right and not merely a transitional phase to  $C_4$  photosynthesis. Lundgren and Christin (2017) also reported that  $C_3$ - $C_4$  taxa are remarkably widespread across geographical and environmental space, maintaining their ability to exist in both typical  $C_3$  and  $C_4$  niches. Because, the physiology of  $C_3$ - $C_4$  species does not strongly restrict the migration of species geographically or into new environments and it is a lineage that converges towards warm habitats to facilitate the

transition to  $C_4$  photosynthesis, effectively bridging the ecological gap between  $C_3$  and  $C_4$  plants. *Glinus* and *M. pentaphylla* with  $C_3$  photosynthesis, *M. nudicaulis* with  $C_3$ - $C_4$  photosynthesis and *M. cerviana* with  $C_4$  photosynthesis have developed different pollination mechanisms to maximize fruit and seed set rate. Genetic variation achieved through insect pollination in all these species, except *M. cerviana*, is essential to broaden their ecological niches since they grow both in dry and moist habitats. In *M. cerviana*, genetic variation achieved through thrips pollination is important to expand and invade dry habitats.

Different authors reported that *Glinus lotoides* and *G. oppositifolius* are commonly used as vegetables. Further, these species and all three *Mollugo* species now studied are used in traditional medicine and in recent times used in Ayurvedic system of medicine to treat different diseases and ailments. In Ethiopia and Tanzania, *G. lotoides* is given threatened status due to its regular harvest for medicinal purpose. *M. cerviana* is widely used as a pot herb in India and its extract is useful as inhibitor of corrosion of mild steel (Hamed *et al.* 1996; Parvathamma and Shanthamma 2000; Sastri 2002; Rajamanikandan *et al.* 2011; Dutta *et al.* 2012; Sahu *et al.* 2012; Arockiasamy *et al.* 2014; Bhavani 2015; Teshome and Feyissa 2015). These various uses indicate that *Glinus* and *Mollugo* species can be promoted as vegetables or medicine. *M. cerviana* can be commercially promoted as a pot herb. Further studies on the steel corrosion inhibition properties of this plant may provide more concrete information for its potentiality as inhibitor of steel corrosion. Except *M. cerviana*, all other plant species are useful as important nectar sources for almost throughout the year for the low flying lycaenid butterflies. It appears that there is a close relationship between these plants and lycaenids, and both benefit from this relationship. Bees also use these plants as a major source of pollen and a minor source of nectar. These plants in general form important forage sources during dry season. Therefore, all these plant species are ecologically and medicinally useful. Further, they are important in soil erosion control and in the initiation of eco-restoration process in dry areas where there is no vegetation and in areas where soil usually contains very little moisture.

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## Floral biology and pollination of carpet weeds, *Glinus lotoides* L. and *Glinus oppositifolius* (L.) Aug. DC. (Molluginaceae)

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### Resumen

*Biología floral y polinización de las malas hierbas Glinus lotoides L. y Glinus oppositifolius (L.) Aug. DC.*

*Glinus lotoides* y *G. oppositifolius* son hierbas rastreras, esparcidas, anuales. En ambas, las flores son muy pequeñas, actinomorfas, bisexuales, protandradas, herkogamosas y facultativamente autógamas. Ambas especies exhiben anthesis sincrónica y flores masivas para atraer a los polinizadores. Las abejas, hormigas, mariposas y trips polinizan las flores. La hercogamia excluye la autogamia espontánea, pero el evento de cierre de la flor facilita esta autogamia. En ambas especies, el fruto es una cápsula con semillas escrotiformes con estrofiolo; anemocoria, ombrohidrocoria e hidrocoria son funcionales. Las semillas germinan inmediatamente si el suelo tiene humedad. Las especies *Glinus* con fotosíntesis C3, autogamia facultativa y polifilia son capaces de crecer en hábitats secos y húmedos.

**Palabras clave:** Autogamia facultativa, Polinización por insectos, Anemocoria, Ombrohidrocoria, Hidrocoria.

### Abstract

*Glinus lotoides* and *G. oppositifolius* are prostrate, spreading, annual herbs. In both, the flowers are very small, actinomorphic, bisexual, protandrous, herkogamous and facultatively autogamous. Both species exhibit synchronous anthesis and display flowers en masse to attract pollinators. Bees, ants, butterflies and thrips pollinate the flowers. Hercogamy precludes spontaneous autogamy but flower closing event facilitates autogamy. In both species, the fruit is a capsule with scrotiform strophioled seeds; anemochory, ombrohydrochory and hydrochory are functional. The seeds germinate immediately if soil has moisture. *Glinus* species with C3 photosynthesis, facultative autogamy and polyphily are able to grow in dry and moist habitats.

**Key words:** Facultative autogamy, Insect pollination, Anemochory, Ombrohydrochory, Hydrochory.

## Introduction

Little is known about the pollination ecology of Molluginaceae. In this family, nectar secreting tissue is present in almost all species. In several genera, showy sepals or petals have evolved, both of which strongly suggest entomophily (Watson & Dallwitz 1992; Kubitzki *et al.* 1993). There is some information on pollination ecology of *Mollugo* L. species but not on *Glinus* L. genus (Robertson 1928; Pax & Hoffmann 1934; Bogle 1970; Lin *et al.* 1993; Ponnuchamy *et al.* 2014). Hence, the present study was contemplated to provide details of pollination biology of *Glinus lotoides* L. and *Glinus oppositifolius* (L.) which usually grow in open habitats with little moisture and agricultural areas. Sastri (1956) reported that *G. lotoides* is widespread throughout the tropics and subtropics, especially in Africa, Asia, Australia and South Europe. Teshome & Feyissa (2015) reported that *G. lotoides* is currently given threatened status due to its regular harvest for medicinal purpose in Ethiopia and Tanzania. Sahu *et al.* (2001) reported that *G. oppositifolius* is widely distributed in the Americas, tropical Asia, tropical Africa and Australia. The objective of the present study is to provide details of phenology and floral biology of *G. lotoides* and *G. oppositifolius* to understand their reproductive aspects.

## Materials and methods

Wild patches of *G. lotoides* and *G. oppositifolius* growing in open habitats of Visakhapatnam and its surroundings in Andhra Pradesh, India (17°42'N, 82°18'E) were selected for study between March 2015 and May 2017. Field trips were conducted to record phenological aspects. Ten inflorescences which were not initiated flowering on five plants were tagged and followed to record anthesis schedule and the timing of anther dehiscence. Twenty five fresh flowers were used to record the floral morphological characters such as petals, stamens, ovules and relative positions of sex organs. Nectar could not be measured and analyzed due to its secretion in minute quantity which was further depleted by thrips during mature bud and flower life. Twenty mature, but undehiscent anthers, (two anthers each per flower/plant from ten plants) were collected and examined for pollen output as per the protocol described in Dafni *et al.* (2005). The calculation of

pollen output per flower and pollen-ovule ratio was done as per the formulas described in Cruden (1977). Ten flowers each from five individuals were used to test stigma receptivity. It was tested with hydrogen peroxide from mature bud stage to flower closure/drop as per Dafni *et al.* (2005). Seventy inflorescences were tagged prior to the initiation of their flowering and followed for three weeks to record fruit and seed set rate in open-pollinations. The fruit and seed morphological characteristics were observed in detail to evaluate their adaptations for dispersal by different means. Fields visits were made during rainy season to note the aspects of seed germination and production of new plants. Anther dehiscence schedule and stigma receptivity period, and herkogamous nature of stamens and stigmas were used to infer the sexual system. Further, how the sexual system facilitates both self- and cross-pollination was explained. Further, observations were also made to evaluate as to how these positions preclude self-pollination when flowers stay open.

Insects foraging at the flowers were observed from morning to evening on four different days for their mode of approach, landing, probing behavior and contact with the floral sexual organs. Bees and ants were identified with the representative specimens available with the Department of Environmental Sciences, Andhra University, Visakhapatnam. Butterflies were identified by consulting the books of Kunte (2007) and Gunathilagaraj *et al.* (1998). For each species, an area of 1 x 1 m of flowering individuals of a single species consisting of approximately 450 flowers was used for foraging activity. The foraging visits of insects were recorded for 10 min at each hour for the entire day and the data was tabulated to record the foraging pattern and the percentage of visits made by different insect categories. The pollen/nectar collection behavior of insects was carefully observed to assess their role in effecting pollination. Ten specimens of each insect species were captured between 1400-1600 h and taken to the laboratory. Each specimen was washed in the drop of ethyl alcohol on a glass slide to separate pollen, then it was stained with aniline-blue and observed under microscope to count the number of pollen grains present. From this, the average number of pollen grains carried by each insect species was calculated to know the pollen carry-over efficiency.

Fruit set rate was calculated by dividing the

total number of fruits set with the total number of flowers sampled and then value obtained was expressed in percentage by multiplying it with one hundred for each flower type separately for both *G. lotoides* and *G. oppositifolius*. The sample size used for 8-12-stamened flowers in *G. lotoides* and for 4-6-stamened flowers in *G. oppositifolius* was provided in Table 4. Seed set rate was calculated by dividing the total number of seeds set with the average number of ovules per flower multiplied by the total number of fruits set and then the value obtained was expressed in percentage by multiplying it with one hundred for each flower type separately for both the species.

## Results

### The Plant

*G. lotoides* and *G. oppositifolius* are low-growing prostrate, spreading, annual herbs which grow in

open sandy soils, cultivated fields and open waste lands. In soils with enough moisture, they produce well developed tap root and survive throughout the year producing flowers and fruits simultaneously or alternately. In both the species, the stem is soft, succulent, pubescent and much-branched carpeting the soil with its foliage. In *G. lotoides*, the basal leaves are borne in a rosette form while the upper ones in verticillate form or rarely arranged opposite, and densely stellate tomentose. In *G. oppositifolius*, the leaves are arranged in pseudo-whorls of 3-6 or opposite to each other.

### Flowering

In *G. lotoides* and *G. oppositifolius*, the flowering is profuse when soil is very damp which occurs during July-October due to monsoonal rains (Figs. 1a, 1f). In *G. lotoides*, the flowers are borne on 1.5 mm long stalks in axillary cymes and each cyme consists of  $4.72 \pm 1.3$  (mean  $\pm$  SD) flowers and each plant produces  $82.5 \pm 33.65$  flowers. In



**Figura 1. a-e** *Glinus lotoides*: a. Fase de floración; b. Flor completamente abierta; c. Estambres y estigma; d. Cierre de flor a las 1800 h del día de la antesis; e. Capsula del fruto con estambres y estigma intactos tras marchitarse. **f-j** *Glinus oppositifolius*: f. Fase de floración; g. Flor; h. Estambres dehiscientes; i. Granos de polen; j. Ovario con tres estigmas. **k-o** *Glinus lotoides*: k. *Haplothrips* sp. alimentándose de néctar; l. *Apis cerana* recogiendo polen; m. *Apis cerana* recogiendo néctar; n. *Camponotus* sp. recogiendo néctar; o. *Lycenid*, *Zizeeria karsandra*, recogiendo néctar.

**Figure 1. a-e** *Glinus lotoides*: a. Flowering phase; b. Fully open flower; c. Stamens and stigma; d. Flower closure at 1800 h on the day of anthesis; e. Fruit capsule with stamens and stigma intact in withered state. **f-j** *Glinus oppositifolius*: f. Flowering phase; g. Flower; h. Dehiscing stamens; i. Pollen grains; j. Ovary with three stigmas. **k-o** *Glinus lotoides*: k. *Haplothrips* sp. feeding on nectar; l. *Apis cerana* collecting pollen; m. *Apis cerana* collecting nectar; n. *Camponotus* sp. collecting nectar; o. *Lycenid* butterfly, *Zizeeria karsandra* collecting nectar.

*G. oppositifolius*, the flowers are borne on 3-7 mm long pedicels in axillary fascicles and each fascicle consists of  $7.5 \pm 1.5$  flowers and each plant produces  $64.52 \pm 41.28$  flowers.

### Flower morphology

In both the species, the flowers are small, odourless, actinomorphic and bisexual (Figs. 1b, 1g). They are  $6.1 \pm 1.28$  mm long,  $7.42 \pm 1.25$  mm wide in *G. lotoides* and  $3.51 \pm 0.5$  mm long,  $8.57 \pm 0.7$  mm wide in *G. oppositifolius*. In both the species, the calyx and corolla are represented by perianth with 5 or rarely 6 tepals. The tepals are succulent, free and arranged in quincuncial aestivation in both the species but they are  $6.02 \pm 0.19$  mm long,  $4.05 \pm 0.2$  mm wide, whitish green adaxially and green abaxially, ovate-oblong and covered with stellate hairs in *G. lotoides* while they are  $4.01 \pm 0.2$  mm long,  $2.03 \pm 0.2$  mm wide, creamy white adaxially and brownish orange abaxially, ovate-oblong and pubescent in *G. oppositifolius*. The stamens are 8 to 12, free, white and arranged in two whorls (Fig. 1c). The stamens of inner whorl close to ovary base are long and form a short tube at the base around the ovary while those of the outer whorl are short. In *G. lotoides*, the stamens of both whorls are usually anti-tepalous and occasionally alterni-tepalous. The flowers with 8-stamens constitute 14.28%, those with 9-stamens 21.42%, those with 10-stamens 42.85%, 11-stamens 19.04% and 12-stamens 2.38%. In *G. oppositifolius*, the stamens are 4 to 6, free but connate at the base, and alterni-tepalous. The flowers with 4-stamens constitute 5%, those with 5-stamens 80% and those with 6-stamens 15%. In both the species, the anthers are H-shaped, white, dithecous and versatile (Fig. 1h). In *G. lotoides*, the staminodes are 5-7, petaloid, white, bifid and usually alterni-tepalous but occasionally in between short stamens; they extend be-

yond the height of long stamens. In *G. oppositifolius*, the staminodes are 5 or 6, petaloid, white, bifid and anti-tepalous. In *G. lotoides*, the ovary ( $5 \pm 0.23$  mm long and  $2.03 \pm 0.17$  mm wide) is green, pentacarpellary and pentalocular syncarpous while in *G. oppositifolius*, the ovary ( $4.07 \pm 0.16$  mm long and  $2 \pm 0.01$  mm wide) is green, tri-carpellary and tri-locular syncarpous. In both the species, the ovules in each locule are arranged on axile placentation. The ovule number varied from  $181.9 \pm 35.28$  to  $242.4 \pm 35.14$  in 8- and 12-stamened flowers in *G. lotoides* and from  $115.5 \pm 7.1$  to  $137.7 \pm 9.9$  in 4- and 6-stamened flowers in *G. oppositifolius* (Table 1). In both the species, the ovule production trend showed that the number of ovules produced gradually increased with a gradual increase in the number of stamens per anther and pollen out per flower but the variation is not significant. The style is absent in both the species but *G. lotoides* has five greenish white stigmas while *G. oppositifolius* has three creamy white stigmas (Fig. 1j). In both the species, the stigmas are free, spreading, papillate, wet and shiny.

### Floral biology

Mature buds open during 1400-1500 h in *G. lotoides* and during 1200-1400 h in *G. oppositifolius*. Individual buds open gradually over a duration of 30 to 40 minutes in *G. lotoides* and 10 to 15 minutes in *G. oppositifolius*. In both species, the anthers dehisce by longitudinal slits during anthesis. In *G. lotoides*, the pollen output per anther varied from  $1,193.85 \pm 70.25$  to  $1,371.85 \pm 65.76$  and from  $10,974.85 \pm 526.08$  to  $14,326.28 \pm 843.03$  per flower in 8- to 12-stamened flowers respectively (Table 1). In *G. oppositifolius*, the pollen output per anther varied from  $1,151 \pm 67.22$  to  $957.33 \pm 49.1$  and from  $4,604 \pm 268.9$  to  $5,744 \pm 295.1$  per flower in 4 to 6-stamened flowers (Table 1). In both the species, the pollen pro-

Flower type	Percentage of occurrence	Mean $\pm$ SD pollen output/anther	Mean $\pm$ SD pollen output/flower	Mean $\pm$ SD No. ovules/flower	Pollen: ovule ratio
<b><i>Gliricidia lotoides</i></b>					
8-stamened	14.20	1371.85 $\pm$ 65.76	10974.85 $\pm$ 526.08	181.9 $\pm$ 35.28	60:1
9-stamened	21.42	1331.85 $\pm$ 91.46	11986.71 $\pm$ 823.21	196.7 $\pm$ 35.78	61:1
10-stamened	42.85	1304.57 $\pm$ 62.65	13045.71 $\pm$ 626.57	221.8 $\pm$ 40.67	59:1
11-stamened	19.04	1221.85 $\pm$ 61.36	13440.42 $\pm$ 674.99	230.2 $\pm$ 34.23	58:1
12-stamened	2.38	1193.85 $\pm$ 70.25	14326.28 $\pm$ 843.03	242.4 $\pm$ 35.14	59:1
<b><i>Gliricidia oppositifolius</i></b>					
4-stamened	5	1151 $\pm$ 67.2	4604 $\pm$ 268.9	115.5 $\pm$ 7.1	40:1
5-stamened	80	1027 $\pm$ 55.5	5133 $\pm$ 277.5	128.6 $\pm$ 7.7	40:1
6-stamened	15	957 $\pm$ 49.1	5744 $\pm$ 295.1	137.7 $\pm$ 9.9	41.9:1

Table 1. Aspectos del polen y óvulos de *Gliricidia lotoides* y *Gliricidia oppositifolius*

Table 1. Pollen and ovule aspects in *Gliricidia lotoides* and *Gliricidia oppositifolius*.

duction trend showed that the pollen output rate gradually increased with a gradual decrease in the number of stamens produced per flower. In *G. lotoides*, the pollen-ovule ratio varied from 58:1 to 61:1 in 8- to 12-stamened flowers respectively. In *G. oppositifolius*, the pollen-ovule ratio is 40:1 and it is constant despite variation in the number of stamens and ovules. In both the species, the pollen grains are white, spheroidal, tricolporate, tri-zonoaperturate, colpal membrane densely granulated, and tectum with scabrate ornamentation (Fig. 1i) but the grain size is  $32.52 \pm 4.28 \mu\text{m}$  in *G. lotoides* and  $29.34 \pm 4.26 \mu\text{m}$  in *G. oppositifolius*. In both the species, the stigma is receptive from anthesis onwards and remains receptive until the noon of the following day. Further, the nectar is secreted in traces during mature bud stage. The tepals together with the staminodes, stamens and stigma close back completely by 1800 h on the same day (Fig. 1d) and remain in place in fertilized flowers until fruit dispersal. But, the unfertilized flowers fall off after 7-10 days in *G. lotoides* and after 6-8 days in *G. oppositifolius*.

In *G. lotoides*, the fully open flowers show different positions of the stamens and the stigmas. The stigmas are situated beyond the height of short stamens but below the height of long stamens. In *G. oppositifolius*, the stamens, staminodes and stigmas are situated at the same height. But, in both the species, the sex organs are spatially separated and precludes spontaneous autogamy during open state of the flower. However, during the closure of the flower, the closely spaced dehiscent anthers of long stamens contact the stigmas facilitating spontaneous autogamy but its occurrence is dependent on the availability of pollen in the anthers.

### Foraging activity of insects

In *G. lotoides* and *G. oppositifolius*, thrips species, *Haplothrips* sp. (Amyot & Serville, 1843) (Thysanoptera: Thripidae) ovi-positing during early stage of floral bud (Fig. 1k). The larvae emerged from the eggs in synchrony with anthesis and nectar production in flowers. The larvae and adults foraged for pollen and nectar. Individual thrips were dusted with pollen during their movements within the flowers and carried 428 to 635 pollen grains of *G. lotoides* and 157 to 253 pollen grains of *G. oppositifolius* on their body setae, wings and legs. In both the species, the flowers were foraged between 1300/1400-1700 h with

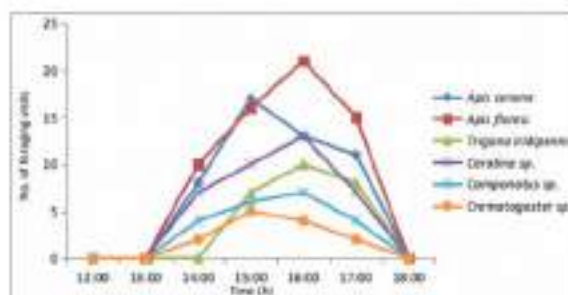


Figura 3. Actividad horaria de recolección de abejas y hormigas en *Glinus lotoides*.

Figure 3. Hourly foraging activity of bees and ants on *Glinus lotoides*.

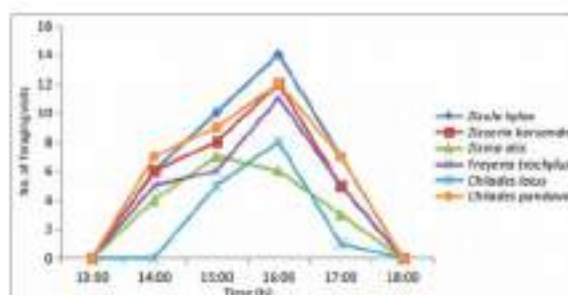


Figura 4. Actividad horaria de recolección de licénidos en *Glinus lotoides*.

