Insect pollination, fruit set and seed yield in *Sesamum indicum* L. (Pedaliaceae)

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Abstract
The investigations were carried out on the flowering phenology and floral biology, flower dynamics, pollen production, pollen viability, pollen: ovule ratio, stigma receptivity, nectar production, flower visitors dynamics, censuses and activity, flower visitors behaviour, pollen load carried out by foragers and role of insect pollination in increasing fruit set and seed yield in *Sesamum indicum* L (Pedaliaceae). The plants cultivated around Arvi Tahasil, Dist. Wardha (M.S.) were selected for studies. In *S. indicum* In *S. indicum*, flowering starts form second week of August and full bloom was during last week of August and ends towards first week of October. Upper leaves lanceolate, entire, the lower often cut at the base into 2 or more serrate segments, glabrous above, puberulous beneath, flower pinkish-purple, corolla pubescent outside. The anther dehiscence takes place during 07.30 hrs to 08.00 hrs. Stigma becomes receptive during the flower opening at about 09.00 hrs. to 08.30 hrs. The pollen production was 3838.23 ± 463.84pollen grains per flower. Percentage of pollen viability in T.T.C. was found to be 94.56% Pollen: ovule ratio was 6069.85.The stigma becomes receptive before anther dehiscence during 09.00 hrs. to 08.30 hrs. Nectar production was observed on the day of flower opening. Nectar quantity was found to be 0.47 ± 0.01µl, 0.76 ± 0.01 µl and 0.10 ± 0.01µl at two hours interval from 09.00 hrs. onwards. The dominant visitors were *A. dorsata*, large size black colour bee (unidentified), small black colour bee (unidentified), *A. florea* and red colour bee (unidentified). Pollen load carried by *A. dorsata*, 35640 *Xylocopa* spp. carried 73923 pollen grains. Experimental pollination showed increase in number and weight of seed during open and bee pollination against the self-pollination. Yield was higher during bee pollination than self and open pollination.

Keywords: Amravati, *Sesamum indicum*, pollinators, *Apis dorsata*, yield.

1. Introduction
Sesamum indicum is an annual herb. It is the principal oil seed crop plant cultivated in Uttar Pradesh, Madhya Pradesh, Rajashtan, Andhra Pradesh, Tamil Nadu and Maharashtra. It is commonly known as "Sesame". It is economically important oil seed crop. It is an entomophilies crop. Many kinds of insects visit the sesamum flower, of them honeybees (Apis sp.) play an important role in pollination. Several investigators studied the pollination requirements in this crop in different countries [1-3]. From India Singh and Dharmawal [4], Rao *et al*. [5], Jitender *et al*. [6] and Jadhav and Ajri [7] studied the role of bees in pollinationd.

The process of reproduction in plants occurs in series of stages from pollen transfer to receptive stigmas, pollen germination, pollen tube growth to the ovary and ovule and fertilization followed by seed maturation [8]. For understanding the knowledge of mode of pollination, it is necessary to study all the aspects of pollination.
The plants with attractive flowers and high reward levels are visited by various insect species. The insect pollinators are much sensitive to floral rewards, floral phenology and floral diversity. Very little work have been carried out in the state of Maharashtra on the role of insects, especially the bees as the pollinators of *Sesamum indicum* crop and the species of insects associated with the pollination. The role of insects in pollination along with all the aspects, which are related with pollination were studies during study period, and the result are represented here.

2. Materials and Methods
Pollination studies on *Sesamum indicum* L. were carried out during the year 2004-2005, 2005-2006, 2006-2007 at two study sites, one at the Pipari (Pargothan) Pulgaon Road, Arvi Dist. Wardha and other at the Patel Farm, Wardha Road Arvi, Dist. Wardha L.0181 to 700301 and East longitude 290221 to 190151). For collection of flowering phenological data, the plants were visited on alternate days. The period from the opening of the first flower up to the opening of the last flower was taken as the flowering period. The peak period of flowering also noted. Morphological characteristics of flower were noted. Timing of anther dehiscence was also noted. The pollen production was evaluated as per the method of Nair and Rastogi [9]. Mature undehisced anthers were crushed in 5 ml of 50% glycerin and pollen grains were counted by taking a drop of the mixture on the slide and observed under microscope. Pollinated flower's stigmas were observed during the flower opening day and consecutively on second and third day. Under compound microscope plucked pollinated stigmas were observed. The stigma along with part of style were kept in actoalcohol (acetic acid: alcohol, 1:1) at 60 °C for one and half hour and washed with distilled water. Then the stigmas were macerated in one percent KOH solution at 60 °C for one hour. The stigmas were washed with distilled water and kept at 60 °C for one hour and again washed twice or thrice with water. The stigmas were then kept at 30 °C in lactic acid for 10 minutes after washing. Then they were stained in cotton blue. Slides were prepared by mounting the material in glycerin by exerting sufficient pressure and examined under the microscope for assessing *in vivo* germination of pollen. The germinated and ungerminated pollen were counted from the pollen deposited on stigma and percentage germination was calculated for in vivo germination.

