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The pollination template in *Jasminum*

Shikha Shrivastava, Neha Rajak and Lekhika Shrivastava
Government VYT PG Autonomous college, Drug, C.G. India.

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ABSTRACT
The study was done to know the retention of flower template in night flowering Jasmine of Central India (Durg) Chattisgarh. The time of antheses were recorded .The Study was done to locate the arrival time of pollinators. The pollinators of these flowers are in shadow, thus the pollinators were identified with the arrival time and the probable reason of arrival of these insects was investigated. Sucrose tests were done with flowers at anthesis time and the insect arrival time. Thus studying their correlation and whole pollinator invitation mechanism or the flower template (Darwin 1877). After detailed study one insect was identified as pollinator. Though this plant never produced viable seeds and its propogation is by vegetative means only. Still it has retained all the invitation machinery for pollinators. Pollination by insects was also confirmed in the experiments.

Introduction

Jasmine is a night flowering plant and easily available in Durg district (Chhattisgarh State of India).In this plant the fruit develops by cross fertilization, thus pollinators becomes an essential part in flower's life cycle. *Jasminum* is a very popular plant all over the world, found in more than one species as *Jasminum adenophyllum, Jasminum dichotomous, Jasminum grandiflorum, Jasminum humile, Jasminum mesnyi, Jasminum odoratissimum, Jasminum officinale, Jasminum parkeri,Jasminum polyanthum,Jasminum sambac* etc..*Jasminum* is a shrub, having erect, cylindrical, climbing, traling stem.

This shrub is some what an untidy straggling climber when young, to 3 m tall and rooting at the nodes.

The fruit is a black berry and surrounded by the sepal.

Jasminum sambac is widely planted and occurring from sea- level up to 800 m altitude. Several double-flowered varieties are recognized, none of which produce viable fruit. The flowers are used in making perfumes and as a flavoring tea. Arabian jasmine is native to India. Jasmine oil is one of the most expensive of all floral oils.

France was once the centre of Jasmine cultivation & distillation, but now it is Egypt, India, and Morocco & South Africa. As reported in Yogi Times (David Crow 2005) The pollinators of jasmine are still in shadow & not much is said about them.

Though the study of flowers & pollinators is not new and their study has been done from time immemorial. Brower and Brown (2006)

The flower pollinator study dates back to Darwin(1877) where he gave the theory of flower template for pollinators.

Some of the important studies worth listing are of (Brower and Brown (2006)) where their study proved that flowers are pollinator species specific. They revealed the existence of 5 cryptic taxa. They also confirmed the recognition of odour types, where the pollinator wasps are very much taxonomically diverse, and the odour components are widely shared within the sex pheromones of these pollinators. Harris and Johnson 2004 studied the mutual interest of plant pollinator relationship with giving importance to the nesting sites of pollinator. Johnson et al., 2010 studied and gave the importance of Bird pollination. Pollinator mostly depended on local habitat, and this theory has been proved by Johnson et al 2010, Goodell et al., 2010, where they studied local habitat dependent pollinator interaction, but in Waguespack and Kinyo, 2009 found that donor diversity does not affect the size & fruit mass. Connal Eardley, 2010 has highlighted the importance of pollinators of plants and have slightly said that pollinators should be conserved on priority basis. In Farwig et al., 2004 studied the dioecious plant species difference in morphology and found that female flowers sometimes, lack pollen and can be less attractive to pollinators than male flowers. They found that female trees produced larger but fewer flowers, in their 270 hrs. observation 17 insects and two bird species visited the flowers. Lonning 1984 had studied the significance of cross fertilization for mutational research.

In 2001 Thompson, 2001 studied the relation between flower display and number of pollinators in *Jasminum fruticans*. He found a positive relation in flowers and pollinators and the pollinators according to him were bee flies, butterflies & Hawk moths, Guitian et al 2008, also studied *jasminum fructicans* in 2008. Knudsen, et al 2006 had done a remarkable work in diversity & distribution & floral scent.

Bhattacharya and Bhattacharya, 1997 studied the in vitro propagation of jasminum officinale with basal media of sucrose & 6 benzyladenine and nepthaleneacetic acid. Raguso and Pichersky, 1995 studied the effect of strong floral fragrance produced by *Clarkia breweri* (onagraceae) and its pollination by moths.