Figure 4. Hourly foraging activity of lycaenid butterflies on *G. lotoides*.

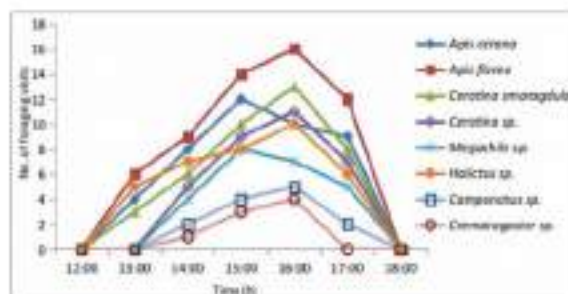


Figura 5. Actividad horaria de recolección de abejas y hormigas en *Glinus oppositifolius*.

Figure 5. Hourly foraging activity of bees and ants on *G. oppositifolius*.

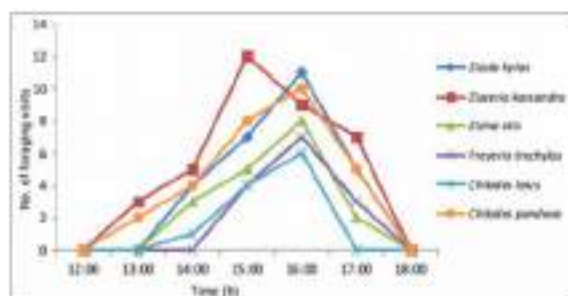


Figura 6. Actividad horaria de recolección de licénidos en *Glinus oppositifolius*.

Figure 6. Hourly foraging activity of lycaenid butterflies on *G. oppositifolius*.

concentrated foraging activity between 1500-1600 h (Figs. 2-5). The foragers included bees which visited for pollen and nectar, ants and butterflies which visited for only nectar.

On *G. lotoides*, the bee foragers were *Apis cerana* (Fabricius, 1793) (Figs. 11, 1m), *Apis florea* (Fabricius, 1793), *Trigona iridipennis* (Smith, 1854) and *Ceratina* sp. (Latreille, 1802). The ant foragers included *Camponotus* sp. (Mayr, 1861) (Fig. 1n) and *Crematogaster* sp. (Lund, 1831). The butterfly foragers included only lycaenids, namely, *Zizula hylax* (Fabricius, 1775), *Zizeeria karsandra* (Moore, 1865) (Fig. 1o), *Zizina otis* (Fabricius, 1787), *Freyeria trochylus* (Forster, 1980), *Chilades latus* (Stoll, 1780) and *Chilades pandava* (Horsfield, 1829) (Table 2). On *G. oppositifolius*, the bee foragers were *A. cerana*, *A. florea* (Figs. 6a, 6b), *Ceratina* sp. (Figs. 6c, 6d), *Ceratina smaragdula* (Bingham 1897) (Fig. 6e), *Megachile* sp. (Cockerell, 1897) and *Megachile* sp. (Latreille, 1802) (Fig. 6f). The ant foragers included *Camponotus* sp. and *Crematogaster* sp. The butterfly foragers included only lycaenids, namely, *Z. hylax* (Fig. 6g), *Z. karsandra* (Figs. 6h, 6i), *Z. otis* (Fig. 6j), *F. trochylus*, *C. latus* and *C. pandava* (Table 2). In both the species, these insects approached the flowers in upright position, landed on the tepals and then probed for forage collection. Bees first accessed anthers to collect pollen and then moved to the flower base to collect nectar, if available in the same and/or differ-

ent visits. Ants were resident foragers and continuously crawled all over the plant and accessed the floral base to collect nectar. Butterflies stretched out their proboscis and inserted into the flower base to collect nectar. All insect species collected forage from several flowers of different cymes of the same or different plants to collect the forage. The bees during pollen collection brushed against the stigmas with their ventral surface effecting sternotribic pollination. Further, the bees and also ants during nectar collection brushed against anthers and stigmas with their dorsal surface effecting nototribic pollination. The butterflies during nectar collection contacted the stamens and stigmas with their proboscis and front side of head and ventral surface of thorax and abdomen effecting sternotribic pollination. Their wings never contacted the stamens and stigma during nectar collection as they kept them in vertical position. On *G. lotoides* flowers, bees made 47%, ants 9% and lycaenids 44% of total foraging visits. On *G. oppositifolius* flowers, bees made 60%, ants 5% and lycaenids 35% of total foraging visits. The body washings of insects collected from the flowers of both *G. lotoides* and *G. oppositifolius* during peak foraging period revealed that all insects carry pollen but bees carry the highest number of pollen grains. Further, the mean number of pollen grains varied with each insect species (Table 3). The nectar secretion in traces and its depletion by thrips during and after anthesis in both the plant



Figure 6. a-f. *Gliricidia oppositifolius*. a: *Apis florea* recogiendo polen; b: *Apis florea* recogiendo néctar; c: *Ceratina* sp. recogiendo polen; d: *Ceratina* sp. recogiendo néctar; e: *Ceratina smaragdula* recogiendo néctar; f: *Megachile* sp. recogiendo néctar; g-j Lycaenidos. g: *Zizula hylax*; h: *Zizeeria karsandra*; i: *Zizeeria karsandra* enestado de cópula; j: *Zizina otis*; k: Semillas de *Gliricidia lotoides*; l: Semillas de *Gliricidia oppositifolius*.

Figure 6. a-f. *Gliricidia oppositifolius*. a: *Apis florea* collecting pollen; b: *Apis florea* collecting nectar; c: *Ceratina* sp. collecting pollen; d: *Ceratina* sp. collecting nectar; e: *Ceratina smaragdula* collecting nectar; f: *Megachile* sp. collecting nectar; g-j Lycaenid butterflies. g: *Zizula hylax*; h: *Zizeeria karsandra*; i: *Zizeeria karsandra* in mating state; j: *Zizina otis*; k: *Gliricidia lotoides* seeds; l: *Gliricidia oppositifolius* seeds.

Order/Family	Insect species	<i>Glinus lotoides</i>	<i>Glinus oppositifolius</i>	Forage sought
<b>Hymenoptera</b>				
Apidae	<i>Apis cerana</i> F.	+	+	Pollen + Nectar
	<i>Apis florea</i> F.	+	+	Pollen + Nectar
	<i>Trigona indipennis</i> Smith	+	-	Pollen + Nectar
	<i>Ceratina smaragdula</i> F.	-	+	Pollen + Nectar
	<i>Ceratina</i> sp.	+	+	Pollen + Nectar
Halictidae	<i>Halictus</i> sp.	-	+	Pollen + Nectar
Megachilidae	<i>Megachile</i> sp.	-	+	Pollen + Nectar
Formicidae	<i>Camponotus</i> sp.	+	+	Nectar
	<i>Crematogaster</i> sp.	+	+	Nectar
<b>Thysanoptera</b>				
Thripidae	<i>Haplothrips</i> sp.	+	+	Pollen + Nectar
<b>Lepidoptera</b>				
Lycaenidae	<i>Zizula hylax</i> F.	+	+	Nectar
	<i>Zizeeria karsandra</i> Moore	+	+	Nectar
	<i>Zizina otis</i> F.	+	+	Nectar
	<i>Freyeria trochylus</i> Freyer	+	+	Nectar
	<i>Chilades laius</i> Stoll	+	+	Nectar
	<i>Chilades pandava</i> Horsfield	+	+	Nectar

**Tabla 2.** Lista de insectos polinizadores de *Glinus lotoides* y *Glinus oppositifolius*.

**Table 2.** List of insect pollinators on *Glinus lotoides* and *Glinus oppositifolius*.

Insect species	Sample size (N)	<i>Glinus lotoides</i>			<i>Glinus oppositifolius</i>		
		Number of pollen grains			Number of pollen grains		
		Range	Mean	S.D.	Range	Mean	S.D.
<i>Apis cerana</i>	10	92-307	208.2	55.45	87-236	161.5	46.6
<i>Apis florea</i>	10	78-252	167.1	56.83	66-214	140.5	42.9
<i>Trigona indipennis</i>	10	43-214	129.2	46.26	--	--	--
<i>Ceratina smaragdula</i>	10	--	--	--	68-164	115.9	24.8
<i>Ceratina</i> sp.	10	35-94	64.1	17.44	41-117	84.2	21.6
<i>Halictus</i> sp.	10	--	--	--	27-63	44.1	9.1
<i>Megachile</i> sp.	10	--	--	--	43-102	74.8	16.1
<i>Camponotus</i> sp.	10	27-58	39.4	8.59	22-45	31.3	7.4
<i>Crematogaster</i> sp.	10	23-46	34.5	6.43	13-34	25.1	6.4
<i>Zizula hylax</i>	10	8-31	21.4	6.29	11-38	26.8	7.2
<i>Zizeeria karsandra</i>	10	15-40	27.8	7.40	9-46	28.8	9.2
<i>Zizina otis</i>	10	11-29	20.8	5.24	14-30	23.1	5.1
<i>Freyeria trochylus</i>	10	10-38	25.4	7.4	7-29	19.3	6.1
<i>Chilades laius</i>	10	12-34	23.3	6.37	10-35	24.8	7.0
<i>Chilades pandava</i>	10	10-42	28.4	8.27	12-41	28.1	7.2

**Tabla 3.** Polen registrado en lavados de cuerpo de insectos obtenidos en *Glinus lotoides* y *Glinus oppositifolius*.

**Table 3.** Pollen recorded in the body washings of insects on *Glinus lotoides* and *Glinus oppositifolius*.

species appeared to be driving the insects to visit as many flowering cymes as possible to quench their thirst for nectar. Such a foraging behavior was considered to be facilitating the promotion of cross-pollination.

### Fruiting ecology and seed dispersal

The pollinated and fertilized flowers grow continually and produce fruits within 8-12 days in *G. lotoides* and 7-10 days in *G. oppositifolius*. In both the species, the stamens and stigmas are persistent and remain inside due to the closure of the flower (Fig. 1e). The tepals bulge gradually and protect the bulging ovary in which the seeds form and mature. In *G. lotoides*, natural fruit set rate varied from 88% to 92% while seed set rate varied from

85% to 93% in 8- to 12-stamened flowers respectively (Table 4). In *G. oppositifolius*, natural fruit set rate varied from 88% to 92% while seed set rate varied from 88% to 91% in 4- to 6-stamened flowers respectively (Table 4). Fruit is a loculicidal 5-valved capsule,  $6.05 \pm 0.75$  mm long and  $4.1 \pm 0.64$  mm wide in *G. lotoides* while it is an ellipsoid loculicidal 3-valved capsule,  $5.5 \pm 0.5$  mm long and  $2.1 \pm 0.2$  mm wide in *G. oppositifolius*. In both the species, the fruit is stalked, membranous and densely pubescent. In *G. lotoides*, the seeds are small, reniform, smooth, 0.7 mm long and 0.5 mm wide, initially red and finally dark brown and have a white aril of funicular origin developed into elongate filiform strophiole (Fig. 6k). In *G. oppositifolius*, the seeds are



Flower type	Number of flowers sampled	Number of flowers set fruit	Fruit set (%)	Seed set (%)
<b><i>Glinus lotoides</i></b>				
8-stamened	50	45	90	85
9-stamened	60	53	88	87
10-stamened	110	98	89	90
11-stamened	45	41	91	92
12-stamened	25	23	92	93
<b><i>Glinus oppositifolius</i></b>				
4-stamened	61	54	88	88
5-stamened	225	206	91	90
6-stamened	105	97	92	91

**Tabla 4.** Proporción entre los conjuntos de frutos y semillas de *Glinus lotoides* y *Glinus oppositifolius*.

**Table 4.** Natural fruit and seed set rate in *Glinus lotoides* and *Glinus oppositifolius*.

small, sub-reniform, granulose, 0.9 mm long and 0.7 mm wide, reddish-brown and have a white aril of funicular origin developed into extensively curved scrotiform strophiole (Fig. 6I). In both the plant species, the seeds are arranged in two rows in each locule. Dry capsules of both the species break open when tepals are wet and expose the seeds. But the seeds remain so and gradually separate and fall to the ground on their own on clear sunny days. In dry season, when the capsules are ripe, the plants die, dry out and become brittle. In this state, the base of the stem breaks off and it is more so when high winds prevail. Then, the plant parts roll readily and fruit and seeds disperse to other areas. On rainy days, drops of water falling on the distal opening after the locules are filled with rain water result in an explosive expulsion of water droplets and seeds. Further, water also disperses seeds fallen on the soil during rainy season. Therefore, seed dispersal is characteristically anemochorous, ombrohydrochorous and hydrochorous in both *G. lotoides* and *G. oppositifolius*.

## Discussion

*G. lotoides* and *G. oppositifolius* are prostrate, spreading, annual herbs that carpet the ground in open sandy soils and agricultural lands. They occur throughout the year and show vegetative, flowering and fruiting phases in different areas. But, their robust growth, profuse flowering and fruiting is confined to wet season. The flowers borne in axillary cymes in *G. lotoides* and axillary fascicles in *G. oppositifolius* stand erect above the foliage and display their prominence. In both the species, the stems produce many branches and each branch produces several cymes or fascicles. Since the plants usually grow as green carpets, the simultaneous display of several flowers from individual plants and from the entire population(s) could enhance their attraction to insect pollinators.

Ronse De Craene (2010) stated that *Glinus* species have five sepals in quincuncial aestivation. In the present study, *G. lotoides* and *G. oppositifolius* have been found to have five tepals as common and six tepals as rare. The study also indicates that the word "tepal" is the appropriate word since it acts as petal adaxially and sepal abaxially. This is further substantiated by two different colours displayed on adaxial and abaxial surface. The tepals are whitish green on adaxial surface and green abaxial surface in *G. lotoides* and creamy white on adaxial surface and brownish orange on abaxial surface in *G. oppositifolius*.

In *Glinus* species, the stamen number is unstable and varies from five to several series which include outer staminodes also (Hoffman 1994). The staminodes are petaloid, represent the stamens that lost their function as producers of fertile pollen and antisepalous replacing a fertile stamen or alternisepalous as an appendage of upper stamen (Weberling 1989; Brockington *et al.* 2013). Ronse De Craene (2010) reported that in *G. lotoides* the alternisepalous whorl is complete with staminodes and odd stamen is opposite to petals. Sharma (1963) mentioned that *G. lotoides* flowers produce rarely more than five stamens while *G. oppositifolius* produce 10-13 stamens in three whorls. He also suggested a tendency for reduction with the loss of the outer stamen whorl in androecium. In the present study, *G. lotoides* flowers had 8-12 antitepalous functional stamens in two whorls and 5-7 alternitepalous staminodes in outer whorl. *G. oppositifolius* flowers had 4-6 alternitepalous functional stamens in one whorl and 5-6 antitepalous staminodes in outer whorl. In both the species, the staminodes are petaloids indicating the fusion of two adjacent stamens that lost the function of producing viable pollen in course of the evolution of flowers. These staminodes are integral features of floral morphology and appear to have evolved to serve as attractants

to pollinators, reduce self-pollination rate and optimize the available nutrients for enhanced reproductive output in water and nutrient deficient habitats. Further, the production of staminodes appears to be an adaptation to reduce pollen production per flower, increase efficiency of pollen dispersal by limiting pollen removal by individual pollinators and enable precise contact between pollinators and pollen presenters or pollinators and stigmas. Therefore, the petaloid staminodes are evolved to perform different roles in the flowers and are unique for *Glinus* within Molluginaceae (Stebbins 1974; Ronse De Craene & Smets 1993, 1995; Ronse De Craene 2013).

Hammer (1995) reported that different populations of Aizoaceae growing in the same habitat exhibit synchrony in flowering time. The period of flowering is usually short and the flowers show repeated opening but this phenomenon is restricted to a certain period of the day. Groen & Van Der Maesen (1999) observed that the mixed populations of Aizoaceae genera, *Bergeranthus* Schwantes, *Faucaria* Schwantes and *Orthopterum* L. Bolus flower simultaneously. These authors suggested that such a synchrony in flowering in these genera in the same habitat collectively enable them to enhance their floral attraction to pollinators. In the present study, it is found that *Glinus* species form mixed and distinct populations in the same and different habitats depending on soil moisture and nutrient conditions. These species exhibit synchrony in flowering by opening flowers in the afternoon. Further, the flowers are too small, lack corolla, tepals not vividly coloured and stay open for a brief period, for three hours in *G. lotoides* and four hours in *G. oppositifolius* for visitation by insects. Therefore, the synchrony in anthesis schedule and massive floral display appear to be imperative for them to attract pollinators during the brief period of open state of flowers.

Peter *et al.* (2004) reported that the temperature and relative humidity are probably important cues determining flower opening in the afternoon. The specific timing of anthesis in the late afternoon is a likely mechanism to filter out generalist pollinators most active at midday, rather targeting specific group of insects, primarily bees, still active in the late afternoon. The present study indicates that afternoon anthesis in *Glinus* species is probably evolved in course of time to avoid competition for pollinators in a pollinator-

deprived environment, especially in habitats where other herbaceous plant species flower simultaneously, show anthesis during forenoon period and attract insect pollinators with their vivid floral colors. *Glinus* species provide sufficient forage for insect pollinators in the afternoon period and accordingly bees, ants and lycaenid butterflies collect forage and pollinate flowers. Since bees and butterflies are active throughout day, they soon switch to fresh forage available in the habitat. *Glinus* species with afternoon anthesis readily provide forage, and bees and butterflies begin to shift to these floral sources and concentrate on forage collection from them. Therefore, afternoon anthesis in *Glinus* species could ensure insect pollination and reciprocate the insect pollinators with pollen and/or nectar.

Watson & Dallwitz (1992) stated that Molluginaceae members are entomophilous. These authors considered nectar secreting tissue and showy tepals in several species as adaptations for entomophily. In *Glinus* species, the floral characters such as the erect position of flowers above foliage, adaxial surface of the tepals, petaloid staminodes and nectar secreting tissue between the ovary base, connate part of staminal filaments and scabrate ornamentation of pollen grains appear to be adaptations for insect pollination. The bees while collecting pollen and butterflies while collecting nectar effect stromatoblastic pollination. Further, the bees and ants while collecting nectar effect nototribic pollination. In both *G. lotoides* and *G. oppositifolius*, the pollen output per anther varies with the number of functional stamens present in the flowers; it increases with a decrease in the stamen number. The pollen output per flower in *G. lotoides* is more than double the amount produced per flower in *G. oppositifolius*. The variation in pollen production in these plant species is partly attributable to the number of stamens produced. The varied amount of pollen output in the flowers of the same and different inflorescences on the same plant drives the pollen collecting bees to visit the flowers across population (s) in search of more pollen collection and such a foraging activity contributes to both self- and cross-pollination. The nectar secreted in traces in both the species and nectar removal by thrips species, *Haplothrips* also drives the nectar collecting bees, ants and lycaenid butterflies to visit flowers across population(s) due to which both self- and cross-pollinations occur. *Glinus* species

appear to be important sources of pollen for bees, especially for honey bees. Saad-Aldeen (2014) also noted that *G. lotoides* is an important pollen source for honey bees in Radom area, South Darfur State, Sudan. Further, these plant species in the study area are important nectar sources for ants and lycaenid butterflies. Among butterflies, lycaenids are the smallest, low-flying and appropriate pollinators for prostrate herbs such as *Glinus* species.

In both *Glinus* species, the flowers are protandrous because there is a brief gap between anther dehiscence and commencement of stigma receptivity. Since both male and female sexes mature almost at the same time, the flowers in these plant species can be stated as homogamous. Further, the stamens and stigmas are spatially separated in both the species; such a situation suggests that the flowers are also herkogamous. Herkogamy does not facilitate the occurrence of spontaneous autogamy despite the flowers being homogamous. However, the thrips emerging from the floral buds during anthesis and their movements in the flowers after anthesis for pollen and nectar collection could result in autogamy. They also bring about geitonogamy due to their migration to different inflorescences on the same plant for forage collection and xenogamy due to their migration to other conspecific plants for forage collection. Further, the movement of tepals together with stamens towards the pistil during flower closure facilitates contact between the sex organs and effects spontaneous autogamy if pollen is still available in the dehiscent stamens. Further, the tiny thrips by moving into the closed flowers from apical portion and from sides may effect geitonogamy or xenogamy. Therefore, *G. lotoides* and *G. oppositifolius* while keeping the options open for spontaneous or vector-mediated selfing exhibit polyphily involving bees, ants, butterflies and thrips as pollinators.