The dehisced anthers from the flower were collected from different sites for in vitro germination. They were tested with various concentrations of sucrose viz. 10, 20, 30, 40, and 50%. In vitro pollen germination percentage was calculated from the pollen counts of germinated and ungerminated pollen grains. Average of five flowers was taken for each treatment.

Pollen: ovule ratio was determined as per Cruden [10]. To assess the pollen viability, tetrazolium test proposed by Loken [11] was used. Stigma receptivity was tested by pollinating flowers at different stages and also by observing the stigmatic surface with the help of hand lens (10X). The quantity of nectar was measured by graduated capillaries at different time on the days of flower opening. The timing of insects visit, time spent on a flower, number of flower visited per bout was noted. Pollen load carried by insect was assessed. The insects were caught by insect catching net and preserved in separate box with naphthalene bolls inside it. Bee specimens were identified from Central Bee Research Institute, Ganeshkhind Road, Pune.

To compare the yield three treatments viz., self- pollination, open-pollination and bee- pollination were carried out. 10 umbels of equal size were selected for each treatment. For self-pollination bagging of the flower before opening was done, for open pollination the umbels were kept unbagged and for bee pollination the umbels were bagged after the visit of bees. At the time of harvest all the umbels were collected separately. The observations on fruit set and its weight in gram were noted.

3. Results

3.1 Flowering phenology and flower biology

Upper leaves lanceolate, entire, the lower often cut at the base into 2 or more serrate segments, glabrous above, puberulous beneath, flower pinkish-purple, corolla pubescent outside. In *S. indicum*, flowering starts form second week of August and full bloom was during last week of August and ends towards first week of October (Table No. 1).

Table 1: Blooming phenology of *Sesamum indicum* during the year.

Year	Study site	First flower	Full bloom	Last flower
2004-2005	A	12th Aug.	20th Aug. to 5th Sept.	28th Oct.
	F	17th Aug.	25th Aug. to 8th Sept.	28th Sept.
2005-2006	A	29th Aug.	10th Sept. to 25th Sept.	10th Oct.
	F	20th Aug.	30th Aug. to 10th Sept.	15th Sept.
2006-2007	A	27th July	10th Aug. to 20th Aug.	5th Sept.
	F	5th Aug.	15th Aug. to 25th Aug.	10th Sept.

3.2 Flower dynamic

In *S. indicum* flower opening takes place during 06.30 hrs till 08.00 hrs. The anther dehiscence takes place during 07.30 hrs to 08.00 hrs. Stigma becomes receptive during the flower opening at about 09.00 hrs. to 08.30 hrs. The upper surface of receptive stigma appears glossy and white colour. The receptivity ceases during 16.00 hrs to 18.00 hrs on the flower opening day.

3.3 Pollen productivity

In *S. indicum* pollen production was found to be 3838.23 ± 463.84, 3361.05 ± 782.70 and 3735 ±775.64 per flower during the flowering period of the years 2004 – 2005, 2005 – 2006, 2006 – 2007 respectively (Table No. 2).

Table 2: Pollen production per flower in *S. indicum*

Year	Mean No. of p.g. per flower	S.D.	S.E.	Range	Total pollen production
2004	3838.23	463.84	146.69	3247.20 -4692.60	3838.23 ± 463.84
2005	3361.05	782.70	247.53	2356.20--4801.50	3361.05 ± 782.70
2006	3735.27	775.64	245.30	2772 -- 5742	3735.27 ± 775.64

3.4 *In-vivo* and *in-vitro* pollen germination

In-vivo pollen germination was found to be 12.98%, 30.41%, and 46.17% on the day of flower opening, second day and third day respectively (Table No. 3). *In-vitro* pollen germination was found to be 83.24%, 88.64%, 59.39%, 43.35% and 34.07% in 10%, 20%, 30%, 40% and 50% sucrose solution respectively (Table No. 4).

Table 3: *In vivo* pollen germination on stigma.

Day	Total no. of pollen Germinated (five flowers)	No. of pollen non germinated	Total No. of pollen	% of germination
1st	28	127	155	16.41
2nd	17	71	88	20.36
3rd	27	79	106	27.46

Table 4: *In vitro* pollen germination in *S. indicum*

S. N.	Percentage of sucrose %	No. of pollen germinated	No. of pollen non germinated	Total No. of pollen	% of germination
1	10%	10	85	95	10.52
2	20%	27	124	151	17.88
3	30%	34	122	156	21.79
4	40%	32	116	148	21.62
5	50%	38	128	166	22.89

3.5 Pollen viability

Percentage of viability in 1% acetocarmine was found to be 94.56% during 2004–2005 and in triphinyl tetrazolium chloride percentage of pollen viability was found to be 96.93% and 87.65% during the flowering season 2005–2006 and 2006–2007 respectively (Table No. 5).