Methodology

The Study was carried out in Durg(C.G.),The central Part of India, For one year from 1.4.09 t0 30.4.10.in Jasmine plants in the house gardens .This is a summer flowering plant, so records of observations were kept on daily basis in summer. To know about the pollinators, it was necessary to observe the bud, its opening time and time of arrival of pollinators. Then only we

Tele: 91-9981159116
E-mail addresses: shikhapk@yahoo.com, rajak.neha@yahoo.com,lekhikashrivastava@gmail.com

could have concluded about the arrival and type of pollinators for jasmine.

Thus we studied type of fertilization, flowering time,an thesis period and sucrose level in jasmine flowers.

To conform cross-fertilization-

To confirm that cross fertilization existed in this flower or not? Following methods were used –

1. The young bud before it bloomed was covered by thin net, so that no insect can enter that particular flower.
2. If fruits will develop in spite of this covering, this proves the self fertilization.
3. If fruits do not develop after covering the flowers, this will prove Cross fertilization.
4. This proves the necessity of insects for cross fertilization.

Observation method to study flowering-

The flowers were observed for whole night and photographed manually until they wore off from the plant. The day break times, opening of flowers, were observed for a week in every season.

Sucrose presence test-

It was also a question that does the arrival of pollinator depends on the sucrose level change in the flower necter? Thus sucrose test in flowers was also done at different time of opening.

Observation

Table -1

In the evening opening flowers the insect arrival time is after 4hrs. Of flower opening exceeding up to 5hrs.

Evening opening flowers

Month	Time of beginning flower opening	Time of insect arrival	Difference in time of insect arrival after flower opening
Feb	8pm.(20:00hrs)	None	None
March	7 pm(19:00hrs)	11 pm(23hrs)	4 hrs.
April	6:30pm(18:30hr)	11 pm(23hrs)	4:30 hrs
May	6 pm(18:00hrs)	11 pm(23hrs)	5 hrs.
June	5:30pm(17:30hr)	None	None

Table -2

Night flower –

The insect arrival time was found only in May when these are maximum number of flower.

Month	Time of flower opening	Time of insect arrival	Difference
Feb	None	No	No
March	None	No	No
April	None	No	No
May	11:30pm(23:30hr)	5.00 am	5:30 hrs
June	No	No	No

Table- 3

Morning flower

These were found only in May. They started flowering from 1:00am and anthesis continued till 4:50am.

The insects arrived here two times –

1. In the morning after 5am.
2. Evening after 5Pm.(17:00hrs)

Month	Time of flower opening	Time of insect arrival	Difference
Feb	No	No	No
March	No	No	No
April	No	No	No
May	4pm(16:00hrs)	5:00pm & 5:00am (17:00hrs& 5 am)	After1hrs./After12 h.
June	No	No	No

In the month of February evening flowering started at 8pm (20:00hrs) and they completely bloomed after 1 to 2 hours at 10:30(22:30hrs) to 11:00 pm(23:00hrs).

In March 7:00pm (19:00hrs) to 7:30pm (19:30hrs) and bloomed about 10:30 pm(22:30); in April flowering started at 6:30pm(18:30hrs) to 7:00 pm(19:00hrs) and bloomed after 2 to 2:30 hour , 10:30-11:00 pm.(22:30-23:00hrs).

In May flowering started at 6:00-6:30 pm (18-18:30hrs) and bloomed after 3-4 hour about 10:30pm(22:30hrs) to 11:00 pm(23:00); in June flowering started at 5:30 -6:00 pm (17:30-18:00hrs) and they bloomed after 5 hour, 11:00 pm.(23:00hrs)

The anthesis period of all evening flowers was between 11:00 pm(23:00hrs) to 2:00 am and most of the insect arrived at anthesis period.

Evening flowers-The budding time of evening flowers was 8pm(20:00hrs) in Feb., 7-7:30(19:00-19:30 pm) in March, 6:30-7pm(18:30-19:00hrs) in April; 6-7pm(18:00-19:00hrs) in May and 5:30-6:30pm(17:30-18:30hrs) in June. Their blooming time was started after 3-5 hours and they had 3-4 hour as anthessis periods. Insects mostly came at that period.

Night flowers-The budding started in these flowers in between 7:30pm(19:30hrs) to 00:30am [Feb-June] and bloomed after 3-4 hour, anthessis period was 3-4 hour and mostly insects came during this period.