In the present study, *Glinus* species show variation in the number of carpels and ovules per flower. The flowers of *G. lotoides* produce five carpels while those of *G. oppositifolius* produce three carpels. Likewise, the number of ovules also varies depending on the number of stamens and pollen output per flower; the ovules are relatively more in *G. lotoides* than in *G. oppositifolius*. This ovule production trend indicates that the pollen output increases with an increase in ovule number in order to provide sufficient pollen to fertilize as

many ovules as possible through spontaneous autogamy or vector-mediated pollination. This situation is reflected in the natural fruit and seed set rates in both plant species. The highest fruit and seed set rates and the lowest pollen-ovule ratios recorded in *G. lotoides* and *G. oppositifolius* indicate that they are facultatively autogamous but breeding test is required for confirmation.

Bittrich (1990) reported that Molluginaceae members produce capsules with many seeds and the capsules dehisce loculicidally to expose seeds. In *G. lotoides*, the capsules open when moistened with the aid of expanding keels. In the present study, in *G. lotoides* and *G. oppositifolius*, the fertilized flowers produce fruits within a week or two. The fruit is a capsule but it is 5-valved in *G. lotoides* and 3-valved in *G. oppositifolius*. In dry season, plants with ripe and dry capsules break off which are then dispersed by wind. Dry capsules break open loculicidally when tepals become wet and then expose the seeds. However, the seeds remain attached to the base of the perianth. In both the species, the seeds exposed from the capsules fall to the ground on clear sunny days. On rainy days, water drops find their way into the fruit through the distal opening and the fruit filled with water expels both water and seeds explosively. Further, seeds fallen on the ground disperse through surface water runoff during rain fall. Therefore, *G. lotoides* and *G. oppositifolius* exhibit anemochory, ombrohydrochory and hydrochory.

*Glinus* species produce seeds with a white aril of funicular origin and the latter develops into elongate, filiform strophiole (Narayana 1962; Hofmann 1973). *Glinus* genus is characterized by its seeds with a filiform appendaged aril and indumentum of often stellate hairs (Ronse De Craene 2013). In this study, *G. lotoides* has been found to produce small reniform, smooth and dark brown seeds and its seeds have a white aril of funicular origin developed into elongate filiform strophiole. In *G. oppositifolius*, the seeds are small, sub-reniform and reddish-brown with a white aril of funicular origin formed into extensively curved scrotiform strophiole. The presence of filiform or scrotiform strophiole in *Glinus* species appears to be an adaptation for seed dispersal by ants (Costea *et al.* 2003). But, ants have not been found to use the strophiole as food and carry seeds of these species. However, further studies if taken up on this aspect may throw more light either to confirm

or refute this observation.

Balcha (2009) reported that *G. lotoides* has short seed viability period. Teshome & Feyissa (2015) also reported that this species propagates by seed but short period of seed viability and poor seed germination percentage are the limiting factors for its invasiveness. The present study showed that both *G. lotoides* and *G. oppositifolius* produce several batches of populations in a year and their seeds germinate as soon as they are dispersed but their germination could be related to soil moisture which plays an important role in breaking the seed coat. Therefore, *Glinus* species appear to have short period of seed viability as reported by Balcha (2009) and Teshome & Feyissa (2015) and also the viability may also be attributable to the extent of genetic variation achieved through vector-mediated pollination.

Rama Das & Raghavendra (1973) noted that *G. lotoides* and *G. oppositifolius* are C3 species. *Glinus* with C3 photosynthesis have developed different pollination mechanisms to maximize fruit and seed set rate. Genetic variation achieved through insect pollination would enable to extend their distribution range by producing populations especially in dry and moist habitats. Since *Glinus* species grow well in these habitats and carpet the soil, they can be considered as important candidate species in soil erosion control and in eco-restoration programs.

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### COMMUNICATION

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## POLLINATION ECOLOGY OF THREE ECOLOGICALLY VALUABLE CARPETWEED HERBS, *MOLLUGO CERVIANA*, *M. NUDICAULIS* AND *M. PENTAPHYLLA* (MOLLUGINACEAE)

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**Abstract:** *Mollugo cerviana*, *M. nudicaulis* and *M. pentaphylla* are annual herbs which usually grow in open dry sandy and sandy/loamy soils, and also occur in moist habitats, especially cultivated lands. The flowers possess five tepals, functional stamens and 3-carpelled ovary with several ovules and three stigmas. *M. nudicaulis* and *M. pentaphylla* are pollinated by insects. *Haplothrips* uses the flowers for breeding and feeding, which affects pollination. These species have specialized floral structural and functional behaviours for self-induced and spontaneous autogamy while keeping the options open for insect pollination after anthesis. They are facultative autogamous, which is reflected in pollen-ovule ratios and natural fruit and seed set rates. Seed dispersal modes include anemochory, ombrohydrochory and hydrochory. The seeds germinate immediately after their dispersal, and soil moisture is important in rupturing the seed coat. These species are best adapted to survive in open dry habitats as they take advantage of any sign of temporary humidity to complete their life cycle quickly.

**Keywords:** Anemochory, facultative autogamy, hydrochory, insect-pollination, ombrohydrochory, soil binder.

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**Author Details:** Mrs. M. SULAKSHANA PhD, has published four research papers on pollination ecology. Prof. A.J. SOLOMON RAJU is working in the Department of Environmental Sciences, Andhra University, Visakhapatnam. He is the recipient of several national and international awards. He has more than 300 research papers in international and national journals.

**Author Contribution:** Both the authors contributed equally overall.

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## INTRODUCTION

Hutchinson (1926) recognized Molluginaceae as distinct from Aizoaceae. Molluginaceae genera previously included in the larger family Aizoaceae have been separated and treated under the Molluginaceae in subsequent classifications of the Angiosperm Phylogeny Group (APG) 1998, APG II of 2003, APG III of 2009 and APG IV of 2016. APG IV classification is the modern molecular-based system of plant taxonomy for flowering plants (angiosperms). The genus *Mollugo* is distributed in tropical to warm temperate parts of North and South America, Europe, Africa and Asia. The generic name is derived from the Latin word "mollis" meaning soft (Short 2002). *M. cerviana* is native to India, Sri Lanka, Pakistan and Bangladesh (Parvathamma & Shanthamma 2000). *M. nudicaulis* is distributed throughout tropical Africa and Asia (Burrows & Willis 2005). *M. cerviana* is a  $C_4$  species, *M. nudicaulis*  $C_3$ - $C_4$  species and *M. pentaphylla*  $C_3$  species; the first species is distributed in hot arid regions from pantropics to temperate regions while the other two species are distributed from pantropical and subtropical regions (Christin et al. 2010). These three species are valuable in traditional medicine for treating different diseases and ailments (Parvathamma & Shanthamma 2000; Rajamanikandan et al. 2011; Sahu et al. 2012).

Little is known about the pollination ecology of Molluginaceae, where nectar secreting tissue is present in almost all species. In several genera showy sepals or petals have evolved, both of which strongly suggest entomophily (Watson & Dallwitz 1992; Kubitzki et al. 1993). *Mollugo verticillata* is pollinated by the syrphid fly *Mesogramma marginata* (Robertson 1928). The most widely spread, weedy species of *Mollugo verticillata*, *M. nudicaulis* and *M. cerviana* are self- and insect-pollinated (Pax & Hoffmann 1934; Bogle 1970). In Taiwan, *M. pentaphylla* is a minor pollen source for *Apis mellifera* (Lin et al. 1993). In southern India, honey bees use *Mollugo* species as pollen source and reciprocate the plants with pollination (Ponnuchamy et al. 2014). The present study examines how *M. cerviana*, *M. nudicaulis* and *M. pentaphylla* are able to reproduce in semi-dry and dry habitats where pollinators are usually scarce. The principal objective of this study is to understand how floral biology, sexual and breeding systems, pollination mechanisms, fruiting ecology and seed dispersal collectively contribute to the success of sexual reproduction in these three species growing in dry habitats.

## MATERIALS AND METHODS

Wild patches of *Mollugo cerviana*, *M. nudicaulis* and *M. pentaphylla* growing in open dry and semi-dry areas of Visakhapatnam and its surroundings (17.686°N & 83.218°E) were selected for study during March 2015–May 2017. Field trips were conducted to record phenological aspects. Ten inflorescences which have not initiated flowering on five plants were tagged and followed to record anthesis schedule and the timing of anther dehiscence. Twenty-five fresh flowers were used to record the floral morphological details. Nectar could not be measured and analyzed due to its secretion in minute quantity which was further depleted by thrips during mature bud and flower life. Twenty mature, but un-dehisced anthers, two anthers each per flower/plant from ten plants were collected and examined for pollen output as per the protocol described in Dafni et al. (2005). The calculation of pollen output per flower and pollen-ovule ratio was done as per the formulas described in Cruden (1977). Ten flowers each from five individuals were used to test stigma receptivity. It was tested with hydrogen peroxide from mature bud stage to flower closure/drop as per Dafni et al. (2005). Seventy inflorescences were tagged prior to the initiation of their flowering and followed for three weeks to record fruit and seed set rate in open-pollinations. The fruit and seed morphological characteristics were observed in detail to evaluate their adaptations for dispersal by different means. Field visits were made during rainy season to note the aspects of seed germination and production of new plants. Based on the timings of maturation of anthers and receptivity of stigmas, the sexual system was defined and also elaborately explained its functionality to achieve self-induced autogamy, spontaneous autogamy, geitonogamy and xenogamy. The positions of stamens and stigmas during and after anthesis were observed to evaluate as to how they facilitate spontaneous autogamy during anthesis and flower closure. Further, observations were also made to evaluate as to how these positions preclude self-pollination when flowers stay open.

Insects foraging at the flowers were observed from morning to evening on four different days for their mode of approach, landing, probing behavior and contact with the floral sexual organs. Bees were identified from representative specimens available with the Department of Environmental Sciences, Andhra University, Visakhapatnam. Butterflies were identified by consulting the books of Kunte (2007) and Gunathilagaraj et al. (1998). The foraging visits of

insects were recorded using 1mx1m area of flowering patch for 10min at each hour for the entire day on four different days and the data were tabulated to record the foraging pattern and the percentage of visits made by different insect categories. The pollen/nectar collection behaviour of insects was carefully observed to assess their role in effecting pollination. Ten specimens of each insect species were captured during 0800–1100 h and brought to the laboratory. Each specimen was washed in ethyl alcohol, stained with aniline-blue on a glass slide and observed under microscope to count the number of pollen grains present. From this, the average number of pollen grains carried by each insect species was calculated to know the pollen carryover efficiency.

## RESULTS

### Phenology

*Mollugo cerviana* is a small, glabrous, slender annual herb. It is common in open dry sandy and semi-dry soils along roadsides, waste places, bare ground and dry river beds (Image 1a). Its presence is usually overlooked due to its very low ground habit, wiry reddish orange stems and thin linear leaves. The stems are numerous, upright, thin and stiff. Leaves are sessile, grey green and linear with acute apex; they arise in whorls on the stem but some are in a rosette at the base. *M. nudicaulis* is a small acaulescent annual herb with a rosette of prostrate leaves while *M. pentaphylla* is small much-branched annual herb with a thin tap root. *M. nudicaulis* (Image 2a,b) and *M. pentaphylla* (Image 4a) are common in open dry sandy and moist soils along roadsides, waste places, bare ground and cultivated lands. In *M. nudicaulis*, the leaves are sessile, succulent, glabrous, obovate to spatulate, margin entire and apex rounded. In *M. pentaphylla*, the stem is thin, angular, glabrous and tinged with brownish red when old. Leaves are petiolate, unequal, succulent, glabrous, obovate to spatulate, margin entire and apex mucronate. The basal leaves are 5 or more in rosette form while those upwards vary from 4 to 1. All three species appear simultaneously in vegetative, flowering and fruiting phases in different populations growing in different habitats throughout the year (Image 1b, 4b). Individual plants, however, have a short life cycle of 3 months from seed germination to seed dispersal. Although they appear throughout the year, they show robust vegetative growth and profuse flowering and fruiting during July–October when soil is damp due to occurrence of rains. In *M. cerviana*, the flowers are borne on 7–8 mm long pedicels in dichotomous and

trichotomous umbellate cymes produced terminally or in leaf axils. The inflorescence arises from the rosette of basal leaves in *M. nudicaulis* and from leaf axils and terminally in *M. pentaphylla*. In *M. nudicaulis*, the dichasial or trichasial cymes are common during dry season while polychasial cymes are common during wet season. In *M. pentaphylla*, polychasial cymes are common during wet season while di- or tri-chasial cymes are common during dry season. Further, the cymes are of spreading type, pedunculate and produce pedicellate flowers; the peduncle is 7–8 mm long pedicel is 4mm long in the former while the corresponding measures for the latter are 5–8 mm and 2–4 mm, respectively. In both, the peduncle and pedicel are wiry and stiff. A polychasial cyme produces  $7.5 \pm 1.5$  flowers in *M. nudicaulis* and  $13.83 \pm 4.9$  in *M. pentaphylla*.

### Flower morphology

In all three species, the flowers are small, odourless, actinomorphic and bisexual. They are  $2.52 \pm 0.4$  mm long,  $1.51 \pm 0.5$  mm wide, whitish green on adaxial side and green on abaxial side in *M. cerviana*;  $3.51 \pm 0.4$  mm long,  $4.03 \pm 0.3$  mm wide, creamy white on adaxial side and light green on abaxial side in *M. nudicaulis*; and  $2.75 \pm 0.4$  mm long,  $1.8 \pm 0.4$  mm wide, white on both adaxial and abaxial side in *M. pentaphylla*. In all, the sepals and petals are represented by a monochlamydeous perianth of 5 elliptic to oblong free tepals. They are  $2.45 \pm 0.4$  mm long,  $1.13 \pm 0.2$  mm wide with white margins in *M. cerviana*;  $3.28 \pm 0.41$  mm long,  $1.82 \pm 0.33$  mm wide, connate base and hooded apically in *M. nudicaulis*; and 2–3 mm long but connate at base in *M. pentaphylla*. The stamens are 5, anti-tepalous and  $1.22 \pm 0.3$  mm long in *M. cerviana*; 3–6 and  $2.27 \pm 0.17$  mm long in *M. nudicaulis*; and 3–5 and  $1.8 \pm 0.17$  mm long in *M. pentaphylla*. In all, the filaments are free, connate at base and tipped with dorsifixed dithecous anthers. In *M. pentaphylla*, the flowers with 3-stamens constituted 60%, those with 4-stamens 33% and those with 5-stamens 7%; these three types of flowers occur on the same plant. The flowers with 6-stamens are very rare. A single plant all with 5-stamened flowers was encountered during the study period and these flowers are prominently larger than other types of flowers. In 3-stamened flowers, one stamen is alterni-tepalous while the other two are anti-tepalous (Image 2e). In 4-stamened flowers, three stamens are alterni-tepalous while the other one is anti-tepalous. In 5-stamened flowers, two stamens are alterni-tepalous while the other three are anti-tepalous (Image 2f). In 6-stamened flowers, three stamens are alterni-tepalous while three



Image 1. *Mollugo cerviana*: a. Habitat with *M. nudicaulis*, b. flowering phase, c. & d. Flowering-opening phase, e. Position of stigmatic lobes and anthers at the same height contacting each other at anthesis, f. Dehiscent anthers, g. Pollen grain, h. Ovary with three stigmas, i. & j. Multi-ovuled ovary, k. Maturing fruit, l. & m. Dehiscent fruit capsule, n. Seeds. © A.J. Solomon Raju.

others are anti-tepalous (Image 2g). In *M. pentaphylla*, the flowers with 3-stamens constituted 91%, those with 4-stamens 7% and those with 5-stamens 2%; all three types of flowers occur on the same plant. In 3-stamened flowers, one stamen is alterni-tepalous while the other two are anti-tepalous. In 4-stamened flowers, three stamens are alterni-tepalous while the other one is anti-tepalous. In 5-stamened flowers, two stamens are alterni-tepalous while the other three are anti-tepalous. In all three species, the ovary is light green, tri-carpellary, tri-locular syncarpous with ovules arranged on axile placentation (Image 1i,j, 2k, 4i). The ovules are  $58.2 \pm 8.16$  in *M. cerviana* but the ovule number varied with change in stamen number in the other two species. In *M. nudicaulis*, they are  $17.45 \pm 3.51$  in 3-stamened flowers,  $19.9 \pm 2.88$  in 4-stamened flowers and  $23.1 \pm 3.70$  in 5-stamened flowers. In *M. pentaphylla*, they are  $16.02 \pm 4.0$  in 3-stamened flowers,  $18.44 \pm 2.0$  in 4-stamened flowers and  $20.11 \pm 2.6$  in 5-stamened flowers. The ovules are D-shaped in *M. cerviana* and *M. nudicaulis*,

and reniform in *M. pentaphylla*. In all, the style is absent but the ovary is terminated with 3 free stigmas (Image 1h, 2j, 4h). The stigmas are minutely denticulate with membranous flaps in *M. cerviana* while they are densely papillose, shiny and spreading in the other two species.

#### Floral biology

In all three species, mature buds open during 0700-0800 h and extend until 0900h in *M. pentaphylla* (Image 2d). Individual buds take 5 to 10 minutes from partial to full opening (Image 1c,d; 4c-e). The flowers are homogamous as the anthers and stigmas attain maturity at the same time during anthesis; the former dehisce by longitudinal slits (Image 1f, 2h) while the latter continue receptivity until the noon of the second day. In *M. cerviana*, the pollen output is  $159.7 \pm 14.5$  per anther and  $798.5 \pm 69.5$  per flower. The pollen-ovule ratio is 14:1. In *M. nudicaulis*, the pollen output varied with change in stamen number. It varied from  $209.6 \pm 17.12$  to  $171.4 \pm 13.44$  per anther and from  $628.8 \pm 51.36$  to



Image 2. *Mollugo nudicaulis*: a. Habit - flowering phase, b. Individual plant in flowering, c. New plants, d. Bud, e. 3-stamened flower, f. 5-stamened flower, g. 6-stamened flower, h. Dehisced anthers, i. Pollen grain, j. Ovary with three styles, k. Ovules, l. Maturing fruit, m. Dehisced fruit capsule, n. Dehisced fruit capsule with seeds intact, o. Seeds. © A.J. Solomon Raju.

Table 1. Pollen aspects in *Mollugo nudicaulis* and *Mollugo pentaphylla*

Flower type	Percentage of occurrence	Mean pollen output/ anther	Mean pollen output/ flower	Mean no. of ovules/ flower	Pollen: ovule ratio
<i>Mollugo nudicaulis</i>					
3-stamened	60	309.6 ± 17.12	628.8 ± 51.36	17.45 ± 3.51	36 : 1
4-stamened	33	384.4 ± 13.12	737.6 ± 52.48	19.90 ± 2.88	37 : 1
5-stamened	7	171.4 ± 13.44	857.0 ± 67.20	23.30 ± 3.70	37 : 1
<i>Mollugo pentaphylla</i>					
3-stamened	91	277.2 ± 13.4	831.6 ± 40.2	16.02 ± 4.0	52 : 1
4-stamened	7	242.6 ± 19.6	970.4 ± 78.4	18.44 ± 2.0	53 : 1
5-stamened	2	213.4 ± 12.9	1067 ± 64.5	20.11 ± 2.6	53 : 1

857 ± 67.2 per flower in 3- to 5-stamened flowers (Table 1). The pollen-ovule ratio is 36:1 in 3-stamened flowers while it is 37:1 in 4- and 5-stamened flowers. In *M. pentaphylla*, it varied from 277.2 ± 13.4 to 213.4 ± 12.9 per anther and from 831.6 ± 40.2 to 1067 ± 64.5 per flower in 3- to 5-stamened flowers (Table 1). The pollen-ovule ratio is 52:1 in 3-stamened flowers while it is 53:1 in 4- and 5-stamened flowers. In both *M. nudicaulis* and

*M. pentaphylla*, the pollen production trend showed that pollen output rate gradually increased with a gradual decrease in the number of stamens produced per flower. The pollen grains are pale yellow, spheroidal, tri-colpate, tri-zonoperturate, granulated, tectum scabrate, 21.9 ± 4.12 µm (image 1g; 2i, 4g). In all three species, the nectar is secreted in traces during mature bud stage. The tepals together with the stamens and stigmas close

back by 1000–1100 h but this event extends until 1200h in *M. nudicaulis*.

#### Pollination mechanism and Pollinators

In all three species, dehiscent anthers collected during anthesis showed some percentage of pollen formed tubes indicating *in situ* germination. It varied from 20% to 25% in *M. cerviana*, from 11% to 21% in *M. nudicaulis* and from 18% to 26% in *M. pentaphylla*. In all, the pollen tubes were also found on the stigma. The pollen germination and formation of tubes both within the dehiscent anthers and on the stigma indicate that the presence of self-induced autogamy. In *M. cerviana*, during and after anthesis, the dehiscent anthers and receptive stigmas contact with each other due to their close proximity and their position at the same height due to which autogamy occurs (Image 1e). In the other two species, during anthesis, one anther in 3-stamened flowers and 2–3 anthers in 4- and 5-stamened flowers contact the stigmas due to their close proximity and their position at the same height (Image 4f). With this situation, the anthers brush against the stigmas causing autogamy. After anthesis, all anthers move away from the stigmas but both the sex organs are situated at the same height facilitating vector-mediated self- or cross-pollination. In all the three species, during the closing of the flower, the stamens and stigmas contact each other very closely assuring autogamy if it did not occur during open state of the flower.