Table 5: Pollen viability in *S. indicum*

Sr. No.	No. of viable pollen	No. of non-viable pollen	Total No. of pollen	Viability Percentage Mean
1	138.00	5.00	143.00	96.50
2	161.80	5.30	167.10	96.82
3	117.30	17.20	134.50	87.21

3.6 Pollen: ovule ratios

The pollen: ovule ratio was found to be 6069.85, 5517.60 and 5656.69 during the successive years of observation (Table No. 6).

Table 6: Pollen: ovule ratios in *S. indicum*

Year	Total pollen production	No. of ovule	Pollen-ovule ratio
2003	3838.23	46	83.43
2004	3361.05	46	73.06
2005	3735.27	48	77.81

3.7 Stigma receptivity

In *S. indicum* stigma becomes receptive during the flower opening at about 09.00 hrs. to 08.30 hrs. The upper surface of receptive stigma appears glossy and white colour. The receptivity ceases during 16.00 hrs to 18.00 hrs on the flower opening day.

3.8 Nectar production

Nectar quantity was measured between morning hrs during 09.00 hrs to 11.00 hrs, 01.00 hrs to 03.00 hrs and 04.00 hrs to 06.00 hrs. The quantity of nectar was found to be 0.47 ± 0.01µl, 0.76 ± 0.01 µl and 0.10 ± 0.01µl respectively (Table No. 7).

Table 7: Total nectar production in *S. indicum*

S.N.	Nectar Amount	Mean	S.D.	S.E.	Range	Total Nectar Amount
9:00 to 11:00 am						
1	4	3.4	3.32	0.27	0.3.0-3.6	3.32+0.12
2	4					
3	4					
4	3					
5	2					
13.00 to 15:00 pm						
1	5	5.60	0.53	0.24	5.0 - 6.6	5.60 ± 0.24
2	5					
3	5					
4	6					
5	6					
16:00 to 18:00 pm						
1	0	0.6	0.56	0.03	0.4-0.6	0.56 ± 0.03
2	1					
3	1					
4	0					
5	0					

3.9 Flower visitor dynamics

In *S. indicum* insect visitors were *A. dorsata*, large size black colour bee (unidentified) small black colour bee (unidentified), *A. florea*, *Xylocopa* spp. and Flying bee (unidentified). The occasional visors were moths, beetles and thrips (Table No. 8).

3.10 Flower visitor behaviour

In *S. indicum*, the flowers are white or pink colour. The visitors especially bees visit the flowers during 07.00 to 12.30 hrs. *A. dorsata*, large size black colour bee (unidentified), small black colour bee (unidentified), *A. florea* and red colour bee (unidentified) visit the flower and stay on flower for 4 to 27 seconds. *Xylocopa* spp. start visiting the flower during 07.10 hrs and remain active upto 17.30 hrs. The occasional visitors were moths, beetles and thrips which stay on flower for long period (Table No. 8). In *S. indicum*, *A. dorsata* carried 35640 pollen grains. Flying bee carried 25126 pollen grains and *Xylocopa* carried 73923 pollens on their body (Table No. 8).

Table 8: Visitor census in *S. indicum.*

Forage type	Forage type	Length of visit (sec.)	Time of visit (hrs)	Pollen load	Flower visited per bout	Visit freq.
A. dorsata	P/N	5-27	07.00 am-05.30 pm	35640	1-4	VF
Black bee (unidentified)	P/N	6-24	07.10am-05-10 pm.	-	1-4	VF
Xylocopa	P/N	6-22	07.10 am-05.20pm	73923	1-3	VF
Red Bee (Unidentified)	P/N	5-18	08.30 am-04.30 pm	25126	1-5	VF
Thrips	P	Reside	08.30 am-05.30 pm	-	1	O

A. dorsata visiting the flower

Moth collecting nectar

Black bee visiting the flower

Beetle visiting the flower

A. florea visiting the flower

Dominant mode of reproduction, fruit set and yield. The fruit set was found to be 00%, 90%, 60%, 70%, 60%; 00%, 95%, 70%, 75%, 70%; 00%, 95%, 55%, 80% and 90% in apomixis, autogamy, allogamy, and pollination by insect and open pollination respectively during 2004 – 2005, 2005 – 2006 and 2006–2007 (Table No.9). The effects of different treatment on the yield in terms of weight of the capsule was found to be 0.582 gm, 0.619 gm and 0.574 gm in self-pollination, 0.782 gm, 0.818 gm and 0.738 gm in insect pollination; 0.674 gm, 0.716 gm and 0.698 gm in open pollination. . (Table No. 10).

