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# THE STUDY OF POLLEN BIOLOGY OF *Bauhinia variegata* L. AT AGRA (U.P.)

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## ABSTRACT

***Bauhinia variegata* L. is a species of flowering plants in the family (Caesalpinaceae), native to southeastern Asia, from southern China to India. It is a small to medium sized tree growing to 10-12 m height, deciduous in the dry season. Pollen biology was studied in plants growing in Agra. Time of anthesis, stigma receptivity, pollen fertility, pollen germination, pollen-ovule ratio and pollination biology etc. were studied.**

**KEYWORDS :** Pollen, stigma, pollen fertility

*Bauhinia variegata* L. (Caesalpinaceae) is a large moderate sized deciduous tree and attains a height up to 10 meter. The stem is thick, slender in shape and cream in color. The plant bears primary as well as lateral branches. The leaf fall is not restricted to a particular time in a year. The floral bud initiation occurs in the month of March. The flowering period starts in the March and extended up to April. The initiation of fruit formation occurs in the month of April; however, fruit is matured in the month of May. Mature fruits remain attach to the plant up to one to two months. After the maturation of fruits, seeds are dispersed by birds.

## MATERIALS AND METHODS

### Pollen Production

Pollen production per flower was calculated by first counting the number of pollen per anthers and multiplying this figure by number of anther per flower (Cruden, 1977). Total number of pollen/anther was measured by a heamocytometer. Mature anthers were crushed in lactophenol glycerine with aniline blue. A known dilution was placed on the grid and 10 replicate counts were made using a haemocytometer (Barrett et al., 1985).

### Pollen Ovule Ratio

The number of pollen grains divided by the number of ovules per flower yield the pollen- ovule ratio (Cruden, 1977).

### Pollen Viability

### (a) 1% Tetrazolium Vhloride (TTC) Test

Anthers were crushed and their pollen grains were dusted from freshly dehisced anthers in 1 % TTC solution prepared in 0.15m, Tris HCl Buffer, pH 7.8. The slides were kept at 33°C in dark and observed after 30-60 minutes.

### (b) In *vitro* Pollen Germination Test

In *vitro* pollen germination studies were also made on pollen grains collected from the freshly dehisced anthers by hanging drop method after Brewbaker and Kwack's, 1963. Composition of nutrient medium at pH 7.3 is given in table 1.

### (c) In *Vivo* Pollen Germination

### Alexander's Stain (1987)

The pistils were fixed in Carnoy's mixture (Absolute alcohol: Chloroform: Glacial acetic acid 6:4:1) for 12 hours. The fixed pistils were transferred to water through a descending series of ethanol and finally to a few ml of the staining mixture for 12 hrs. The stained pistils were transferred to the clearing and softening mixture for 24 hours at 45°C. The material was washed twice in lactic acid then mounted in mounting medium (table 2)

### Pollination Biology

Pollination mechanism under different environmental conditions was studied. Observations on types of pollinators their population and visitation rates were recorded. Pollinators were also fixed in 70 percent alcohol and identified. Pollination efficiency of different pollinators was checked by observing the body part of pollinators under microscope (Kearns and Inouye, 1993).

**Table 1 : Composition of Brewbaker and Kwack's Medium**

| S.No. | Constituents | Quantity/100ml |
|---|---|---|
| 1. | Sucrose | 10gm |
| 2. | $Ca(NO_3).2H_2O$ | 30gm |
| 3. | $MgSO_4.2H_2O$ | 20mg |
| 4. | $H_3BO_2$ (Borate) | 10mg |
| 5. | $KNO_3$ | 10mg |

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**Table 2 : Constituents of Staining Mixture, Clearing Softening Solution and Mounting Medium**

| S.No. | Constituents | Quantity |
|---|---|---|
| 1. | **Staining Mixture:** | |
| (i) | Lactic acid | 78ml |
| (ii) | Malachite green (1% aq.) | 4ml |
| (iii) | Acid fuchsin (1% aq.) | 6ml |
| (iv) | Aniline blue (1% aq.) | 4ml |
| (v) | Organge G (1% in 50% ethanol) | 2ml |
| (vi) | Chloral hydrate | 5g |
| 2. | **Clearing and softening solution:** | |
| (i) | Lactic acid | 78ml |
| (ii) | Phenol | 10g |
| (iii) | Chloral hydrate | 10g |
| (iv) | Orange G (1% in 50% ethanol) | 2ml |
| 3. | **Mounting Medium:** | |
| (i) | Lactic acid | 50ml |
| (ii) | Glycerol | 50ml |

**Table 3 : Floral Biology of *Bauhinia variegata* L.**

| S.No. | Parameters | Observations | |
|---|---|---|---|
| | | Morning | Evening |
| 1. | Time of anthesis | 5.00–6.30am | 5.00-10.00pm |
| 2. | Time of anther dehiscence | 5.30–6.30am | 5.30–7.00pm |
| 3. | Time of stigma receptivity | 5.30–6.30am | 6.00–9.00pm |

**Table 4 : UV Absorption Spectra of Stigmatic Exudates of *B. variegata***

| S.No. | Wave length (nm) | Absorbance |
|---|---|---|
| 1. | 350.0 | 2.430 |
| 2. | 226.0 | 0.150 |
| 3. | 218.0 | 0.002 |

**Table 5 : Pollen Fertility in *Bauhinia variegata* L.**

| S.No. | Medium (Stain) | Pollen fertility (%) |
|---|---|---|
| 1. | Alexander stain | 80-90 |
| 2. | T.T.C. (1%) | 80-85 |
| 3. | FCR | 75-80 |

**Controlled Pollination Studies**

(a) Flower buds of suitable stage i.e. the oldest bud prior to anthesis and anther dehiscence were selected for controlled pollination studies.