#### Thrips pollination

*Haplorthrips* sp. (Thysanoptera: Thripidae) used the flower buds of all three species for breeding and

flowers for feeding. The larvae emerged from the eggs in synchrony with anthesis and nectar production in flowers. The larvae and adults foraged for pollen and nectar. Individual thrips were dusted with pollen during their movements within the flowers. They carried pollen on their body setae, wings and legs. The pollen carried by them varied from 87 to 176 pollen grains in *M. cerviana*, from 69 to 158 in *M. nudicaulis* and 89 to 217 in *M. pentaphylla*. The thrips dispersed the pollen on free denticulate and membranous stigmas of *M. cerviana* and on free densely papillose spreading stigmas of *M. nudicaulis* and *M. pentaphylla* due to their active movement, rubbing of abdomen against the stigmatic surface, cleansing of their body parts with their hind legs and also by their wing combing mechanism. In all, the homogamous flowers were found to facilitate self-pollination in the same or different flowers of the same plant. As the plant occurs as small or large populations, thrips could fly to migrate to the flowers of other closely spaced plants and effect cross-pollination by feeding on the forage.

#### Insect pollination

The flowers of *M. cerviana* were never visited by any insects. The flowers of *M. nudicaulis* were foraged by bees, flies and butterflies while those of *M. pentaphylla* by bees and butterflies. Bees and flies foraged for pollen and nectar while butterflies for nectar only during 0800–1100 h with concentrated foraging activity during 0900–1000 h (Figs. 1–4). The bees, *Apis cerana* (Image 3a, 5a), *A. florea* (Image 3b, 5b), *Trigona iridipennis* (Image 3c, 5c), *Ceratina* sp. (Image 3d, 5d) visited the flowers of both *M. nudicaulis* and *M. pentaphylla*; the former was

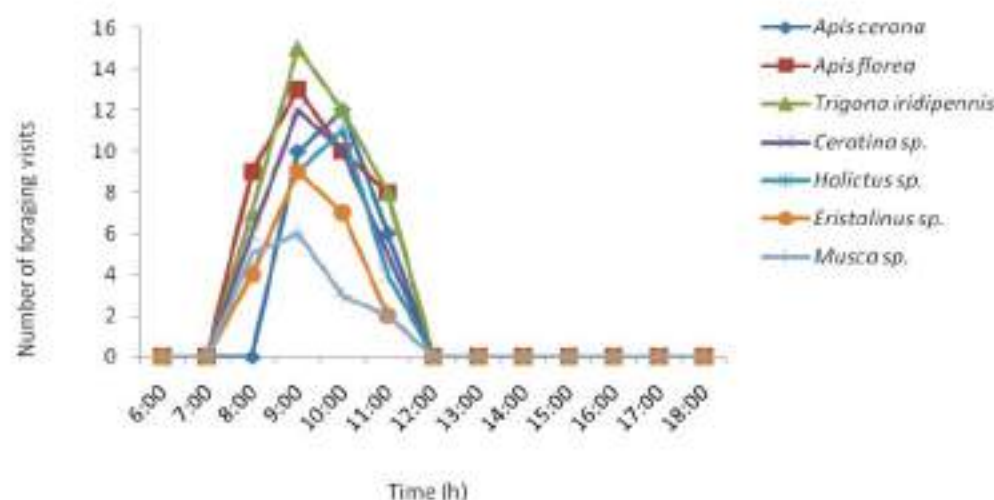


Figure 1. Hourly foraging visits of bees and flies on *Mollogo nudicaulis*

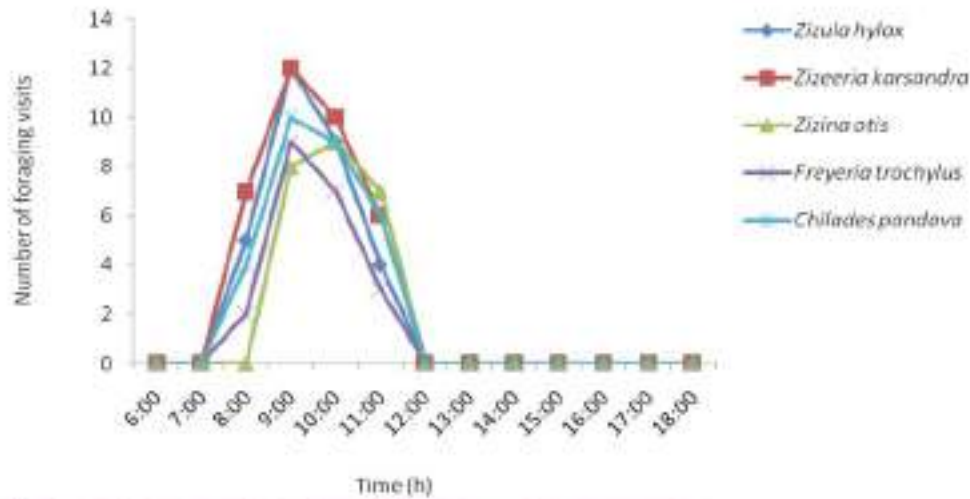


Figure 2. Hourly foraging activity of lycaenid butterflies on *Mollugo nodicaulis*

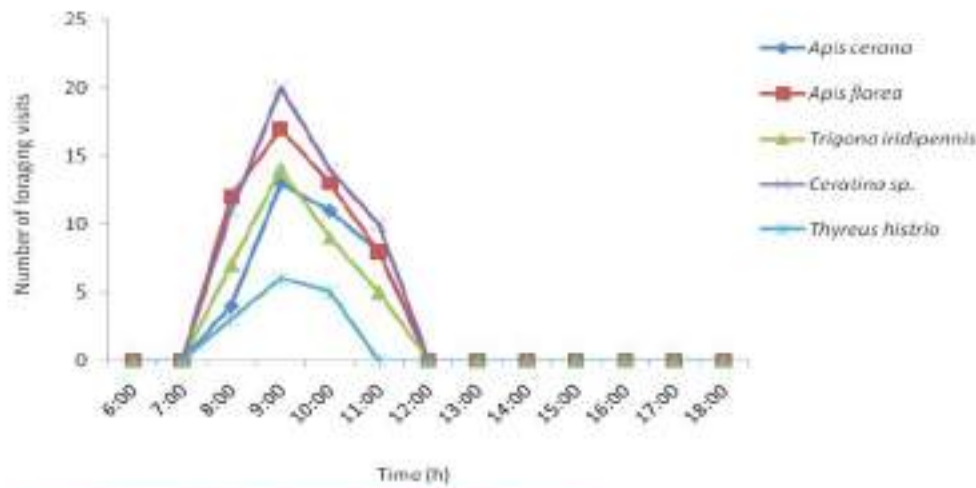


Figure 3. Hourly foraging activity of bees on *Mollugo pentaphylla*

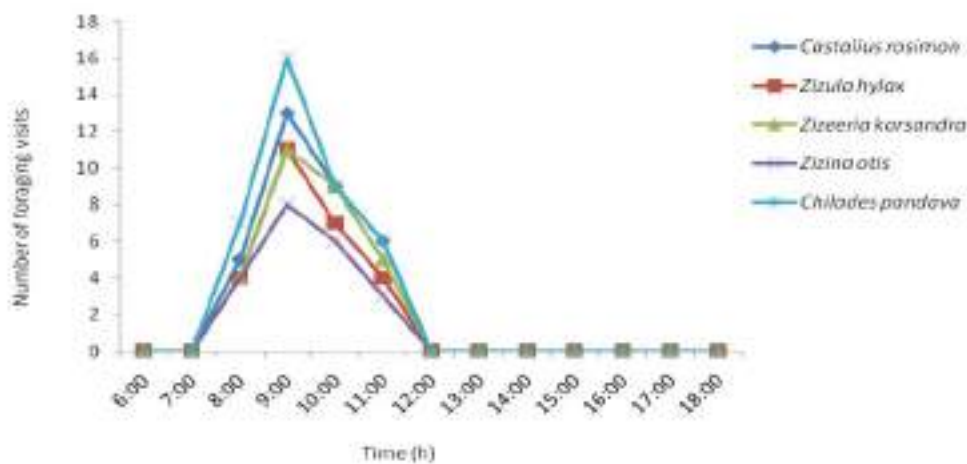


Figure 4. Hourly foraging activity of lycaenid butterflies on *Mollugo pentaphylla*



Image 3. *Mollugo nudicaulis*: a. *Apis cerana*, b. *Apis florea*, c. *Trigona iridipennis*, d. *Ceratina* sp., e. *Halictus* sp., f. *Eristalinus* sp., g. *Musca* sp., h–j. Lycaenids - h. *Zizula hylax*, i. *Zizeeria karsandra*, l. *Zizina otis*, j. *Chilades pandava*. © A.J. Solomon Raju.

also visited by *Halictus* sp. (Image 3e) and the latter also by *Thyreus histrio* (Image 5e). The flies recorded on *M. nudicaulis* were *Eristalinus* sp. (Image 3f) and *Musca* sp. (Image 3g). The butterflies represented only lycaenids - *Zizula hylax* (Image 5g), *Zizeeria karsandra* (Image 3h, 5h), *Zizina otis* (Image 3i) and *Chilades pandava* (Image 3j) (Table 2) foraged on the flowers of both plant species. *M. nudicaulis* was also visited by *Freyeria trochylus* and *M. pentaphylla* also by *Castalius rosiman* (Image 5f). All these insects approached the flowers in upright position, landed on the tepals and then probed for forage collection. Bees first accessed anthers to collect pollen and then moved to the flower base to collect nectar, if available in the same and/or different visits. Flies and butterflies stretched out their proboscis and inserted into the flower base to collect nectar. All insect species collected forage from several flowers of different cymes of the same or different plants to collect the forage. The bees during pollen collection brushed against the stigmas with their ventral surface effecting strobilic pollination. Further, these insects during nectar collection brushed against anthers and stigmas with their dorsal surface effecting nototribic pollination. The flies and butterflies during nectar collection contacted the stamens and stigmas with their proboscis and occasionally front side of head and ventral surface of thorax and abdomen effecting strobilic pollination. The butterfly wings never contacted the stamens and stigma during nectar collection as they kept them in vertical position. In *M. nudicaulis*, bees made 49%, flies

Table 2. List of insect foragers on *Mollugo nudicaulis* and *Mollugo pentaphylla*

Order/Family	Insect species	<i>Mollugo nudicaulis</i>	<i>Mollugo pentaphylla</i>
<b>Hymenoptera</b>			
Apidae	<i>Apis cerana</i> F.	+	+
	<i>Apis florea</i> F.	+	+
	<i>Trigona iridipennis</i> Smith	+	+
	<i>Ceratina smaragdula</i> F.	-	-
	<i>Ceratina</i> sp.	+	+
	<i>Thyreus histrio</i>	-	+
Halictidae	<i>Halictus</i> sp.	+	-
Megachilidae	<i>Megachile</i> sp.	-	-
Formicidae	<i>Camponotus</i> sp.	-	-
	<i>Crematogaster</i> sp.	-	-
<b>Diptera</b>			
Syrphidae	<i>Eristalinus</i> sp.	+	-
Muscidae	<i>Musca</i> sp.	+	-
<b>Lepidoptera</b>			
Lycaenidae	<i>Castalius rosiman</i> F.	-	+
	<i>Zizula hylax</i> F.	+	+
	<i>Zizeeria karsandra</i> Moore	+	+
	<i>Zizina otis</i> F.	+	+
	<i>Freyeria trochylus</i> Freyer	+	-
	<i>Chilades latus</i> Stoll	-	-
	<i>Chilades pandava</i> Horsfield	+	+

Table 3. Pollen recorded in the body washings of insects on *Mollugo nudicaulis* and *Mollugo pentaphylla*

Insect species	Sample size (N)	<i>Mollugo nudicaulis</i>			<i>Mollugo pentaphylla</i>		
		Number of pollen grains			Number of pollen grains		
		Range	Mean	S.D.	Range	Mean	S.D.
<i>Apis cerana</i>	10	73–204	133.5	37.5	82–246	159.2	51.8
<i>Apis florea</i>	10	61–183	126.1	33.31	68–217	145.1	43.56
<i>Trigona indigena</i>	10	57–95	63.4	14.4	31–86	62.1	13.3
<i>Carattina</i> sp.	10	54–62	47.8	8.27	24–51	38.2	9.07
<i>Halictus</i> sp.	10	41–87	69.8	12.2	—	—	—
<i>Thyreus histrio</i>	—	—	—	—	19–43	30.6	7.60
<i>Eristalis</i> sp.	10	26–50	38.2	7.26	—	—	—
<i>Murco</i> sp.	10	13–38	27.8	7.5	—	—	—
<i>Centistes roselman</i>	10	—	—	—	15–41	29.5	6.38
<i>Zizula hyala</i>	10	—	21.3	5.47	9–30	20.6	5.27
<i>Zizeria karsandra</i>	10	13–32	23.8	5.57	13–45	27.8	9.49
<i>Zizina otis</i>	10	16–40	28.1	6.48	10–36	23.7	6.63
<i>Freyeria trachylus</i>	10	8–31	24.4	7.19	—	—	—
<i>Chilades pandava</i>	10	15–36	28.5	6.27	8–43	30.7	8.8

11% and lycaenids 40% of total foraging visits. In *M. pentaphylla*, bees made 56% and lycaenids 44% of total foraging visits. The body washings of insects collected from the flowers during peak foraging period revealed that all insects carry pollen but bees carry the highest number of pollen grains. Further, the mean number of pollen grains varied with each insect species (Table 3). The nectar secretion in traces and its depletion by thrips during and after anthesis appeared to be driving the insects to visit as many flowering cymes as possible to quench their thirst for nectar. Such a foraging behavior was considered to be facilitating the promotion of cross-pollination.

#### Fruiting ecology and seed dispersal

In all three species, fruits mature within 8–12 days (Image 6a). The stamens and stigmas are persistent and remain inside due to the closure of the flower. The tepals bulge gradually and protect the bulging ovary in which the seeds form and mature (Image 1k, 6b). The natural fruit set is 91.27% in *M. cerviana*, 86–89 % in *M. nudicaulis*, and 83–88 % in *M. pentaphylla*. Seed set rate is 61.94% in *M. cerviana*, 88–92 % in *M. nudicaulis*, and 83–86 % in *M. pentaphylla* (Table 4). Fruit is a loculicidal 3-valved broadly-ellipsoid capsule, stalked and membranous and densely pubescent in all three species but it is densely pubescent in *M. cerviana* and *M. nudicaulis* while it is glabrous in *M. pentaphylla*. It is  $2.35 \pm 0.36$  mm and  $1.85 \pm 0.23$  mm wide in *M. cerviana*,  $3.4$

$\pm 0.4$  mm long and  $2.33 \pm 0.39$  mm wide in *M. nudicaulis*, and  $2.67 \pm 0.4$  mm long and  $1.97 \pm 1.4$  mm wide in *M. pentaphylla*. In all three species, the seeds are arranged in two rows in each locule. In *M. cerviana*, the seeds are tiny, brown, shiny, D-shaped and faintly striate dorsally (Image 1n). The seed coat is studded with minute granular excrescences with reticulate ornamentation. In *M. nudicaulis* and *M. pentaphylla*, the seeds are tiny, black, slightly shiny, reniform and concentrically ridged (Image 2a, 6e). The seed coat is closely packed with uniformly distributed, pebble-like, lyrate and chipped areoles. Dry capsules break open when fruit pericarp and tepals are dry and expose the seeds (Image 1f,m; 2l,n; 6c). But the seeds remain so and gradually separate and fall to the ground on their own on clear sunny days. On rainy days, the water droplets falling on the dehiscent capsules washout seeds to the ground. Water also acts as an efficient dispersal agent for seeds that fall during the rainy season. Seeds do not have adaptations for wind dispersal, but wind disperses dry cymes and dehiscent capsules short distances and subsequently fall to the ground from capsules. Thus, seed dispersal modes include ombrohydrochory, hydrochory and anemochory. The seeds produced from plants growing in cultivated lands have the potential to be dispersed as a cereal grain contaminant and in effect agricultural produce movement contributes to seed dispersal and expansion of its distribution (Image 2c; 6f,g).





Image 4. *Mollugo pentaphylla*: a. Habit, b. Flowering phase, c-e. Different stages of anthesis, f. Position of anthers and stigmas at the same height, g. Pollen grain, h. Ovary with three stigmas, i. Ovules. © A.J. Solomon Raju.

Table 4. Natural fruit and seed set rate in *Mollugo nudicaulis* and *M. pentaphylla*

Flower type	Number of flowers sampled	Number of flowers set fruit	Fruit set (%)	Seed set (%)
<i>Mollugo nudicaulis</i>				
3-stamined	320	286	89	88
4-stamined	85	73	86	81
5-stamined	40	35	88	82
<i>Mollugo pentaphylla</i>				
3-stamined	250	230	88	83
4-stamined	150	130	87	84
5-stamined	75	62	83	86

## DISCUSSION

*Mollugo* species are annual herbs which usually grow in open dry sandy and sandy and loamy soils but also occur in moist habitats, especially in cultivated lands. In this study, it is found that *M. cerviana*, *M. nudicaulis* and *M. pentaphylla* with their low ground habit populate the soil and for this reason, they are often called as carpet weeds. Of these, *M. cerviana* does not cover the soil extensively due to its wiry stems and thin, linear leaves. *M. nudicaulis* without any stem covers the soil with a rosette of prostrate leaves. *M.*

*pentaphylla* with branched stems carpets the soil with its basal rosette form of leaves and upper spatulate leaves. All the three plant species grow throughout the year displaying vegetative, flowering and fruiting phases in different populations. Their robust growth, profuse flowering and fruiting, however, is confined to the wet season. Individual plants complete their life cycle within three months from seed germination to seed dispersal. Similarly, Owens & Lund (2009) reported that *M. cerviana* is a herbaceous ephemeral species and completes its life cycle in a very short time. In the present study, it is found that the inflorescence is a dichotomous or trichotomous umbellate cyme in *M. cerviana* while it is di- or tri- or poly-chasial cyme in *M. nudicaulis* and *M. pentaphylla*. In the last two species, di-/tri-chasial cymes are common during dry season while poly-chasial cymes are common during wet season, suggesting that the branching of inflorescences and the production rate of flowers is regulated by the soil moisture and nutrient environment. *M. cerviana* and *M. pentaphylla* produce inflorescences in leaf axils and terminally while *M. nudicaulis* produces inflorescences from the axils of rosette of leaves due to lack of stems. Since all the three plant species usually grow as green carpets, the simultaneous display of several flowers from individual plants and from the entire population(s) enhances their attraction to insect pollinators.



Image 5. *Mollugo pentaphylla*: Foragers: a. *Apis cerana*, b. *Apis florea*, c. *Trigona iridipennis*, d. *Ceratina* sp., e. *Thyreus histrio*, f-h. Lycaenid butterflies – f. *Costalvus rosimon*, g. *Zizula hyala*, h. *Zizeeria karsandra*. © A.J. Solomon Raju.



Image 6. *Mollugo pentaphylla*: a. Fruiting phase, b. Maturing fruits, c. Dehiscent fruit capsule, d. Dehiscent fruit capsule with seeds intact, e. Seeds, f. & g. New plants. © A.J. Solomon Raju.

The floral descriptions of *Mollugo* species provided by different authors are not accurate. The present study provides details of the floral descriptions, especially of perianth, androecium and gynoecium in *M. cerviana*, *M. nudicaulis* and *M. pentaphylla* as these are important from the pollination of point view. In these species, perianth typically consists of five tepals which serve the function of calyx (sepals) and corolla (petals). In

*M. cerviana* and *M. nudicaulis*, the abaxial surface of the perianth serves the role of calyx while the adaxial surface of the perianth serves the role of corolla due to display two different colours on each surface. But, in *M. pentaphylla*, the perianth is white on both abaxial and adaxial surface. The study shows that *M. cerviana* with perianth acting as both calyx and corolla is unable to attract any insect pollinators in pollinator-deprived

habitat or pollinator-available habitat. Such a situation explains that *M. cerviana* is not dependent on insect foragers for pollination. *M. nudicaulis* with perianth displaying light green on its abaxial surface and creamy white on its adaxial surface, and *M. pentaphylla* with perianth displaying white colour on both adaxial and abaxial surface attract insect foragers. Eckardt (1974) and Stannard (1988) reported that the sister genera of *Mollugo*, *Corbichonia* and *Lophiocarpus* have only four stamens of which three alternate with sepals and one is opposite a sepal. Batenburg & Moeliono (1982) reported that the presence of one stamen opposite a sepal is unusual in these genera and indicate that this stamen is derived from an original condition with five alternisepalous stamens by a fusion of two stamens under the influence of a reduced tetramerous ovary which is similar to a process occurring in *Mollugo*.