Sucrose Test

The above test was done on the flowers which opened at 6:30am, 6:00pm (18:00hrs) &12:00pm, midnight, so that the difference in sucrose level may be determined. Fifteen flowers of each group were crushed, filtered and the traditional sucrose test was done on the filterate. The presence of sucrose was indicated by the purple colour in the test tube. The sucrose level difference was detected by the difference in purple color intensity in colorymetery. Thus in colorimeter test we found equal amount of sucrose in all test tube,for all different opening time flowers. Simultaneously some flowers of different species like *Calotropis procera* which invites lots of ants were also tested for sucrose and the difference in purple colour was observed clearly which states the high amount of sucrose in these flowers.

Discussion

The experiments conducted were to know whether this flower is fertilized by cross fertilization on not .Thus the flowers before they open up i.e. in the bud condition were surrounded and covered by thin net, each bud separately so that no insect or pollinator can reach the flower, and the result was, these covered flowers did not produce fruit, while the uncovered flowers produced the fruits. Thus confirming the hypothesis that there is cross fertilization in these flowers, though the fruits in this plant are of inferior quality & does not participate in propagation of the plant. This plant is propagated by cuttings of ripe wood i.e. by vegetative methods. Previously in Eardley and Foster, 2001 have also given the Importance to pollinators in his detailed study of plant pollinator relationship.

Evening Flowers →[Table-1]

Their opening time and the time of anthesis was recorded from Feb to June (for 5 months).

The result, as stated in the observation that as the day length is increasing from Feb to March then from winter to summer, the day length is increasing along with the time of opening of flowers is repeatedly being reported early, like 8pm (20:00 hrs) in Feb and 6pm(18:00 hrs) in May and 5.30pm(17:30hrs) in June.

As, it is well known that this is a summer flowering plant so it can be presumed that the increase in day length and increase in day & night temperature works as a cue for the time of anthesis.

This is proves the study done by Tanaka et al(1989) where they studied the opening of flower is the response to thermonasty or photonasty and found that the temperature of night before opening is critical in determining whether flower opening under natural conditions depends on thermonasty or photonasty.

In these flowers the pollinator's arrival starts from 11 pm(23:00hrs) sharp, right from Feb to June. The insect arrival time is constant irrespective of flowers opening time or anthesis.

Night Flowers →[Table-2]

Though very few night flowers started to bloom in late February, but full bloom & large number of flowers and size gradually increased at the onset of summer and in June maximum number of flowers was seen,but as Lunav et al 1996 found that size of flowers does not matter much for pollinators.

The time of anthesis also increased in summer and along with the size of flowers too.

The flowers opened in May at 11pm (23:00hrs). Anthesis in June took 2hrs. The insect arrival time was recorded from Feb to June and insects arrived in the flowers only in May that too after 11pm (23:00hrs), the number of insects visiting each flower was recorded; it was always around five species.

Morning Flowers →[Table-3]

Morning opening flowers were observed only in May when the summer in Chhattisgarh is at its height and the day temperature was around 48^{o} C in mid day. These flowers took the longest time for anthesis from 1am to 5am and the insects arrived around 5am.

A study by Tanaka et al., 1989 found the flower opening time is dependent on the response to change in temperature, but not the constant temperature, or change in light keeping the temperature constant. Therefore the temperature of night is more important and the critical temperature is the temperature just before opening of flower.

Again the highest number of insect were around 7 – 5 in each flowers.

It was clearly seen that the maximum no. of flower, and good quality flowers were closely related to day temperature, along with other necessary factor whether they are morning opening night opening or evening flowers.

Thus the question arises again that are all these insects are pollinators ?.Because Blatt and Manning 2006 has observed 17 pollination system for 1 flower species & subspecies of Iridaceae of Sahara. But in India that too in Chhattisgarh there are already various types of pollinators. Right from variety of birds to large variety of insects.

While Schueller 2004 observed self pollination where there is pollinator scarcity and introduced humming bird as pollinators.

The Jasmine flowers were closely observed and only 3 insect variety visited Jasmine regularly and only one was seen with proboscis inside the flower and after few days the fertilized seeds were seen. Thus giving the assumption that this is the pollinators

The time of pollinator arrivals was not seen to be directly related to the opening time of flower.