(b) The floral buds were opened carefully, causing minimum disturbances to the floral parts, all the anthers were excised (emasculation) with forceps.

(c) The emasculation buds were bagged.

(d) On the day of natural pollination, the bag was carefully opened and little pollen from freshly dehisced anthers was rubbed on the receptive surface of stigma.

(e) The pollinated flowers were bagged.

(f) After 8-10 days from pollination, bags were removed and each pollinated flower was observed, all dried and abscised flower were counted as unsuccessful pollination. The flowers which shows fruit formation was counted as successful pollinators.

## OBSERVATIONS

**Floral Biology**

The observations on floral biology are described separately in the following paragraphs:

**Anthesis**

It is clear from table 3 that flowers open early in the morning at 5.00-6.30 am. However, some flowers were also open in the evening at 5.00 10.00 p.m.

**Time of Anther Dehiscence**

Table 3 shows that the dehiscence of anther at about 5.30-6.30 am in the morning. It is interesting to note that pollen grains dehisced by longitudinal slits, while in the evening, open flowers anthers are dehisced at 5.30-7.00pm.

**Time of Stigma Receptivity**

It is also clear from table 3 that stigma received the pollen grains between 5.30-6.30 am in the morning this time the stigma becomes receptive. However, in the evening open flowers stigma becomes receptive at 6.00-9.00pm.

**Ultra-Violet Absorption Spectra of Stigmatic Exudates**

table 4 shows that the UV absorption spectra of stigmatic exudates of *B. variegata*. The highest peak was exhibited at the wave length of 350.0 nm with the absorbance of 2.430. The other two lower peaks are seen at the wave length of 226.0 and 215.0 nm with absorbance of 0.150 and 0.002 respectively.

The composition of exudates varies greatly from species to species. It generally contains varying proportion of lipids, carbohydrates, phenolics and proteins (Heslop-Harrison et al., 1979).The functions of different components of exudates are not clear. The phenolic compounds play an important role in pollen germination, pollen nutrition and in selective promotion or inhibition of pollen germination on the stigma (Sedgley, 1985).

**Table 6 : In *vitro* Pollen Germination in *Bauhinia variegata* L.**

| S.No | Chemicals (Medium) | Concentration (%) | Germination(%) | Pollen tube length (µm) |
|---|---|---|---|---|
| 1. | Brewbaker and Kwack's medium | | 50 | 850.0 |
| 2. | Sucrose | 10 | 40 | 590.0 |
| | | 15 | 60 | 880.50 |
| | | 20 | 45 | 675.0 |

**Pollen Fertility**

table 5 shows the extent of pollen fertility during the entire flowering period of *Bauhinia variegata*. It is clear from data in the Table that during flowering period the pollen fertility was 80-90 percent as tested by Alexander stain and was 80-85 percent as tested by 1% TTC. However, pollen fertility was 75-80% as observed by FCR test.

**IN *Vitro* Pollen Germination**

The percentage of in *vitro* pollen germination and pollen tube growth in Brewbaker and Kwack's medium and in different concentrations of sucrose solution. These are described separately in the following paragraphs:

**Brewbaker and Kwack's Medium**

The data on Brewbaker and Kwack's medium is shown in table 6. It is evident from Table that pollen germination in Brewbaker and Kwack's medium is 50.00 percent. The average pollen tube length recorded as 850µm.

**Sucrose**

It is also clear from the table 6 that the highest pollen germination (60%) with longest pollen tube length (880.50µm) was recorded in 15% sucrose solution as compared to 10% and 20% sucrose solutions. There are 40% pollen germination recorded in 10% sucrose solution. The average pollen tube length recorded in this concentration was 590µm. However, in 20% sucrose solution, the pollen germination percentage is 45 % with 675 µm tube length.

**IN *vivo* Pollen Germination**

In *vivo* pollen germination percentage and pollen tube length in *Bauhinia variegata* is shown in table 7. It is evident from the table that the maximum pollen load on stigma is 360-400. However, the number of germinated pollen grains is only 180-200 with 210.0 µm long pollen tubes. While the number of non-germinated pollen was recorded as 180-200. Therefore, these pollen tubes are very short and failed to grow into the long style and thus ovules remain unfertilized.