Ronse-De-Craene (2010) reported that in Molluginaceae including *Mollugo*, the androecium consists of generally of five stamens alternating with the sepals. In *Mollugo*, the number of stamens ranges from five in *M. cerviana* to three in *M. nudicaulis*. The present study shows that *M. cerviana* flowers produce a fixed number of 5 stamens and all are opposite to tepals suggesting that there is no process evolving to produce flowers with 3 or 4 stamens. *M. nudicaulis* produces flowers with 3-6 stamens while *M. pentaphylla* produces flowers with 3-5 stamens on the same plant. In these species, 3-stamened flowers have one stamen between two tepals and two stamens opposite to tepals, the 4-stamened flowers have three stamens alternate to tepals and one stamen opposite to a tepal, and the 5-stamened flowers have two stamens alternate to tepals and three stamens opposite to tepals. In *M. nudicaulis*, the 6-stamened flowers have three stamens alternate to tepals and three stamens opposite to tepals. The study indicates that all the three plant species produce trimerous ovary with three stigmas irrespective of the number of stamens produced in the flowers. In *M. nudicaulis* and *M. pentaphylla*, the production of 5-stamened flowers appears to be a residual trait still functional because these flowers are occasionally or rarely produced. In *M. nudicaulis*, the rarity of 6-stamened flowers may be a trait of polyploidy. In *M. nudicaulis* and *M. pentaphylla*, the two stamens opposite to tepals in 3-stamened flowers appear to be derived from the pair-wise fusion of four stamens (Ronse-De-Craene 2010) and the stamen opposite a tepal in 4-stamened flowers appear to be derived from the fusion of two stamens as in *Corbichonia* and *Lophiocarpus*, *sensu* Batenburg & Moeliono (1982).

In *M. nudicaulis* and *M. pentaphylla*, the variation in pollen output levels due to variation in stamen number in 3-5(6) stamened flowers make bees and/or flies to fly from flower to flower in quick succession to collect pollen from the same or different conspecific plants and effect both self- and cross-pollination.

Hammer (1995) reported that different populations of Aizoaceae growing in the same habitat exhibit synchrony in flowering time. The period of flowering is usually short and the flowers show repeated opening but this phenomenon is restricted to a certain period of the day. Groen & van der Maesen (1999) observed that the mixed populations of Aizoaceae genera, *Bergeranthus*, *Faucaria* and *Orthopterum* flower simultaneously. These authors suggested that such a synchrony in flowering in these genera in the same habitat collectively enable them to enhance their floral attraction to pollinators. In the present study, it is found that *Mollugo* species form mixed and distinct populations in the same and different habitats depending on soil moisture and nutrient conditions. These species exhibit synchrony in flowering by opening flowers during morning time. Further, the flowers are too small, lack corolla, tepals not vividly coloured and stay open for a brief period of three hours for visitation by insects. Therefore, the synchrony in anthesis schedule and massive floral display appear to be imperative for them to attract pollinators during the brief period of open state of flowers.

Peter et al. (2004) reported that the temperature and relative humidity are probably important cues determining flower opening in the afternoon. In the present study, the anthesis during morning time in *Mollugo* species is attributable to their predominance in open, dry habitats where herbaceous flora usually do not grow. With synchrony in anthesis schedule, these species provide sufficient forage but insect foragers collect forage only from *M. nudicaulis* and *M. pentaphylla*. Bees and lycaenid butterflies visit and pollinate both the plant species while flies additionally visit and pollinate *M. nudicaulis*. Bees and butterflies are generalists which visit a wide range of flowers and hence are polylectic. Since *Mollugo* species keep the flowers open only for a brief period, the polylectic foragers soon switch over to other plant species which provide forage in the nearby habitats. The morning anthesis in *Mollugo* species ensures insect pollination and reciprocates the insect pollinators with pollen and/or nectar. The total absence of insect foraging activity on *M. cerviana* could be attributable to its common occurrence in pollinator-excluded or deprived habitats and production of tiny flowers which can be overlooked or unnoticed by

foragers.

Watson & Dallwitz (1992) stated that Molluginaceae members are entomophilous. These authors considered nectar secreting tissue and showy tepals in several species as adaptations for entomophily. Robertson (1928) reported that *Mollugo verticillata* is pollinated by syrphid fly, *Mesogramma marginata*. Pax & Hoffmann (1934) and Bogle (1970) stated that the showy sepals or petals evolved in several genera of Molluginaceae suggest entomophily. *Mollugo verticillata*, *M. cerviana* and *M. nudicaulis* are the most widely spread, weedy species and adapted for self- and insect-pollination. In the present study, it is found that in *Mollugo* species, the floral characters such as the erect position of flowers above foliage, adaxial surface of the tepals and nectar secreting tissue between the ovary base and connate part of staminal filaments appear to be adaptations for insect pollination. In *M. nudicaulis*, the bees while collecting pollen, and flies and butterflies while collecting nectar effect sternotribic pollination. Further, the bees while collecting nectar effect nototribic pollination. In *M. pentaphylla*, the bees while collecting pollen effect nototribic pollination. The bees and also butterflies while collecting nectar effect sternotribic pollination. In *M. nudicaulis* and *M. pentaphylla*, the pollen output per anther varies with the number of stamens present in the flowers; it increases with a decrease in the stamen number. The pollen output per flower in *M. pentaphylla* is more than in *M. nudicaulis*. The variation in pollen production in these plant species is partly attributable to the number of stamens produced. The varying amount of pollen output in the flowers of the same and different inflorescences on the same plant drives the pollen collecting bees to visit the flowers across population(s) in search of more pollen and such a foraging activity contributes to both self- and cross-pollination. The nectar secreted in traces in both the species and nectar removal by thrips species, *Haplithrips* also drives the nectar collecting bees, flies and lycaenid butterflies to visit flowers across population(s) due to which both self- and cross-pollinations occur. *M. nudicaulis* and *M. pentaphylla* appear to be important sources of pollen for bees, especially for honey bees. Further, these plant species in the study area are important nectar sources for lycaenid butterflies. Among butterflies, lycaenids are the smallest, low-flying and appropriate pollinators for prostrate herbs, *M. nudicaulis* and *M. pentaphylla*.

Bhargava (1934) and Kshirsagar (1960) reported *in situ* pollen germination in *M. nudicaulis* and *M. pentaphylla*. Johri et al. (1992) noted that self-pollination seems to occur in these species as pollen tubes reached

the ovules of ovaries in un-opened flowers and pollen grains with pollen tubes occur both inside the anther and on the stigma of the same flowers. But, these authors did not mention the time of the occurrence of these events in unopened flowers. In the present study, all three *Mollugo* species show certain percentage of pollen germination only in the dehisced anthers and also the pollen tube formation on the stigma during anthesis process which occurs in individual flowers over a period of five to ten minutes. Such *in situ* pollen germination and the occurrence of pollen tubes on the stigma during the process of anthesis facilitates self-induced autogamy to some extent. In *M. cerviana*, the close proximity of dehisced anthers of all five anthers to the stigmas facilitate the occurrence of spontaneous autogamy. In *M. nudicaulis* and *M. pentaphylla*, the close proximity of one dehisced anther in 3-stamened flowers and 2-3 dehisced anthers in 4- and 5-stamened flowers facilitate the occurrence of spontaneous autogamy. The minutely denticulate stigmas with membranous flaps in *M. cerviana* and densely papillose spreading stigmas in *M. nudicaulis* and *M. pentaphylla* capture pollen easily from the dehisced anthers to result in pollination. Further, in all the three *Mollugo* species, the thrips emerging from the floral buds during anthesis and their movements in the flowers after anthesis for pollen and nectar collection result in autogamy. They also bring about geitonogamy due to their migration to different inflorescences on the same plant for forage collection and xenogamy due to their migration to other conspecific plants for forage collection. In these plant species, the movement of tepals together with stamens towards the pistil during the flower closure facilitates contact between the sex organs and effects spontaneous autogamy if pollen is still available in the dehisced stamens. Further, the tiny thrips have the possibility to carry pollen from other flowers, enter the closed flowers from the apical portion and laterally, and deposit the same on the stigmas effecting either geitonogamy or xenogamy. Therefore, all the three *Mollugo* species have specialized floral structural and functional behaviours for self-induced and spontaneous pollination while keeping the options open for insect pollination after anthesis.

In the present study, all three *Mollugo* species have three carpels with variation in ovule number per flower which is highest in *M. cerviana* and lowest in the other two *Mollugo* species. In *M. nudicaulis* and *M. pentaphylla*, the ovule number also varies depending on the number of stamens and pollen output per flower. This ovule production trend indicates that the pollen output increases with an increase in ovule number in

order to provide sufficient pollen to fertilize as many ovules as possible. This situation is reflected in the natural fruit and seed set rates in both the plant species. The highest fruit and seed set rates and also the lowest pollen-ovule ratios recorded in *Mollugo* species now studied indicate that they are facultatively autogamous.

Bittrich (1990) reported that in Molluginaceae, *Adenogramma* is the only genus which produces one-seeded nutlets. All other genera produce capsules with many seeds which become exposed by loculicidal dehiscence. Soerjani et al. (1987) reported that *Mollugo pentaphylla* is hydrochorous. In the present study, the *Mollugo* species produce fruits within a week or slightly more than a week. The fruit is a 3-valved broadly ellipsoid capsule which breaks open and exposes the seeds on clear sunny days; the seeds subsequently fall to the ground. On rainy days, water drops find their way into the fruits which are then filled with water. In effect, the fruits expel both water and seeds explosively. Further, wind disperses the dry cymes together with dry dehisced capsules to short distances and subsequently the seeds fall to the ground from the capsules. The seeds that reach the ground through these modes are further disseminated through surface water runoff during rain fall. Therefore, *Mollugo* species now studied exhibit anemochory, ombrohydrochory and hydrochory.

Narayana (1962) and Hofmann (1973) noted that *Mollugo* species produce seeds with a primordium-like swelling on the funiculus and this structure is considered to be a vestigial aril. In the present study, it is found that *M. cerviana* produces tiny, brown, shiny, D-shaped seeds with faintly striate dorsal surface. The seed coat is studded with minute granular excrescences with reticulate ornamentation. *M. nudicaulis* and *M. pentaphylla* produce tiny, black, slightly shiny, reniform and concentrically ridged seeds. The seed coat is closely packed with uniformly distributed, pebble-like, lyrate and chipped areoles. Since the seeds of these plant species lack any aril or strophiole-like structure that usually serves as food for ants, the possibility for myrmecochory is ruled out. Wagner et al. (1999) noted that *Mollugo* species produce fruit capsules and inside seeds that lack means of external attachment for dispersal by animals. The present study is also in agreement with this report as all the three *Mollugo* species now studied do not have external structures that aid in the dispersal of seeds by animals. Therefore, seed dispersal by animals is totally ruled out.

Bittrich & Ihlenfeldt (1984) reported that *Mollugo* seeds germinate by means of an operculum. *M. cerviana* and *M. pentaphylla* propagate by seeds and reseed

themselves, often forming colonies. The present study showed that *Mollugo* species produce several batches of populations in a year and their seeds germinate as soon as they are dispersed but their germination is related to soil moisture which plays an important role in breaking the seed coat. As therophytes, these species are best adapted to survive in open dry habitats as they take advantage of any sign of temporary humidity that allows them to complete their life cycle quickly. Jurado et al. (1991) reported that *M. cerviana* does not form dense cover that inhibits other vegetation and compete well in crowded conditions. The present study also indicates that all the three *Mollugo* species do not grow in shaded habitats or form dense populations that inhibit other vegetation but *M. nudicaulis* and *M. pentaphylla* share insect pollinators along with other simultaneously flowering herbaceous taxa in certain habitats.

Brockington et al. (2009) reported that *Mollugo cerviana* is the only known  $C_4$  species in Molluginaceae. Edwards & Walker (1983) noted that the genus *Mollugo* contains  $C_2$ ,  $C_4$  and  $C_2$ - $C_4$  species. Christin et al. (2010) reported that *M. cerviana* being a  $C_4$  species is distributed in hot arid regions of tropical and temperate latitudes. *M. nudicaulis* is a  $C_2$ - $C_4$  species while *M. pentaphylla* is a  $C_2$  species but both are distributed in tropical and subtropical regions of the world. Raghavendra et al. (1978) reported that *M. nudicaulis* produces some leaves with  $C_2$  characteristics and some other leaves with  $C_4$  characteristics according to their position on the stem. Sage et al. (1999) documented that  $C_2$ - $C_4$  photosynthesis is believed to be a relatively rare condition in plants and only a few dozen species have been identified so far, many of which belong to *Flaveria* (Asteraceae). The present study shows that *M. pentaphylla* with  $C_2$  photosynthesis usually occurs in dry habitats displaying the sparse growth of a few other prostrate or erect herbs and the presence of insect pollinators although they grow in cultivated lands that enable herbaceous flora, especially weeds and insect pollinators thrive well. Their occurrence in habitats with scanty or robust vegetation indicates that  $C_2$  photosynthesis does not facilitate them to grow in habitats without any vegetation or insect pollinators. On the contrary, *M. cerviana* with  $C_4$  photosynthesis grows only in dry habitats which are almost devoid of other vegetation and also devoid of pollinator fauna. This finding is in line with the statement by Lundgren et al. (2015) that  $C_4$  species are usually abundant in warm but not in cool environments and this photosynthetic pathway is physiologically advantageous for their niche broadening in warm environments. *M. nudicaulis* with  $C_2$ - $C_4$  photosynthesis is versatile

to flourish well both in dry habitats and cultivated areas with scanty and robust vegetation comprising of herbaceous flora that support insect pollinators. The  $C_3$ - $C_4$  photosynthetic pathway enables this species to grow in warm and cool habitats which in turn enables it to be widespread and abundant. Vogan et al. (2007) reported that of all  $C_3$ - $C_4$  intermediates, *M. nudicaulis* and *M. verticillata* are the most widespread and also abundant. These species are found in hot, ruderal habitats where competition is low and the potential for photorespiration is high. Their ability to survive in such habitats is likely due to their  $C_3$ - $C_4$  pathway. Their ecological success demonstrates that  $C_3$ - $C_4$  intermediacy is a successful photosynthetic pathway in its own right and not merely a transitional phase to  $C_4$  photosynthesis. Lundgren & Christin (2017) also reported that  $C_3$ - $C_4$  taxa are remarkably widespread across geographical and environmental space, maintaining their ability to exist in both typical  $C_3$  and  $C_4$  niches. Because, the physiology of  $C_3$ - $C_4$  species does not strongly restrict the migration of species geographically or into new environments and it is a lineage that converges towards warm habitats to facilitate the transition to  $C_4$  photosynthesis, effectively bridging the ecological gap between  $C_3$  and  $C_4$  plants. *M. pentaphylla* with  $C_3$  photosynthesis, *M. nudicaulis* with  $C_3$ - $C_4$  photosynthesis and *M. cerviana* with  $C_4$  photosynthesis have developed different pollination mechanisms to maximize fruit and seed set rate. Genetic variation achieved through insect pollination in all these species, except *M. cerviana*, is essential to broaden their ecological niches since they grow both in dry and moist habitats. In *M. cerviana*, genetic variation achieved through thrips pollination is important to expand and invade dry habitats.

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## POLLINATION ECOLOGY OF THE SPECIES *MOLLUGO CERVIANA* (L.) SER. (MOLLUGINACEAE)

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**KEYWORDS:** *Mollugo cerviana*, soil binder, facultative autogamy, anemochory, ombrohydrochory, hydrochory.

### ABSTRACT

*Mollugo cerviana* is an annual herb which usually grows throughout the year in open dry sandy and sandy-loamy soils, but also occurs in moist habitats, especially in cultivated lands. *Haplothrips* uses the flowers for breeding and feeding; the feeding activity affects pollination. The flowers have specialized floral structural and functional behaviours for self-induced and spontaneous autogamy while keeping the options open for insect pollination after anthesis; it is facultative autogamous which is reflected in pollen-ovule ratio and natural fruit and seed set rates. Seed dispersal modes include anemochory, ombrohydrochory and hydrochory.

**ZUSAMMENFASSUNG:** Bestäubungsökologie von *Mollugo cerviana* (L.) Ser. (Molluginaceae).

*Mollugo cerviana* ist ein einjähriges Kraut, das in der Regel das ganze Jahr über auf offenen trockenen, sandigen und sandig-lehmigen Böden wächst, aber auch in feuchten Lebensräumen, vor allem in Kulturlandschaften, vorkommt. *Haplothrips* verwendet die Blüten zur Aufzucht und Fütterung; die Nahrungsaufnahme beeinflusst die Bestäubung. Die Blüten haben sich auf ein floral strukturelles und funktionelles Verhalten selbstinduzierter und spontaner Autogamie spezialisiert, während die Möglichkeiten für Insektenbestäubung nach der Anthese offen bleiben; die Pflanze ist fakultativ autogam, was sich im Pollen-Samen-Verhältnis und den natürlichen Frucht- und Samensatzraten widerspiegelt. Ihre Samenausbreitungsmodi umfassen Anemochorie, Ombrohydrochorie und Hydrochorie.

**REZUMAT:** Ecologia polenizării la *Mollugo cerviana* (L.) Ser. (Molluginaceae).

*Mollugo cerviana* este o plantă anuală care crește în mod normal pe soluri uscate, nisipoase și nisipo-argiloase, pe tot parcursul anului, dar se dezvoltă și în habitate umede, în special în locuri cultivate, *Haplothrips* folosește florile pentru reproducere și hrănire, Aportul alimentar afectează polenizarea. Florile se specializează în comportamentul structural și funcțional floral pentru autogamia auto-indusă și spontană, în timp ce posibilitățile de polenizare a insectelor rămân deschise după anteză; este autogamă facultativă, ceea ce se reflectă în raportul dintre semințele de polen și rata naturală a ratelor de fructe și semințe. Modurile de propagare a semințelor includ anemochoria, ombrohydrochoria și hidrochoria.



## INTRODUCTION

Pollination is an important part of plants' life (Solomon Raju, 1998), a key element for mangrove flora ecology and conservation (Aluri, 2013). It is a successful tool for maximizing the gene flux (Almeida-Soares et al. 2010). The genus *Mollugo* is native to tropical and a warm temperate part of North and South America, but it is also distributed in Europe, Africa and Asia. The name derives from the Latin word "mollis" meaning soft (Short, 2002). *M. cerviana* is native to India, Sri Lanka, Pakistan and Bangladesh (Parvathamma and Shanthamma, 2000). It is a C<sub>4</sub> species distributed in hot arid regions from pantropics to temperate regions (Christin et al., 2010). It is valuable in medicine for treating different diseases and ailments (Parvathamma and Shanthamma, 2000; Rajamanikandan et al., 2011; Sahu et al., 2012).

Scientists know little about the pollination ecology of Molluginaceae. In this family, nectar-secreting tissue is present in almost all species. In several genera, showy sepals or petals have evolved, both of which strongly suggest entomophily (Watson and Dallwitz, 1992; Kubitzki et al., 1993). Syrphid fly, *Mesogramma marginata* pollinates *Mollugo verticillata* (Robertson, 1928). The most widely spread, weedy species of *Mollugo verticillata*, *M. nudicaulis*, and *M. cerviana* are self- and insect-pollinated (Pax and Hoffmann, 1934; Bogle, 1970). In Taiwan, *M. pentaphylla* is a minor pollen source for *Apis mellifera* (Lin et al., 1993). In South India, honey bees use *Mollugo* species as a pollen source and reciprocate the plants with pollination (Ponnuchamy et al., 2014). This study was done to investigate how *M. cerviana* is able to reproduce in semi-dry and dry habitats with scarce pollinators. The objective of the present study is to know how various aspects pollination ecology contributes for the reproductive success through seed mode in dry habitats.

## MATERIAL AND METHODS

*Mollugo cerviana* wild patches grow in open dry and semi-dry areas of Visakhapatnam and its surroundings (17°42'N latitude and 82°18'E longitude) were selected for study during March 2015-May 2017. Field trips were conducted to record phenological aspects. Ten inflorescences which have not initiated flowering on five plants were tagged and followed to record anthesis schedule and the timing of anther dehiscence. Twenty five fresh flowers were used to record the floral morphological details. Nectar could not be measured and analysed due to its secretion in minute quantity which was further depleted by thrips during mature bud and flower life. Twenty mature, but un-dehisced anthers, two anthers each per flower/plant from ten plants were collected and examined for pollen output as per the protocol described in Dafni et al. (2005). The calculation of pollen output per flower and pollen-ovule ratio was done as per the formulas described in Cruden (1977). Ten flowers each from five individuals were used to test stigma receptivity. It was tested with hydrogen peroxide from mature bud stage to flower closure/drop as per Dafni et al. (2005). Seventy inflorescences were tagged prior to the initiation of their flowering and followed for three weeks to record fruit and seed set rate in open-pollinations. The fruit and seed morphological characteristics were observed in detail to evaluate their adaptations for dispersal by different means. Fields visits were made during rainy season to note the aspects of seed germination and production of new plants. Based on the timings of maturation of anthers and receptivity of stigmas, the sexual system was defined and also elaborately explained its functionality to achieve self-induced autogamy, spontaneous autogamy, geitonogamy, and xenogamy. The positions of stamens and stigmas during and after anthesis were observed to evaluate how they facilitate spontaneous autogamy during anthesis and flower closure. Further, observations were also made to evaluate as to how these positions preclude self-pollination when flowers stay open. The flower buds were used by thrips for breeding and feeding and in this context their role in pollination was observed.