The invitation for pollinators continue from 1hr. to 12hrs of anthesis

So what may be the invitation? This was investigated in 3 heads

1. Visual
2. Odour
3. Food

Visual → The opening of the flower in itself is a visual stimulus because pure white silvery flowers in dark night are it self an invitation. But only the color signal is not enough to guide the insect, because if only this has been the signal then the insects must have arrived as soon as the flower opens up, but their arrival time is an average of 4hrs. after their opening .Only in May they are arriving 1hrs after the opening of flower. The color contrast here at this time is white against the dusky background and this is not as bright as it is in night, so this stimulus can not be said to work alone. Diverse pollinator assemblages may impose complex selection and thus limits specialization pollinator as Thompson 2001 has studied the floral display for Jasminum fructicans.

Flower commonly have glands called, nectaries on various parts that attract insects looking for Nutritious nectar.Birds & Bees have colour vision, enabling them to seek colourful flowers. Some flowers have patterns called nector guides, that show pollinators where to look for nector; they may be visible only under ultraviolet light, which is visible to Bees & some other insect. Swihart, (1971) have studied the color discrimination in Butterfly,Dronefly(Ilse 1949) and bees (Guirfa et al., 1995)and an inclination towards brightness while Bees (Lehrer et al 1995) were found to prefer flower like shapes.

Food → The flower or any part of the flower is not providing food for these pollinators, except the sucrose secreted in the nectar. Thus the amount of the nectar secreted was also compared and if was found that the amount of sucrose is similar in all the time types of flowers. Thus this is assumed that it is not affecting the arrival time of insects.

Odour →

1. Flowers also attract by odour and some of those odours are pleasant to our sense of smell. Not all scents are appealing to humans, a number of flower are pollinated by insects that are attracted to rotten flesh, and also like dead animals (eg. Rafflesia).

The sense of smell is neurologically our most powerful & primitive sense. Olfaction is deeply & directly linked to emotional memories, sexual vitality and sensual well being which in turn are basis of immunity & longevity.

2. It is the mixture of essential oil & aroma compounds. The nectars secreted by the plant also varies in amount ,as Schueller, 2004. (19) has observed that Nicotiana glance produced large amount of nectar with 20% sucrose, But compared to it, jasmine is a small flower with scanty amount of nectar which is fragrant & where only presence & absence of sucrose was observed. This flower is famous for its odour. There was no fool proof method to measure the odour, so volunteers were asked to smell the odour from the flowers at different distances and rate them. Thus it was concluded that the maximum odour present at the furthers distance (4 mtrs) was only after around 2 hrs of anthesis.

This is for human nose. But as insect's pollination may be more sensitive to these stimuli, thus they could have smelled it far earlier thus the main difference of these arrival is after 1mm and maximum to 12mm.

But flowers pollinated by night visitors, including Bat & Moths are likely to concentrate on scent to attract pollinators, and such flowers are white. Thus we can understand jasmine which is the best example of entomophilus; bearing white flowers, pattern less nector guides and with very strong and pleasant smell.

Similarly the white flower of orchid *mystacidium venosum*, of South Africa contains a dilute sucrose dominated nector which is secreted during afternoon and early evening. Odour dominated by jasmine lactose is emitted in evening flowers .The experiments by Colin 2006 has proved that the odour is the strongest reason to attract the insects for pollination , because the odours components share the sex pheromones structure.

In *unginea moritima* three co existing pollination mechanisms are found in wind, self pollination and insect pollination syndrome .They offer abundant exposed nector as well as pollen. The *apis mellifera, polistes gallicus* and *vespa orientalis* were found to be insect pollinators. As Farwryand Voight 2004 found 17 insects & 2 bird species visiting flowers in 270 hrs. Thompson 2001 found bee & butterflies but we did not see any of them near the flower. Hawk moths were also found as pollinators by them.

In Jasmine, the pollination was established after the visit of ants, and scarab beetles, Dafni and Dukes 1986. Harris and Johnson 2004 have previously given importance to the nesting sites of insects as pollinator but we could not find the nesting site of pollinator in the study. But, as the habitats of insects are very important as was found by Johnson et al., 2010 Goodell et al 2010, the habitat around jasmine shrubs was found very much favourable for these insects.

Thompson 2001 found that there is a significant interaction between insect type & years for single population, for 3 years the number of visits was positively related to number of open flowers. But we did not find any such relation.