Table 9: fruit set

Treatment Sample size no. of flowers	Fruit set Fruit set % In No. of Flower		Fruit set Fruit set % in No. of flower		Fruit set Fruit set % in No. of flower	
Apomixis	00	00	00	00	00	00
Autogamy 20	18	90	19	95	19	95
Allogamy	12	60	14	70	11	55
Inscet Pollination	14	70	15	75	16	80
Open Pollination	12	60	14	70	18	90

Table 10: Effect of different pollination treatment on the yield (in grams).

Treatment	2004-05	2005-06	2006-07
Self-pollination	0.582 g	0.619 g	0.574 g
Hand pollination	-	-	-
Insect pollination	0.782 g	0.818 g	0.738 g
Open pollination	0.674 g	0.716 g	0.698 g

4. Discussion

In S. indicum flower pinkish-purple, corolla pubescent outside having pollen and nectat as a reward. Bees, A. florae, *A. dorsata*, were found to be the visitors in *S. indicum*. The bees land on the flower to collect pollen and nectar (Table No. 8). Jadhav and Ajri [7] and Jitender *et al*. [6] also reported that *A. florae*, *A. dorsata*, and other insects visits the flower of *S. indicum*. Singh and Dharamwal [4] have reported that *A. dorseta* was the major insect species responsible for the pollination of this crop. However, during the present study *A. cerana indica* was not noticed on seasum crop in this region. The results reported by the Jadhav and Ajri [7] and Jitender *et al*. [6] are comparable with the present findings. As far as the activities of the honey bees are concerned, it was observed that A. florae and *A. dorsata* were most active between 11.00 to 12.00 hrs, respectively. These findings are also in agreement with those reported by Singh and Dharamwal [4]. From the observations on flower visitors; the bees are found to be dominant and important pollinators in *S indicum*. The pollen production per flower in *S. indicum* was found to be 3838.23 ± 463.84. Cruden [10] stated that the pollen: ovule ratio is a better indicator of breeding system. In *S. indicum* pollen: ovule ratio was found to be higher than that suggested by Cruden [10]. The variation may be due to some factors not directly associated with the breeding system.

The stigma receptivity is an important factor in achieving the process of fertilization and plays an important role in successful completion of post pollination events. In *S. indicum* stigmas become receptive after flower opening and emains receptive for whole day. Change in flower colour and closing of flower indicates the loss of stigma receptivity [12-13]. The secretion of nectar synchronizes with activity of pollinators, Karoly [14] stated that for many self-compatible insect pollinated species, the out crossing rate is likely to result from the interaction of plants traits and local pollination ecology. The number of visitors visits the blossom of *S. indicum* either to collect the pollen or nectar or both. The dominant visitors were *A. dorsata*, large size black colour bee (unidentified) small black colour bee (unidentified), *A. florea*, *Xylocopa* spp. and Flying bee (unidentified). The occasional visors were moths, beetles and thrips. Deodikar and Suryanarayana [15] also reported the role of insect visitors in *S. indicum*. During the visit honey bees collect the pollen in the pollen basket attached to their hind legs. The time spent on flower is defined by reward level [16]. Pyke [17] also reported that pollinator visits to flower are related to the amount of nectar present in them. The foraging rate of different flower visitors and time spent differs widely [18].

5. Conclusion

The pollen production in *S. indicum* was found to be 3838.23 ± 463.84, 3361.05 ± 782.70 and 3735 ±775.64 per flower during the flowering period of the years 2004 – 2005, 2005 – 2006, 2006 – 2007 respectively which assures the higher fruit and seed set. Availability of more pollen grains and abundance of flower visitors is again found to be responsible for higher yield. From the observations, the percentage of viability was found to be maximum, which play an important role in fruit and seed set. During present investigation, in *S. indicum* quantity of nectar was found to be more during the period of insect activity. It is concluded from the above observations that the insect visits synchronizes with the time of nectar secretion reflecting the mutualistic relation between flower and insect visitor. *In vitro* pollen germination was found to be more in 50% sucrose. From the observations, the percentage of pollen viability was found to be 96.82% Pollen viability also plays an important role in fruit and seed set.

In *S. indicum A. dorsata*, large size black colour bee (unidentified) small black colour bee (unidentified), *A. florea*, *Xylocopa* spp. and Flying bee (unidentified) were regular visitors. Bees are found to be dominant and important pollinators.

The effect of bee pollination on fruit and seed set significant than self and open- pollination. The fruit set was found to be maximum during insect pollination; this indicated that insect plays an important role in pollination.

6. References

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