## RESULTS

**Phenology.** The species is a small, glabrous, slender annual herb common in open dry sandy and semi-dry soils along roadsides, waste places, bare ground and dry river beds (Fig. 1a). Due to its very low ground habit, wiry reddish orange stems and thin linear leaves its presence is usually overlooked. The stems are numerous, upright, thin and stiff. Leaves are sessile, grey-green and linear with acute apex; they arise in whorls on the stem, but some are in a rosette at the base. The plant appears simultaneously in vegetative, flowering and fruiting phases in different populations growing in different habitats throughout the year (Fig. 1b). An individual plant, however, has a short life cycle of three months from seed germination to seed dispersal. Although it appears throughout the year, it shows robust vegetative growth and profuse flowering and fruiting during July-October when the soil is damp due to the occurrence of rains. The flowers are borne on seven-eight mm long pedicels in dichotomous and trichotomous umbellate cymes produced terminally or in leaf axils.



Figure 1a-n: *Mollugo cerviana*: a. Habitat with *Mollugo cerviana* and *M. nudicaulis*, b. *Mollugo cerviana* in flowering phase, c. and d. Flowering-opening phase, e. Position of stigmatic lobes and anthers at the same height contacting each other at anthesis, f. Dehiscent anthers, g. Pollen grain, h. Ovary with three stigmas, i. and j. Multi-ovuled ovary, k. Maturing fruit, l. and m. Dehiscent fruit capsule, n. Seeds.

**Flower morphology.** The flowers are small ( $2.52 \pm 0.4$  mm long,  $1.51 \pm 0.5$  mm wide), whitish green on adaxial side and green on abaxial side, odourless, actinomorphic and bisexual. A monochlamydeous perianth of five herbaceous scarious, elliptic to oblong,  $2.45 \pm 0.4$  mm long,  $1.13 \pm 0.2$  mm wide long free tepals with white margins represent sepals and petals. The stamens are five, anti-tepalous, free but connate at base, white,  $1.22 \pm 0.3$  mm long with dorsifixed, golden yellow, less than one mm long and dithecous anthers. The ovary is light green, tri-carpellary, tri-locular syncarpous with  $58.2 \pm 8.16$  D-shaped ovules arranged on axile placentation (Figs. 1i-j). The style is absent but the ovary is terminated with three free stigmas (Fig. 1h). The stigmas are minutely denticulate with membranous flaps.

**Floral biology.** Mature buds open during 07,00-08,00 h. Individual buds take five to 10 minutes from partial to a full opening (Figs. 1c-d). The flowers are homogamous as the anthers and stigmas attain maturity at the same time during anthesis; the former dehisce by longitudinal slits (Fig. 1f), and the latter continue receptivity until the noon of the 2nd day. The pollen output is  $159.7 \pm 14.5$  per anther and  $798.5 \pm 69.5$  per flower. The pollen-ovule ratio is 14:1. The pollen grains are pale yellow, spheroidal, tri-colpate, tri-zonoaperturate, granulated, tectum scabrate, and  $21.9 \pm 4.12$   $\mu$ M (Fig. 1g). The nectar secretes in traces during mature bud stage. The tepals with the stamens and stigmas close back by 10,00-11,00 h.

**Pollination mechanism and pollinators.** 20-35% of pollen grains found in dehisced anthers collected during anthesis possess pollen tubes indicating *in situ* germination. Further, the pollen tubes are also present on the stigma. The pollen germination and formation of tubes both within the dehisced anthers and on the stigma indicate the presence of self-induced autogamy. During and after anthesis, the dehisced anthers and receptive stigmas contact with each other due to their close proximity and their position at the same height due to which autogamy occurs (Fig. 1e). Further, the stamens and stigmas contact each other very closely during the closing of the flower assuring autogamy if it did not occur during the open state of the flower. Any insects never visited the flowers. *Haplothrips* sp. (Thysanoptera: Thripidae) used flower buds for breeding and flowers for feeding. The larvae emerged from the eggs in synchrony with anthesis and nectar production in flowers. The larvae and adults foraged for pollen and nectar. Pollen dusts individual thrips during their movements within the flowers. They carried 87 to 176 pollen grains on their body setae, wings and legs. The thrips dispersed the pollen on free denticulate and membranous stigmas due to their active movement, rubbing of abdomen against the stigmatic surface, cleansing of their body parts with their hind legs and also by their wing combing mechanism. The homogamous flowers were found to facilitate self-pollination in the same or different flowers of the same plant. As the plant occurs as small or large populations, thrips could fly to migrate to the flowers of other closely spaced plants and effect cross-pollination by feeding on the foliage.

**Fruiting ecology and seed dispersal.** Fruits mature within 8-10 days. The stamens and stigmas are persistent and remain inside due to the closure of the flower. The tepals bulge gradually and protect the bulging ovary in which the seeds form and mature (Fig. 1k). Natural fruit set is 91.27% and seed set is 61.94%. Fruit is a loculicidal three-valved broadly-ellipsoid capsule, stalked, membranous, and densely pubescent,  $2.35 \pm 0.36$  mm and  $1.85 \pm 0.23$  mm wide. The seeds are arranged in two rows in each locule. They are tiny, brown, shiny, D-shaped and faintly striate dorsally (Fig. 1n). The seed coat is studded with minute granular excrescences with reticulate ornamentation. Dry capsules break open when fruit pericarp and tepals are dry and expose the seeds (Figs. 1l-m). But the seeds remain and gradually separate and fall to the ground on their own on clear sunny days. On rainy days, the water droplets falling on the dehisced capsules washout seeds to the ground. Further, water acts as an efficient

dispersal agent for the dispersal of seeds fallen on the soil during rainy season. Seeds do not have adaptations for wind dispersal. But, wind disperses the dry cymes together with dry dehisced capsules to short distances and subsequently the seeds fall to the ground from capsules. Therefore, seed dispersal modes include ombrohydrochory, hydrochory and anemochory. The seeds produced from plants growing in cultivated lands have the potential to be dispersed as a cereal grain contaminant and in effect agricultural produce movement contributes to seed dispersal and expansion of its distribution.

### DISCUSSION

This study finds that *Mollugo cerviana* with its low ground habit populates the soil and for this reason is often called carpetweed. The plant grows throughout the year displaying vegetative, flowering and fruiting phases in different populations. However, the wet season confines its robust growth, profuse flowering and fruiting individual plants complete their life cycle within three months from seed germination to seed dispersal. Similarly, Owens and Lund (2009) reported that *M. cerviana* is a herbaceous ephemeral species and completes its life cycle in a very short time. This study finds that the inflorescence is a dichotomous or trichotomous umbellate cyme in *M. cerviana* suggesting that the soil moisture and nutrient environment regulate the branching of inflorescences and the production rate of flowers.

The floral descriptions of *Mollugo* species provided by different authors (Goncalves, 1978; Matthew, 1995; Pullaiah, 2000; Pullaiah and Mohammed, 2000; Bora and Kumar, 2003) are not accurate and/or complete. The present study provides details of the floral descriptions, especially of perianth, androecium and gynoecium in *M. cerviana* as these are important from the pollination of point view. In this species, perianth typically consists of five tepals which serve the function of calyx (sepals) and corolla (petals). The abaxial surface of the perianth serves the role of calyx while the adaxial surface of the perianth serves the role of corolla due to the display of two different colors on each surface. However, the perianth acting as both calyx and corolla is unable to attract any insect pollinators in pollinator-deprived or pollinator-available habitat. Such a situation explains that *M. cerviana* is not dependent on insect foragers for pollination. Ronse De Craene (2010) reported that in *Mollugo*, the androecium generally consists of five stamens alternating with the sepals. *M. cerviana* flowers produce a fixed number of five stamens, and all are opposite to tepals suggesting that there is no process evolving to produce flowers with three or four stamens. Further, the plant produces trimerous ovary with three stigmas; each carpel with a variation in ovule number. Despite the absence of vector-mediated pollination, the plant produces high fruit and seed set rates indicating that this plant is facultative autogamous.

Peter et al. (2004) reported that the temperature and relative humidity are probably important cues determining flower opening in the afternoon. In the present study, the anthesis during morning time in *M. cerviana* is attributable to its predominance in open, dry habitats where herbaceous flora usually does not grow. The absence of insect foraging activity on *M. cerviana* could be attributable to its common occurrence in pollinator-excluded or deprived habitats and production of tiny flowers which can be overlooked or unnoticed by foragers.

Bittrich (1990) reported that in Molluginaceae, *Adenogramma* is the only genus which produces one-seeded nutlets. All other genera produce capsules with many seeds which become exposed by loculicidal dehiscence. Soerjani et al. (1987) reported that *Mollugo pentaphylla* is hydrochorous. In the present study, *M. cerviana* produces fruits within or slightly more than a week time. The fruit is an ellipsoid 3-valved capsule and it breaks open to disperse seeds during sunlight days. But, on rainy days, the fruits when filled with water expel seeds and water violently. Wind also disperses dry cymes along with dry dehisced capsules to short distance and then seeds find their way into the ground. The seeds disseminated through these modes further dispersed by rain water during rainfall. Therefore, *M. cerviana* species is anemochorous, ombrohydrochorous and hydrochorous.

Narayana (1962) and Hofmann (1973) noted that *Mollugo* species produce seeds with a primordium-like swelling on the funiculus and this structure is considered to be a vestigial aril. *M. cerviana* produces tiny, brown, shiny, D-shaped seeds with a faintly striate dorsal surface. Minute granular excrescences with reticulate ornamentation stud the seed coat. Since the seeds of these plant species lack any aril or strophiole-like structure that usually serves as food for ants, the possibility for myrmecochory is ruled out. Wagner et al. (1999) noted that *Mollugo* species produce fruit capsules and inside seeds that lack means of external attachment for dispersal by animals. This study agrees with this report because *M. cerviana* lacks external structures to aid seed dispersal by animals and hence there is no possibility of seed dispersal by animals.

Bittrich and Ihlenfeldt (1984) reported that *Mollugo* seeds germinate by means of an operculum. *M. cerviana* propagates by seeds and reseeds itself, often forming colonies. It produces several batches of populations in a year, and the seeds germinate as soon as they disperse, but their germination is related to soil moisture which plays an important role in breaking the seed coat.

As a therophyte, this species it is best adapted to survive in open dry habitats as it takes advantage of any sign of temporary humidity that allows it to complete its life cycle quickly. Jurado et al. (1991) reported that *M. cerviana* does not form a dense cover that inhibits other vegetation and compete well in crowded conditions. The present study also indicates that *M. cerviana* does not grow in shaded habitats or form dense populations that inhibit other vegetation.

Brockington et al. (2009) reported that *Mollugo cerviana* is the only known C<sub>4</sub> species in Molluginaceae. Christin et al. (2010) reported that *M. cerviana* being a C<sub>4</sub> species is distributed in hot, arid regions of tropical and temperate latitudes. The present study also shows that *M. cerviana* with C<sub>4</sub> photosynthesis grows only in dry habitats which are almost devoid of other vegetation and also devoid of pollinator fauna. This finding is in line with the statement by Lundgren et al. (2015) that C<sub>4</sub> species are usually abundant in warm but not cool environments and this photosynthetic pathway is physiologically advantageous for their niche broadening in warm environments. In *M. cerviana*, genetic variation achieved through thrips pollination is essential to expand and invade dry habitats.

### CONCLUSIONS

*Mollugo cerviana* as an annual facultative autogamous therophyte grows throughout the year in open dry sandy and sandy-loamy soils, and also in moist habitats. The flowers have specialized floral structural and functional behaviors for self-induced and spontaneous autogamy while keeping the options open for insect pollination after anthesis but the insects never visited the flowers. Seed dispersal is polychorous involving anemochory, ombrohydrochory and hydrochory. The seeds germinate immediately after dispersal, but soil moisture is required to rupture the seed coat. The plant is best adapted to survive in open dry habitats as it takes advantage of any sign of temporary humidity to complete its life cycle quickly and acts as a soil binder and also moisture accumulator in the root zone.

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# Balancing The Pond Environment Through Probiotics For Enhancing Environmental Conditions In A Shrimp Farm To Achieve Improved Margins.

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## Abstract

Development of shrimp farming in the state of Andhra Pradesh, India grew at a phenomenal rate during the years 1990-1995. In 1990, a total of 6,000 ha was under shrimp farming and this rose to 88,300 ha during 1997-98. Presently about 78,700 ha is under culture which accounts for more than 50% of the brackish water area potentially available in the state. East Godavari district has an area of 10,807 sq. km, with a population of 51,54,296 (2011 census). It is a major rice producing district with 52% of the total area of the district under rice cultivation. This district ranks fourth in shrimp farming with a production capacity of 40368 tonnes. Generally, farmers culture tiger shrimp (*Penaeus monodon*) because of its high market value.

In Andhra Pradesh, India the shrimp farmers facing a challenge continuously to improve farm efficiency. The key parameter here is water quality, and that is a big challenge to maintain the right balance of pond environment. It requires an accurate estimation of inputs such as feed, algal production, waste effluent and environmental impacts of aquaculture operations. The farmers need to minimize the release of waste output by making improvements in feed quality as well as feed efficiency with better feed conversion ratios (FCR).

Managing the release of feed waste into the pond is also through better farm production practices and feed management. The goal is good margins through optimal survival rates and reduced disease-related losses. When we cannot manage a balanced pond environment, disease outbreaks occur. The worse scenario is high shrimp mortality. But first, it is important to understand the farm environment- from how much water exchange is required and probiotics to use, and how to control the feeding program (feeding rate and frequency). Probiotics can be applied directly into the pond water or supplemented in feeds.

Keywords: Probiotics, Photosynthetic Bacteria, Uni Light, Feed conversion ratios

## Introduction

PSB (Photosynthetic bacteria), just as the name suggests, are a special and unique class of microorganisms that has the ability to convert light energy into chemical energy using their light-absorbing pigments and reaction centres. These bacteria contain a compound known as bacteriochlorophyll which works similarly as chlorophyll in plants and enables them to perform the process of photosynthesis. Scientists are putting great importance in the study of these intriguing PSB (Photosynthetic bacteria) as they believe that the study of their photosynthesis and evolution might unlock some of the locked mysteries of how the world evolved and might help understand the process of potential life survival in uninhabitable.

There are mainly three types of photosynthetic bacteria: Chlorobiaceae, Chromatiaceae and Rhodospirillaceae

We can say that the process of photosynthesis in PSB (Photosynthetic bacteria) resembles the photosynthesis of plants as not everything is known and discovered by the scientists about PSB yet. In the photosynthesis process, the light energy from the sun is converted into chemical energy on the cell membrane of bacteria that acts as a shield to protect and cover the bacteria. Depending on the surface area, the reaction centres are available on cell membranes in the form of tubes, sacs or sheets. These are used to absorb light energy. The energy is used to produce Carbohydrates for the bacteria as their food source which later on contributes to all the materials of the microorganism.

Chlorobiaceae bacteria are green bacteria and also sulphur bacteria that perform anoxygenic photosynthesis. It works as an obligate photoautotroph and uses reduced sulphur species as electron donors. Rhodospirillaceae is purple bacteria in rod-shape, whose length can vary. These purple colour Rhodospirillaceae bacteria are eye-catching and easily identifiable. Hydrogen gas is used as an electron donor by these purple bacteria and can also use malate or succinate. There is another purple microorganism known as Chromatiaceae which are short gram-negative rods. These bacteria use sulphide and sulphur as their electron donor.

#### **Review of Literature on Photosynthetic Bacteria:**

Photosynthetic features were found in purple bacteria before the 19th century as they showed movement and growth when it came into contact with light. A German botanist S. Winogradsky found that some purple bacteria used hydrogen sulphide to sulphate with intracellular deposition of sulphur. In 1930, C.B. Van Niel demonstrated anoxygenic photosynthesis is the primary mode of energy-releasing metabolism in green and purple bacteria. On the other hand, S., a German botanist observed that some purple bacteria can utilize hydrogen sulphide to sulphate (1930) defined various metabolic versions of and demonstrated that it is the characteristic mode of energy-yielding metabolism in both purple and green bacteria. Hydrogen is used as donors in place of water which helped to reduce CO<sub>2</sub> to NADPH<sub>2</sub> and atmospheric nitrogen is also reduced to ammonia. In 1975, Parson and Cogdell isolated functional complexes from photosynthetic bacteria. It was found that the reaction centre from the purple and non-sulphur bacteria contains 4 molecules of chlorophyll, 2 molecules of bacteriopheophytin, 1 or 2 molecules of ubiquinone, and 1 atom of ferrous iron along with three polypeptides. The various photosynthetic bacteria are further classified into 35 different groups wherein group 10 consists of anoxygenic phototrophic bacteria (purple and green bacteria) and group 11 consists of oxygenic phototrophic bacteria (cyanobacteria). There is another kind of oxygenic bacteria was discovered recently which was placed under Prochlorophyta that is like a bridge between Chlorophyta and Cyanophyta.

#### **Application of PSB (Photosynthetic Bacteria)**

When it comes to man-made ecosystems like tanks, ponds or aquariums, in order to have control over the processes and procedures of the aquatic life, PSB is used. It helps in the health management of the aquaspace and benefits the aquatic life like shrimps, or fish. It also helps degrade organic waste and keeps the aquarium clean. Due to the tropical weather of Asia, the purple non-sulphur bacteria are normally preferred to be used in hot spring environments, marine, freshwater, and soil.

The bacterial cell wall of these bacteria is more digestible. Also, vitamins, proteins, biological cofactors, and carotenoids are found in abundance in these bacteria. A little addition of these PSB helps stimulate the growth of fish and shrimp. It also improves the production of scallop seed and increases the fish larvae survival rate.

Different experiments were performed which shows that using PSB reduces the damage to larvae production and their survival rate is much higher. Many PSB products claim to have multiple effects such as improve water quality, prevent diseases and increases growth rate, all the same time. The use of PSB can help maintain an aquarium or pond in better condition and can increase shrimp production too.

#### **Materials & Methods**

The key target of the research on probiotics for aquaculture is to enhance the environmental conditions in a farm, towards a balanced pond condition for the best growth of fish and shrimp. PSB is very efficient and has the following features for aquaculture. It can live and work very well in pond sediments, enhancing decomposition of organic matter and reducing nitrogen compounds. It easily removes hydrogen sulphide (H<sub>2</sub>S) in pond sludge. The bacteria only requires sunlight for energy and carbon source to eliminate nitrites. Products from organic waste decomposition are suitable for algae growth and facilitate stable water conditions in ponds. It inhibits proliferation of microbes and pathogens, altering the pond microbial populations when it becomes the dominant species in the pond water.

## Results

### Changes in nitrite, ammonia and *Vibrio* levels

Trials were conducted in the Laboratory. Three treatment and three control ponds, each of 3,000m<sup>2</sup> at the farm in Sakhineptipalli having coordinates 16.4243° N, 81.7185° E were used. Stocking density was 220 post larvae (PL)/m<sup>2</sup> and the duration of the trial was 90 days.

For the first 2 months, the probiotic dosage was 3L/1,000m<sup>3</sup> (20L/acre)/ week, applied at a frequency of twice a week. Therefore, each treatment pond used 9L/week of the probiotic. Direct application into pond water was at 8-10am during sunny mornings. After the second month, the probiotic was incorporated into the feed at IL PSB/30kg shrimp feed and fed twice/week. The pond water parameters studied were temperature, salinity, dissolved oxygen, ammonia-nitrogen (NH<sub>3</sub>-N), nitrate (NO<sub>3</sub>), H<sub>2</sub>S, zooplankton, phytoplankton, benthos and pond bottom conditions. Shrimp growth performance parameters included growth rate, survival rate, FCR and harvest yields.

Measurements of H<sub>2</sub>S, NH<sub>3</sub>-N, and NO<sub>3</sub> in treatment ponds after 90 days of culture were 30% lower. This demonstrated the efficiency of the probiotics to maintain good water quality. However, the critical issue was to have a stable environment as a large algal bloom would cause a huge difference in the day and night levels of ammonia and pH. After 3 days, pond water was lighter, and algae density became more stable. A comparison of the pond bottom sludge also showed an improvement.



Shrimp Pond in Sakhinetipalli Village

Probiotics can inhibit *Vibrios*. The changes were shown over the 90 days of culture with 20 times lower density of *Vibrios* in the treatment ponds as compared to that in control ponds (Table 3). We know that early mortality syndrome (EMS), white faeces syndrome (WFS) and running mortality syndrome (RMS) will likely occur when density of *Vibrio* spp. rises. If we can control the *Vibrio* populations to a stable level, we can avoid WFS etc. With regards to survival rates, it was 10% higher in the treatment ponds as compared to that in the control ponds.