The pollinators → Many insects ants ,(Fig.1) beetles (Fig.2) , hawkmoths, were found on the flower But only Hummingbird Hawkmoth was found to be the pollinator because no other insect went inside the flower tube.As it is well known that non viable fruits are produced in this plant.A study by Holland et al 2009 stated that donor diversity did not effect seed or fruit mass and thus exluding donor diversity as factor for fruit quality and have included environmental effects.Of all the pollinators ants have been proved to be a major pollinator(Gomez 1999) but in this case ants were visitors for these flowers but are not the pollinators.

Fig.1 ants Fig.2 beetle

The Hummingbird Hawk-moth (*Macroglossum Stella arum*) an arthropodan, Insecta is well known for its long proboscis and its hovering habit. An audible humming noise makes it look like a small humming bird. It flies during the day, especially in bright sunshine, but also at dusk, dawn, and even in the rain, which is uncommon.

Life cycle of hawkmoth is of 4 different stages as egg, larvae, pupa and adult, above 200 eggs may be laid by one female, each on separate plant and they hatch's 6 to 8 days after hatching.

The larval stage takes place 20 days to become pupae and they form cocoon than after some days cocoon emerge as adult.

Larvae were not found on jasmine, only visiting adults were found on flowers .

The adult may be seen at any time of the year as two or more broods are produced each year.

Thompson 2001 also found Hawkmoths as a major pollinator and referred it to be pollinating the Jasmine flowers But it was never stated that is this only pollinator of jasmine or not.

But in the present study Humming bird Hawkmoths were the only pollinators and visitors were also only insects. Other pollinators as birds, butterflies and bats (Johnson et al 2010,Farwig et al 2004) were totally absent from jasmine pollination system, Though many species of birds and butterflies are quite common in the area and bats were also present in the nearby area.

As for the absence of butterflies we can assume that the flower is mostly night flowering and the butterflies are diurnal so they do not come to Jasmine. But if the flowers are opened up in early morning 5am, and can be seen near other flowers there was no reason or explanation that it was not found on the morning flowering jasmine.

The study of Almut(1997) has reported that hawkmoths feed from flowers of all colour and forms.Though it does finds the flower template of Darwin (1877) Influencing. Almut (1997)found that it is the new imigo of Howkmoth that had to find its first flower by means of flower template. Lunav et al., 1996 found large chromaticity contrast between colour of flower and its background leads to high frequency of approaches.This seems to be true in the case of jasmine which is a good contrast against dark night or dusky background in evening or early morning.It is well known that in India sun takes a bit of time to set so there are 2-3 hours of evening forming a dusky background. The background colour does effects the choice behavior of Howkmoths.(Almut 1997).Hawkmoths are also preferred pollinators in Gladium (Luyt 2001), (Alexandersson and Johnson 2002)because of their long tongues, to fecilitate to reach the nector.But short tongued Hawkmoths were seldom seen carrying the pollen .But in the case of jasmine it is only one species of Hawkmoth and which is successfully pollinating the flowers.

Thus the *Jasminum sambac* seems to carry complete flower template as Colour contrasrt (Almut 1997),Flower like pattern(Lehrer et al., 1995),Odour (Ilse, 1949) and Necter(Guirfa et al 1995) for the Hawkmoth, though the entomophilous pollination is not necessary for this plant as it is not producing viable fruits and is only vegetatively propogated.

Fig.3 Bud Fig.4 anthesis commences

Conclusion→ During observation we found that every bud took 3-4 hour(Fig.3 and Fig.4) to open up and anthesis period of flowers becomes very important for the arrival of insects at that period.

Thus we can conclusively say that –

1. *Jaminum sambac* in our studies was found to be entomophilous i.e. insect pollination.

2. *Jasmine sambac* retains the flower template inspite of the fact that it produces non viable seeds.

We can clearly observe that the pollinator's arrival time is minimum 1hrs. After the anthesis and the invitation remain till after 12 hrs. of opening time .

1. There is a Direct co relation between the opening time & insect pollinator arrival time of jasmine as in – Tables 1, 2, 3.
2. The pollinators of jasmine were found to be only adult Hummingbird hawk moth *Macroglossum stellarum* species.

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End Note-

Authors and co-authors-

Shikha Shrivastava designed the whole experiment while analysing and interpretation of data and has also done the final approval of version to be published.

Neha Rajak and Lekhika Shrivastava carried out the observations,sucrose test and photography together.

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