Figure. 1 A B

**Figure 1.** Changes in pond water colour and quality, before and after 3 days of PSB application, measured at 15.30 pm.

A. Before probiotic application-deep green colour; pH 9.0 and ammonia 1 ppm.

B. Three days after probiotic application, light green colour; pH 8.5, ammonia 0 ppm

	UNI Light				Control			
	T1	T2	T3	Mean±SD	C1	C2	C3	Mean±SD
SR%	86.2	90.3	81.5	86±0.04a	82.7	82.2	69.7	78.2±0.07b
FCR	1.18	1.02	1.32	1.17±0.15a	1.22	1.08	1.55	1.28±0.24a

Superscripts with different letters within rows are significantly different (p<0.05)

**Table 1.** Improvements in survival rate and feed conversion ratio (FCR) with application of Uni Light PSB in the treatment ponds

However, pond and farming conditions will vary from one location to another, depending on whether there are constraints with water exchange and weather conditions. For example, water turbidity is higher with adverse conditions such as during the rainy season. Therefore, we need to develop suitable protocols for the probiotic for all culture systems and provide the best solutions for farmers.

	Treatment ponds	Control ponds
Stocking density (PL/m <sup>2</sup> )	220	220
Days of culture	90	90
Harvest size (g)	15.85	14.24
Survival (%)	86%	78.20%
FCR	1.17	1.28
<i>Vibrio</i> (CFU/mL)	1.2x10 <sup>2</sup>	3.8x10 <sup>3</sup>
Income from sales (USD)	2,27,159	1,83,113
Probiotic cost (USD)	574	0
Margin (%)	56%	43%

Table 2. Comparison of pond parameters in the treatment of ponds with Uni Light PSP applications and controlled ponds at a farm in Sakhinetipalli

Days of Culture (DOC)	Total bacteria	Ponds with addition of Uni-Light			Control Ponds		
	(CFU/mL)	T1	T2	T3	C1	C2	C3
30	<i>Aeromonas</i>	1.4 x 10 <sup>2</sup>	1.0 x 10 <sup>2</sup>	1.1 x 10 <sup>2</sup>	1.5 x 10 <sup>2</sup>	1.3 x 10 <sup>2</sup>	1.2 x 10 <sup>2</sup>
	<i>Pseudomonas</i>	1.2 x 10 <sup>2</sup>	1.1 x 10 <sup>2</sup>	1.1 x 10 <sup>2</sup>	1.3 x 10 <sup>2</sup>	1.5 x 10 <sup>2</sup>	1.2 x 10 <sup>2</sup>
	<i>Vibrio</i>	3.4 x 10 <sup>2</sup>	3.8 x 10 <sup>2</sup>	2.5 x 10 <sup>2</sup>	1.3 x 10 <sup>2</sup>	2.0 x 10 <sup>3</sup>	4.2 x 10 <sup>3</sup>
60	<i>Aeromonas</i>	25.7 x 10 <sup>2</sup>	31.5 x 10 <sup>2</sup>	27.1 x 10 <sup>2</sup>	57.8 x 10 <sup>2</sup>	5.7 x 10 <sup>3</sup>	85.3 x 10 <sup>2</sup>
	<i>Pseudomonas</i>	21.4 x 10 <sup>2</sup>	40.1 x 10 <sup>2</sup>	29.6 x 10 <sup>2</sup>	72.8 x 10 <sup>2</sup>	31.2 x 10 <sup>3</sup>	54.7 x 10 <sup>2</sup>
	<i>Vibrio</i>	1.7 x 10 <sup>2</sup>	3.3 x 10 <sup>2</sup>	2.1 x 10 <sup>2</sup>	4.8 x 10 <sup>2</sup>	3.5 x 10 <sup>3</sup>	3.3 x 10 <sup>3</sup>
90	<i>Aeromonas</i>	34.3 x 10 <sup>2</sup>	57.2 x 10 <sup>2</sup>	45.0 x 10 <sup>2</sup>	69.5 x 10 <sup>2</sup>	2.5 x 10 <sup>3</sup>	61.8 x 10 <sup>2</sup>
	<i>Pseudomonas</i>	27.2 x 10 <sup>2</sup>	43.4 x 10 <sup>2</sup>	1.2 x 10 <sup>2</sup>	3.8 x 10 <sup>3</sup>	74.5 x 10 <sup>2</sup>	81.8 x 10 <sup>2</sup>
	<i>Vibrio</i>	1.2 x 10 <sup>2</sup>	2.7 x 10 <sup>2</sup>	35.2 x 10 <sup>2</sup>	1.2 x 10 <sup>3</sup>	2.7 x 10 <sup>3</sup>	3.2 x 10 <sup>3</sup>

Table 3. Inhibition of *Vibrio* spp. with application of PSB probiotic over the 90-day production cycle

## Discussion

When faced with an environmental imbalance, PSB probiotics can help improve and maintain water quality and solve some of the disease and quality issues. For example, shrimp with yellow or black gills will command a lower market price. The application of the PSB probiotic improved pond bottom conditions and mitigated such problems. The benefits are 10% higher margins than from a pond without the use of the probiotic. This is the result of better harvest with higher growth and survival rates and improvements in FCR.

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# Benefits of Renewable Energy Sources to the Environment

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## ARTICLE DETAILS

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## ABSTRACT

All non-renewable energy sources have great impact on Environment. Non-Renewable energy sources (fossil fuel, Petrol, Coal, natural gas, oil, etc.) will become great threat to us in the future. We have to explore another alternative to produce electricity. Renewable energy sources (solar, wind, Geothermal, Biomass, Hydrothermal, Ocean, etc.) will be that problem. With electricity became a basic requirement from day to day, renewable sources will become a good solution not only for our requirement but also to the environment. This review paper briefly describes, Why should we have to prefer renewable energy sources than non-renewable energy sources?. The effects we will face in the future by using non-renewable sources and how we will be benefited by renewable energy sources.

## 1. Introduction

Carbon is the main element in fossil fuels. For this reason, the time period that fossil fuels formed (about 360-300 million years ago) is called the Carboniferous Period. Over time, the dead plants were crushed under the seabed. Rocks and other sediment piled on top of them, creating high heat and pressure underground. In this environment, the plant and animal remains eventually turned into fossil fuels i.e. coal, natural gas, petroleum. These are abundant and inexpensive but impossible to reuse. When they are burnt, they produce large amount of carbon dioxide into the atmosphere. This gas is the greatest defaulter in producing global warming. The carbon dioxide keeps the heat in the atmosphere of the earth. This process is called the "greenhouse effect." Life on Earth requires heat to survive, but it is based on a balanced carbon budget. To protect Environment, control pollution and the potential for global warming may encourage wider access to alternative energy sources such as renewable energy source [1]. All these sources are renewable process of energy generation and do not cause environmental pollution.

## 2. Environmental impact of non-renewable energy source

There are two types of sources for producing electric power energy.

- Conventional Sources (Non renewable sources)- fossil fuel, Petrol, Coal, natural gas, Oil
- Non Conventional Sources (renewable sources)- solar, wind, Hydro, Geothermal, Biomass, Hydrothermal, Ocean, Tidal Energy.

For industrialized nations like the United States, without fossil fuels, we can not imagine modern life. The majority of our

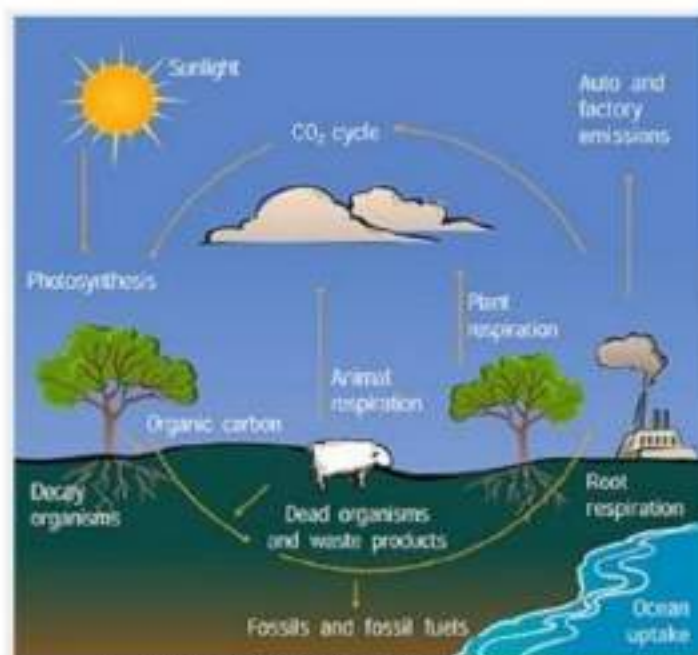
transport and heating, and the power stations we use to generate electricity depend on burning coal, oil or natural gas.. However, fossil fuel production also carries severe economic, environmental and social costs.. Whenever we burn a fossil fuel, we are producing more carbon dioxide, toxic gases into the atmosphere, resulting in potentially devastating long-term contamination of land, air and water resources.

Acid rain refers to precipitation that carries sulfur and nitrogen compounds from the atmosphere to the ground. Negative environmental consequences include damage to vegetation and declining aquatic populations. Decreased agricultural production is also likely, since acid rain depletes the soil of nutrients that crops need to grow.

The temperature in the atmosphere increases due to the greenhouse effect. The result is an increase in global temperatures, the U.S. Environmental Protection Agency states. This phenomenon of climate change is associated with floods and heavy rain falls in many regions, as well as more frequent droughts and severe heat waves.

Fossil fuels are a major contributor to health-harming air pollution. The combustion process also creates nitrogen oxides that lead to the creation of smog, These materials can cause bronchitis and pneumonia, decrease resistance to respiratory infections and irritate the lungs. Power plant and transportation-related activities are about equally responsible for nitrogen oxide emissions

Coal-fired power plants also release pollutants that contaminate nearby soils. The transportation of oil raises the risk of spills that leave oceans and waterways uninhabitable for years to come [2].



Fossil fuel combustion is the part of carbon cycle

To protect the environment from these dangerous pollutants, we need cheap, natural and friendly environment production of Electricity resources are required. For this reason, the importance of renewable resources has increased. Renewable energy sources are sources that constantly renew throughout the human lifespan.

They are also referred to as Green Energy or Clean Energy as they do not emit carbon-di-oxide or other greenhouse gases. The major sources of renewable energy source are Solar, Wind, and Biomass Hydropower, Geothermal, and Tidal energy

All India the total installed capacity due to renewable energy sources is 62646.90MWh on 30-12-2018 [3].

While there are no global warming emissions associated with generating electricity from solar energy, there are emissions associated with other stages of the solar life-cycle, including manufacturing, materials transportation, installation, maintenance, and decommissioning and dismantlement.

The wind is one of the cleanest and most sustainable ways to generate electricity as it produces no toxic pollution or global warming emissions. Wind is also abundant, inexhaustible, and affordable, which makes it a viable and large-scale alternative to fossil fuels.

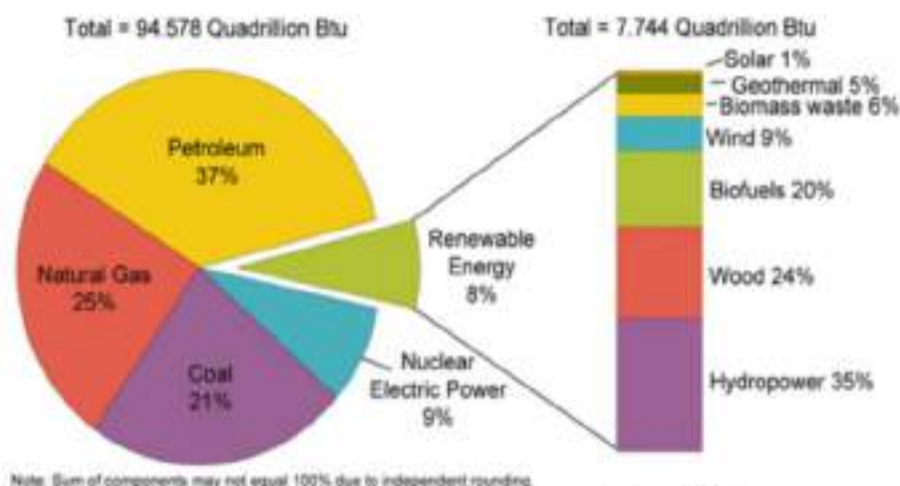
### 3. Nuclear Power

Nuclear power is energy released from the radioactive decay of elements, such as uranium, which releases large amounts of energy. Nuclear power plants produce no carbon dioxide and, therefore, are often considered an alternative fuel (fuels other than fossil fuels). Currently, world production

of electricity from nuclear power is about 19.1 trillion KWh, with the United States producing and consuming about 22% of that. Nuclear power provides about 9% of the electricity in the United States (Figure 7).

There are environmental challenges with nuclear power. Mining and refining uranium ore and making reactor fuel demands a lot of energy. Also, nuclear power plants are very expensive and require large amounts of metal, concrete, and energy to build. The main environmental challenge for nuclear power is the wastes including uranium mill tailings, spent (used) reactor fuel, and other radioactive wastes. These materials have long radioactive half-lives and thus remain a threat to human health for thousands of years. The half life of a radioactive element is the time it takes for 50% of the material to radioactively decay. The U.S. Nuclear Regulatory Commission regulates the operation of nuclear power plants and the handling, transportation, storage, and disposal of radioactive materials to protect human health and the environment.

By volume, the waste produced from mining uranium, called uranium mill tailings, is the largest waste and contains the radioactive element radium, which decays to produce radon, a radioactive gas. High-level radioactive waste consists of used nuclear reactor fuel. This fuel is in a solid form consisting of small fuel pellets in long metal tubes and must be stored and handled with multiple containment, first cooled by water and later in special outdoor concrete or steel containers that are cooled by air. There is no long-term storage facility for this fuel in the United States.



Note: Sum of components may not equal 100% due to independent rounding.

Figure 7. U.S. Energy Consumption by Energy Source, 2009

Renewable energy makes up 8% of U.S. energy consumption. Source: U.S. Energy Information Administration

There are many other regulatory precautions governing permitting, construction, operation, and decommissioning of nuclear power plants due to risks from an uncontrolled nuclear reaction. The potential for contamination of air, water and food is high should an uncontrolled reaction occur. Even when planning for worst-case scenarios, there are always risks of unexpected events. For example, the March 2011 earthquake and subsequent tsunami that hit Japan resulted in reactor meltdowns at the Fukushima Daiichi Nuclear Power Station, causing massive damage to the surrounding area.

The report outlined three broad strategies to shift to a circular economy. The use of products should be maximized, such as through car-sharing or keeping vehicles for longer, it said. Recycling and reducing waste are also key, as is using natural, low-carbon materials in construction, like bamboo and wood instead of cement, it said.

#### 4. Green ambitions — on renewable energy targets

In a surprising statement this month, Union Power Minister R.K. Singh said India would overshoot its target of installing 175 gigawatts of capacity from renewable energy sources by 2022. India was on track, he said, to hit 225 GW of renewable capacity by then. This is a tall claim, considering India has missed several interim milestones since it announced its 175 GW target in 2015. The misses happened despite renewable capacity being augmented at a blistering pace, highlighting how ambitious the initial target was. Technological and financial challenges remain: both wind and solar generation could be erratic, and India's creaky electricity grid must be modernised to distribute such power efficiently. Meanwhile, wind and solar tariffs have hit such low levels that suppliers are working with wafer-thin margins. This means small shocks can knock these sectors off their growth trajectories. The obstacles have capped capacity addition to 69 GW till date, with India missing its 2016 and 2017 milestones. To hit its 2022 target of 175 GW, 106 GW will have to be added in four years, more than twice the capacity added in the last four.

In the solar sector alone, which the government is prioritising, policy uncertainties loom large. Manufacturers of photovoltaic (PV) cells have demanded a 70% safeguard duty on Chinese PV imports, and the Directorate General of Trade

Remedies will soon take a call on this. But any such duty will deal a body blow to solar-power suppliers, who rely heavily on Chinese hardware, threatening the growth of the sector. There is also the problem of the rooftop-solar segment. Of the current goal of 100 GW from solar energy by 2022, 40 GW is to come from rooftop installations, and 60 GW from large solar parks. Despite being the fastest-growing renewable-energy segment so far — rooftop solar clocked a compound annual growth rate of 117% between 2013 and 2017 — India only hit 3% of its goal by the end of 2017, according to a Bloomberg New Energy Finance report. The reason? Homeowners aren't warming up to the idea of installing photovoltaic panels on their terraces because the economics does not work out for them. Compared to industries and commercial establishments, a home typically needs less power and will not use everything it generates. So, homeowners need to be able to sell electricity back to the grid, which in turn needs a nationwide "net-metering" policy. As of today, only a few States have such policies, discouraging users elsewhere. Such challenges can be overcome with the right incentives, but they will take time to kick in. The good news is that even if India hits the 175 GW target, it stands to meet its greenhouse-gas emission goal under the Paris climate agreement. This in itself will be a worthy achievement. Overshooting this target will be a plus, but until the government tackles the policy challenges, it must hold off on implausible claims.

#### 5. Conclusion

Burning fossil fuels gives out toxic gases like carbon dioxide. Trees, eco life, infrastructure and monuments get damaged due to pollution caused by this toxic gases. Burning fossil fuels also increases the greenhouse effect because of increased carbon dioxide emissions. Scientists are increasingly worried about global warming and the melting of polar ice-caps, rising sea levels, and changing weather patterns around the world. 7 million people die every year due to pollution. We argued that clean and low carbon energy is the only logical choice for our future. Renewable energy sources play a major role to modify these deficiencies. Due to this, There is no pollution or ecological balance problem. Several renewable energy sources are financially and economically competitive for certain applications.

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# Parasitic copepods in Carangid fishes from Manginapudi Coastal Waters Machilipatnam, A.P, India

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## Abstract

Carangid, any fish of the family Carangidae (order Perciformes), which contains more than 200 species of marine fishes, including such well-known forms as the jacks and pompanos. Carangids are swift, predatory, usually silvery fishes found throughout the world in warm and tropical regions. They are primarily marine, but some live in brackish water or may invade fresh water.

The members of the family vary greatly in form, from elongated and streamlined to very deep-bodied and thin from side to side. In general, however, they bear the following features in common: two dorsal fins, the first of which may be reduced to a few small spines; anal and second dorsal fins usually high in front; first two anal spines separated from the third; pectoral fins slim and often sickle-shaped; tail base very slender; tail strong, either forked or crescent-shaped; scales small; and a lateral line (a series of small sense organs along the sides of the body) often partly or wholly covered with large, hard, keeled scales (scutes).

In the present study, 68 fishes were infested out of 544 specimens examined from six different species of Carangid fishes which were collected from Manginapudi coastal waters. Eight species of parasitic copepods were found on gill filaments, body surface and nasal capsule regions. The maximum prevalence was recorded in *Carangoides malabaricus* (22.5 %) and minimum was noticed in (2.4 %) *Selaroides leptolepis*. The intensity of infection ranged from 1 to 1.2. Thus, considerable variation in the respiratory area was observed owing to the attachment of parasites in the infected fishes. *Caligus sp.* and *C. epidemicus* parasites were attached to body surface and only one *Sphyriid sp.* parasites were found in nasal capsule region. It is very difficult to estimate the actual harm to fish caused by the presence of parasites; if this is uneasy in cultured fish, it is almost impossible in feral fish populations. It should also be emphasized that the presence of a parasite does not necessarily imply manifestation of a disease. In aquaculture, some parasites are able to reproduce rapidly and heavily infect a large proportion of fish which may lead to diseases with significant economic consequences.

## Keywords

Carangoides, Copepod parasites, Mode of attachment, Respiratory surface area, Gill rack count, Manginapudi

## Introduction

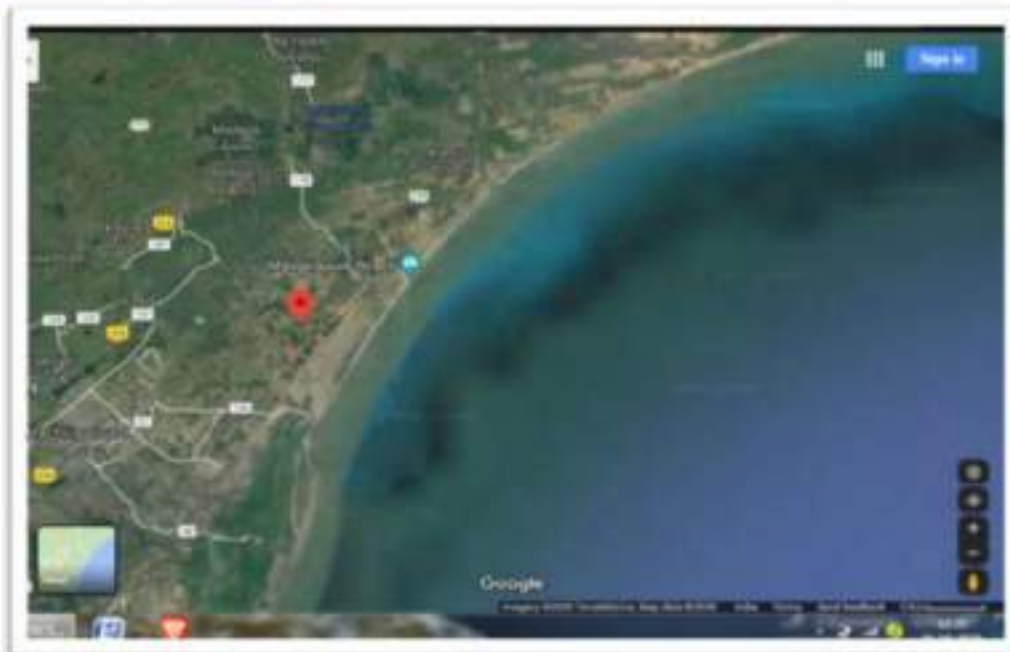
Many of the carangids are small, but some grow to a large size. The greater amberjack (*Seriola dumerili*), for example, reaches a length and weight of about 1.8 m (6 feet) and 70 kg (150 pounds). The members of the family are known by various common names. There are the moonfish, pompano, pilot fish, runner, jack (*qq.v.*), and others. One of the most unusual-looking carangids is the lookdown (*Selene vomer*), with an exceptionally thin body and high "forehead." The first rays of the second dorsal fin extend into filaments that reach to the tail. Many of these fishes are valued for food or sport. Certain species, however, such as the greater amberjack and several jacks, may at times carry a toxic substance in their flesh and, when eaten, cause ciguatera, a form of poisoning.



### *Carangoides malabaricus*

The diversity of parasitic copepods reported from deep mesopelagic and bathypelagic fish hosts is extremely low. Parasitic copepods are commonly found in cultured and wild marine fishes. In the aquaculture industry throughout the world, these parasitic copepods, particularly the family Caligidae, are important as pathogens causing heavy mortality or acting as disease inducers, by creating a portal for entry of bacterial or other pathogens (Johnson et al. 2004).

The gills are a favourite site for the attachment of several parasitic copepods. They damage the gills by feeding on the delicate tissue of the gill lamellae or on the blood circulating within the lamellae, leading to a loss of respiratory surface area (Lester and Hayward 2006). There is extensive gill damage and severe haemorrhage, with inflammation and exsanguinations associated with the attachment and feeding of the copepod (Lester and Hayward 2006). This, nowadays, has become a major problem in identification and treatment of parasites and diseases in the rapidly developing mariculture industry (Roza et al. 2002). More recently (Anil et al. 2019) recorded 16 species of parasitic copepods from the gill region of Sea bass in Manginapudi estuarine waters. But there is no detailed study on the infestation of copepod parasites in Carangid fishes of Manginapudi waters. The present study is the investigation on occurrence and infestation of copepod from Carangid species from Manginapudi coast.



Manginapudi Coast Coordinates: ( $16^{\circ}13'34.3''\text{N}$   $81^{\circ}12'15.8''\text{E}$ )

## Materials and methods

During a routine observation of the Carangid fishery in the Manginapudi ( $16^{\circ}13'34.3''\text{N}$   $81^{\circ}12'15.8''\text{E}$ ) an interesting incidence of parasitisation in Carangid fishes was observed. Fishes were thoroughly checked for parasitic infection in the body surface, fins, head, gill filaments, oral cavities and other tissue also examined. Each fish was examined microscopically for the presence of parasitic crustaceans based on a method described by (Kabata 1985; Anil et al. 2019). The collection and preservation methodology for crustacean parasites was followed by Pritchard and Kruse (1982). Copepod identification was based on morphological features according to Yamaguti (1963), Kabata (1979), Pillai (1985), Sirikanchana (2003), Ho and Kim (2004). Prevalence and mean intensity of each parasitic species were determined as in Margolis et al. (1982).

## Respiratory surface area

The influence of infestation in respiratory surface area of the gill arch of infected and uninfected fish were carefully dissected out and blotted to remove the moisture. The imprint drawing of each gill arch on millimeter graph was used to calculate the surface area of the gill arch. The surface area of each tracing was determined by counting the number of small squares and the total area was obtained. The value was taken and doubled to consider the total functioning of the gill arch. The total surface area of the gill arch of both infected and uninfected fish was compared and then area was considered as reduction of respiratory area due to infestation.

## Gill rack count

The average gill rack count of the 1st, 2nd and 3rd gill arch of infested fishes were taken. The data collected were tabulated and variation in the gill raker count as a function of infestation was recounted Results

## Infestation of fishes

In the present study, 68 fishes were infested out of 544 specimens examined from six different species of Carangid fishes which were collected from Manganapudi coastal waters (Table 1). Eight species of parasitic copepods were found on gill filaments, body surface and nasal capsule regions. These eight species belong to three genera Bomolochidae, Caligidae and Sphyrriidae. *Caligus* sp. (26) was found in highest number followed by *C. epidemicus* (17), *Holobomolochus chilensis* (13), *Parabomolochus bellones* (10) and *Bomolochus* sp. (8) which infested 23, 16, 11, 7, 8 species of Carangid fishes respectively (Table 2). While *Nothobomolochus* sp., *P. cuneatus* and *Sphyrriid* sp. were found in minimum (1) of Carangid fishes. The prevalence and intensity



of copepod parasites on Carangid fishes are presented in Table 3. Maximum prevalence was recorded in *C. malabaricus* (22.5 %) and minimum of (2.4) was noticed in *Selaroides leptolepis* (Fig. 1).

#### Mode of attachment of parasitic copepods

The distribution of copepod parasites in different species of Carangid fishes was reported. Maximum infection was recorded in *C. malabaricus* in the gill region and minimum was recorded in *Carangoides sp.* (gill region) and *S. leptolepis* in the nasal capsule Respiratory surface

Variation in the respiratory surface area of fish owing to the infestation of copepod parasites (*Caligus sp.*, *C. epidemicus*, *Bomolochus sp.*, *H. chilensis*, *P. bellones*, *P. cuneatus*, *N. sp.* and *Sphyriid sp.*) were studied (Fig. 2). Detailed study of respiratory surface area due to the infestation of copepods in Carangid fishes was carried out (Table 4). The maximum numbers of copepods (38) was noticed in the first gill arch and minimum numbers of copepods (5) was found in the fourth gill arch and 16 numbers of copepods were found in the second gill arch and seven numbers of copepods were found in the third gill arch. Thus, considerable variation in the respiratory area was observed owing to the attachment of parasites in the infected fishes. *Caligus sp.* and *C. epidemicus* parasites were attached body surface (10) and only one *Sphyriid sp.* parasites were found in nasal capsule region.

The infested fish had extremely pale gills, indicating the gill rakers were seriously lost, apical damage and out off gill lamellae were deployed. Some secondary gill lamellae were fused or thickened. Gill lamellae of the first and second arches of gill were found to be eroded due to parasites and the damage was found to be concentrated towards posterior position. Several damage have observed in the host of fishes, gill damage was major effect when a large section of filaments was destroyed and gill arch broken.

#### Discussion

*Caligus fortis* was first reported by Kabata (1965) from the nostrils of a yellow spotted trevally *C. fulvoguttatus* (Forsskal) reported as *C. emburyi* (Whitley) by Kabata (1965) caught off Green Island, Queensland. It was subsequently found in the nasal cavities of an unidentified jack (*Caranx sp.*) collected from Trivandrum, India by Prabha and Pillai (1986).

**Table.1 Parasitic copepods in Carangid fishes**

S.No	Name of the Host	No of Fishes Infested	Copepods	No of parasites Collected
1	<i>Carangoides malabaricus</i>	23	<i>Caligus sp.</i>	26
		5		5
		7		10
		1		1
2	<i>Alepes sp.</i>	8	<i>Bomolochus sp.</i>	8
3	<i>Gnathanodon speciosus</i>	11	<i>Holobomolochus chilensis</i>	13
4	<i>Carangoides sp</i>	1	<i>Nothobomolochus sp.</i>	1
5	<i>Selaroides leptolepis</i>	1	<i>Sphyriid sp.</i>	1
6	<i>Parastromateus niger</i>	11	<i>Caligus epidemicus</i>	12

**Table.2 Attachment site of parasite in carangid fishes**

S.No	Host	Parasites	Site of attachment
1	<i>Carangoides malabaricus</i>	<i>Caligus sp.</i>	Gill, body surface
		<i>Caligus epidemicus</i>	Gill, body surface
		<i>Parabomolochus bellones</i>	Gill
		<i>Parabomolochus cuneatus</i>	Gill
2	<i>Alepes sp.</i>	<i>Bomolochus sp.</i>	Gill
3	<i>Gnathanodon speciosus</i>	<i>Holobomolochus chilensis</i>	Gill
4	<i>Carangoides sp</i>	<i>Nothobomolochus sp.</i>	Gill
5	<i>Selaroides leptolepis</i>	<i>Sphyriid sp.</i>	Nasal capsule
	<i>Parastromateus niger</i>	<i>Caligus epidemicus</i>	Gill, body surface



*Carangoides malabaricus*  
(Bloch & Schneider 1801)



*Alepes sp.*



*Carangoides sp*



*Selaroides leptolepis*



*Parastromateus niger*



*Gnathanodon speciosus*

Table.3 Occurrence of Copepod parasites in carangid fishes

S.No	Name of the Host	No of Fish examined	No of Fish Infected	No of Parasites (%)	Prevalence (%)	Mean Intensity
1	<i>Carangoides malabaricus</i>	160	36	42	22.5	1.2
2	<i>Alepes sp.</i>	94	8	8	8.5	1
3	<i>Gnathanodon speciosus</i>	124	11	13	8.9	1.2
4	<i>Carangoides sp</i>	36	1	1	2.8	1
5	<i>Selaroides leptolepis</i>	42	1	1	2.4	1
6	<i>Parastromateus niger</i>	88	11	12	12.5	1.1
	Total	544	68	77	12.5	1.1

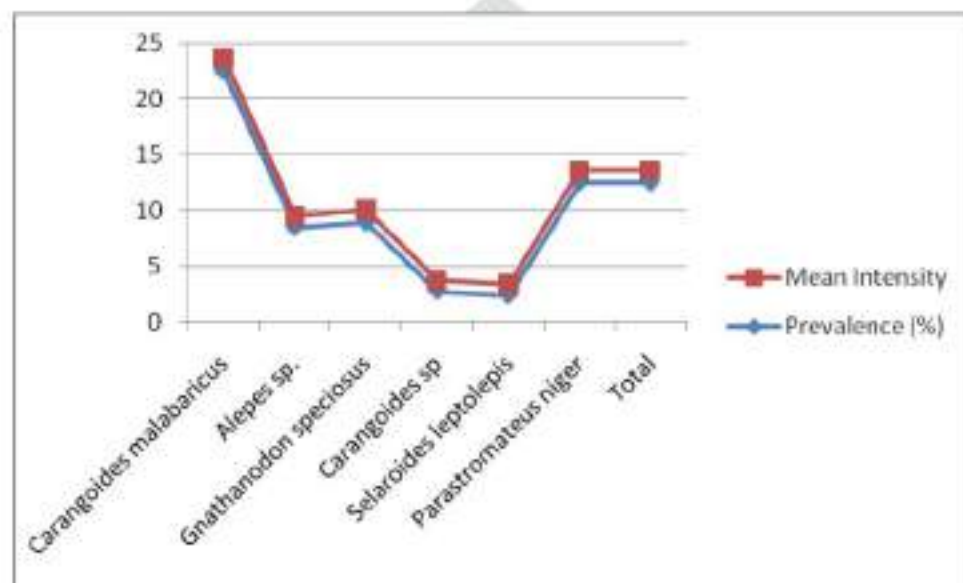


Fig. 1 Prevalence and mean intensity of copepod parasites in carangid fishes

In this study, *Sphyrriid sp.* was found in the Nasal capsule of *S. leptolepis*. *Caligus robustus* was circum-global in distribution, occurring on the carangid fishes in the tropical and sub-tropical oceans (Cressey 1991). However, it seems to be rare off Taiwan. *C. robustus* has a broader distribution than *C. fortis*. It has been reported from off Sri Lanka by Bassett-Smith (1898) and Kirtisinghe (1964), from Jamaica by Wilson (1913), from off Mauritania by Brian (1924), from the Gulf of Mexico by Bere (1936) and Causey (1953), from off India by Pillai (1985), and from off Borneo, the Celebes and the Philippines by Cressey (1991). The present study has reported the infestation of parasitic copepods on gills and Nasal capsule of Carangid fishes from Manginapudi coastal environments. Parasitic copepods especially *C. epidemicus* having a broader distribution than *Caligus spp.* were reported in the Manginapudi waters. According to Boxshall and Halsey (2004), Caligid, Ergasilid, and Lernanthropid copepods are known as common parasites of shallow water fish. Copepods of the family Ergasilidae are mostly known as freshwater parasites, and only few species are known from the brackish water or marine environment (Boxshall and Halsey 2004). The other collected copepod families (Bomolochidae, Caligidae, Lernanthropidae, Lernaeopodidae, Pennellidae, Siphonostomatoida and Tetraodontidae,) are mainly or exclusively known as marine fish parasites (Hallett and Roubal 1995; Boxshall and Halsey 2004; El-Rashidy and Boxshall 2012; Ho and Lin 2012; Ozak et al. 2012) *Sinergasilus polycolpus* and *Sinergasilus major* are over distributed on their respective hosts. Other parasitic copepods, such as Caligid copepods (Hallett and Roubal 1995) have been reported to be over disposed in their host populations. In this study, *C. epidemicus* were found in maximum number of

*C. malabaricus* and *P. niger* fishes. Three species of parasitic copepods, one each from the Siphonostomatoid families Lernanthropidae and Lernaeopodidae and one from the Cyclopoid family Bomolochidae, are redescribed based on material collected from the gills of four fish species belonging to the family Clupeidae caught from coastal waters off Alexandria, Egypt (El-Rashidy and Boxshall 2010).

Caligidae currently accommodates 33 genera, 445 species, more than 75 % are members of *Caligus* (239 spp.) and *Lepeophtheirus* (107 spp.) (Ho 2000). *Caligus* spp. is dominant on marine teleost fishes (Kabata 1979). In the present study, Caligidae has been found on body and gills of Carangid fishes. Many fish genera in this study had same parasites as found in India (Pillai 1985). Many factors have been suggested to influence the aggregation of parasite burdens (Quinnell et al. 1995). However, host resistance and behaviour are considered as important in generating variable parasite burdens (Tanguay and Scott 1992), and host susceptibility is proposed to explain the higher infection levels of *E. briani* in bream *Abramis brama* and tench *Tinca tinca* (Alston and Lewis 1994). *C. malabaricus* was infested with 4 copepod species and showed the highest percentage parasitic infestation followed by other Carangid species of parasitic infestation, respectively.



*C. epidemicus*



*Caligus* sp



*Bomolochus* sp.



*Nothobomolochus* sp.



*P. cuneatus*



*Sphyrriid* sp.



*H. chilensis*



*P. bellones*

Within the present study two species of Caligidae copepods were recorded from Manginapudi; five of them belonging to Bomolochidae and one species probably represents a Sphyrriidae genus. Yuniar et al. (2007)

reported *Mugil cephalus*, *Scatophagus argus*, *Eleutheronema tetradactylum*, and *Johnius coitor* had a species-rich copepod fauna. Six parasitic copepods were recorded from *M. cephalus*. Even though this fish species has a wide distribution and has been well studied for copepod parasites (e.g., Paperna and Overstreet 1981; El-Rashidy and Boxshall 1999), several copepods from the study represent new host records. Parasitic copepod *Pseudocycnus appendiculatus* at their gill filaments and this report documents a new record of the Andaman Sea, Thailand Purivirojkul et al. 2011). Six species of copepods belonging to the Lernanthropidae were found parasitic on the gill filaments of six species of marine fishes of Taiwan (Ho et al. 2011). A new species of *Ergasilus boleophthalmi* parasitic on the gills of two gobiid fishes *Boleophthalmus dussumieri* and *Bathygobius fuscus* from Shatt Al-Basrah Canal, Iraq, was described (Thamir et al. 2011). In the present study eight species of parasitic copepods were recorded from Carangid fishes.

A parasitic copepods study of Algerian teleost fish, report 25 copepod species belonging to eight families harvested from the gills of 14 fish species (Boualleg et al. 2011). *C. elongatus* has been recorded from more than 100 host species, both teleosts and even elasmobranchs, belonging to 47 families (Williams and Williams 1996). Yuniar et al. (2007) reported, seven out of eight fish species were infested with *Caligus spp.* The results of the present study also agree with the earlier works. In the present study it is reported that eight out of six fish species were infested belonging to three genera of copepods. According to Moller and Anders (1986), pranzia stages were recorded to infest a high number of different fish species. Most copepods from Segara Anakan were host-specific, with 19 species infesting only a single host fish species (Yuniar et al. 2007). The present study result also shows that *C. epidermicus* have the character of broad host specificity. It infects two different Carangid fish species but the host specificity of *Bomolochus sp.*, *H. chilensis*, *Nothobomolochus sp.* and *Sphyriid sp.* was very narrow. Both the species are found to infest only the host fishes of *Alepes sp.*, *Gnathanodon speciosus*, *Caligus sp.* and *S. leptolepis*. Host parasite relation is the outcome of the interaction of three factors: the host, the parasite and the environment (Moller 1985).

Prevalence and intensity of parasitic copepods on fish can vary with habitat, season, and host size (Hudson et al. 1994). The prevalence of infection in Saginaw Bay was not as high as in the Alabama ponds, where 100 % of the fishes were infected (Hayden and Rogers 1998). Mugridge et al. (1982) found 50–250 parasites/fish in British ponds and suggested that the reduced growth rate of roach may be caused by *Neoergasilus japonicus*. Ponyi and Molnar (1969) noted severe infections of *N. japonicus* in Hungary but provided no details on the intensity or effects. Effects of a parasitic copepod on the larval growth of the Chilean triplefin *H. chilensis* (Tripterygiidae) based on the microstructure of the sagittal otoliths (Palacios-Fuentes et al. 2012). There are reports that the low prevalence of *L. branchialis* in offshore areas might be attributed to the fact that infected fish remain close to shore (Sproston and Hartley 1941; Kabata 1958). In this study it is reported that the prevalence was maximum in *C. malabaricus* and minimum was noticed in *S. leptolepis* and mean intensity of parasitic copepods on fish vary from 1 to 1.2.

First gill arch preference has been previously reported for microcotylids (El Hafidi et al. 1998), as well as naobranchiids (Roubal, 1999), and it is known that abiotic factors affect the abundance of some monogeneans and copepods (Barker and Cone, 2000). Although less oxygenated and less ventilated than the posterior arches (Hughes and Morgan 1973), gill arch I is where the current flow is minimal (Paling, 1967) and thus, where monogeneans may be the least precariously attached as suggested by El Hafidi et al. (1998). The parasitic copepod *Haemobaphes diceraus* was found localized on the isthmus of two specimens of the walleye pollock *Theragra chalcogramma*. In both cases, the parasite directly penetrated the heart, without entering the blood vessels (Yu and Poltev 2010). In the present case, maximum reduction in respiratory surface area was noticed in the first gill compared to other gill arches. The explanation should be considered with caution, since specimens of *Meta microcotyla macracantha* can secure them by coiling around gill filaments (Baker et al. 2005). Further, some other microcotylids do not exhibit such a preference for the first arch (Lyndon and Vidal-Martinez 1994; Geets et al. 1997). A preference for gill arch I among naobranchiids has neither been investigated nor explained in previous studies. Kabata (1988) reported the adult naobranchiids display a secure mode of attachment, by firmly embracing the individual gill filaments using their modified second maxillae, it is not excluded that larvae are precariously attached when they first settle on the gills. In the

present investigation highest number of copepods was attached in gill filament. These parasitic copepods with neutral interactions have occurred in the first two arches and have decreased in the third and fourth gill arches.

Fish parasites are an integral part of water ecosystem and they are common in natural and cultured populations of fish. In natural conditions, most parasites do not tend to severely injure their hosts and cause mortalities which affect the population size at detectable levels. It is very difficult to estimate the actual harm to fish caused by the presence of parasites; if this is uneasy in cultured fish, it is almost impossible in feral fish populations. It should also be emphasized that the presence of a parasite does not necessarily imply manifestation of a disease. Diseases caused by parasites are much more frequently manifested in cultured fish, which suffer from artificial conditions and numerous stress factors that influence their ability to effectively protect themselves against parasitic infections. In aquaculture, some parasites are able to reproduce rapidly and heavily infect a large proportion of fish which may lead to diseases with significant economic consequences.

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