**Pollen Production and Pollen- Ovule Ratio**

Table 8 clearly indicates that average number of pollen grains produced per anther was 1400-1600 however pollen grains per flower was 7000-8000 during maximum flowering period. It is also evident from the table that the pollen ovule ratio was 1400:1-700:1.

Pollen ovule ratio also indicates that the species is xenogamous, although geitonogamy and autogamy (only induced, not spontaneous) were also recorded. The species is self-compatible.

**Pollination Biology**

The results of open and experimental pollination of *B. variegata* are presented in Table 9 and there is 38 40 % fruit set and 20-24 percent fruit set was observed under open pollination. On the other hand, results on self pollination fruit set percentage are only 15-20 percent. However, the fruit set percentage enhanced by cross pollination

**Table 7 : In Vivo Pollen Germination in *Bauhinia variegata* L.**

| S.No. | Parameters | Observations |
|---|---|---|
| 1. | Pollen load on stigma | 360 – 400 |
| 2. | Germinated pollen grains | 180 – 200 |
| 3. | Non-germinated pollen grains | 180 – 200 |
| 4. | Pollen tube length (µm) | 210.00 |

**Table 8 : Pollen Production and Pollen Ovule Ratio in *B. variegata***

| S.No. | Parameters | Observations |
|---|---|---|
| 1. | No. of pollen grains/anther | 1400-1600 |
| 2. | No. of pollen grains/flower | 7000-8000 |
| 3. | No. of ovules/flower | 5-10 |
| 4. | Pollen ovule ratio | 1400:1 700:1 |

**Table 9 : Percentage of Fruit Set Under Different Modes of Pollination in *Bauhinia variegata* L.**

| S.No. | Parameters | Fruit set (%) | Seed set (%) |
|---|---|---|---|
| 1. | Open | 38-40 | 20-24 |
| 2. | Self | 15-20 | 10-15 |
| 3. | Cross | | |
| | (i) Xenogamy | 34-35 | 20-25 |
| | (ii) Geitonogamy | 35-45 | 25-40 |

**Table 10 : Floral Visitors and Pollinators of *Bauhinia variegata* L.**

| S.No. | Zoological Name | Common Name | Vector Order | Syndrome term | Nature |
|---|---|---|---|---|---|
| 1. | *Apis cerana* | Honey bees | Hymenoptera | Palaenophily | PC |
| 2. | *Apis mellifera* | Honey bees | Hymenoptera | Palaenophily | PC |
| 3. | *Papilio polytes* | Mormon butterfly | Lepidoptera | Palaenophily | PC |
| 4. | *Xylocopa iridipennis* | Bamboo carpenter bee | Hymenoptera | Palaenophily | PC |
| 5. | *Polistis orientalis* | Wasps | Hymenoptera | Palaenophily | NR |
| 6. | *Macroglossum stellatarum* | Hawknoth | Lepidoptera | Palaenophily | NR |
| 7. | *Blissus leucopterus* | Chinch bug | Hemiptera | Palaenophily | NR |
| 8. | *Calypte halenae* | Humming bird | Trochiliformes | Ornithophily | PC & NR |
| 9. | *Psittacula krameri* | Parrot | Psittaciformes | Ornithophily | NR |

PC = Pollen collector; NR = Nectar robber

**Table 11 : Time of Visitation and Visitation Rate of Pollinators in *Bauhinia variegata* L.**

| S.No. | Pollinators | Visitation time | Nature |
|---|---|---|---|
| 1. | *Apis cerana* | 7.00 am – 10.00am | PC |
| 2. | *Apis mellifera* | 7.00 am –9.00 am | PC |
| 3. | *Papilio polytes* | 12.00am – 5.00pm | PC |
| 4. | *Xylocopa iridipennis* | 6.00pm – 10.00pm | PC & NR |
| 5. | *Polistis orientalis* | 5.00pm – 9.00pm | NR |
| 6. | *Macroglossum stellatarum* | 8.00 am – 9.00am | NR |
| 7. | *Blissus leucopterus* | 8.00am – 8.45am | NR |
| 8. | *Calypte halenae* | 9.00 am – 11.00am | NR |
| 9. | *Psittacula krameri* | 10.00-12.00 am | NR&PC |

PC = Pollen collector; NR = Nectar robber

(xenogamy and geitonogamy). The percentage of fruit is 34-35 percent by Xenogamy and 35-45 percent by geitonogamy respectively. It is also clear from Table that there is 34-40 seed set percentage on open pollination and 10-15 seed set percentage by self pollination. There are considerable differences was obtained by xenogamy and geitonogamy. The percentage of seed set is 20-25 percent by Xenogamy and 25-40 percent by geitonogamy respectively.

**Floral Visitors**

It is evident from Table…. that the floral visitors of *B. variegata* are Honey bees *(Apis cerana, Apis mellifera*), Mormon butterfly (*Papilio polytes*) Bamboo carpenter bee (*Xylocopa iridipennis*), wasps (*Polistis orientalis*) *Macroglossum stellatarum* (Hawk moth), *Blissus leucopterus* (Chinch bug) and *Calypte halenae* (Humming bird) and *Psittacula krameri* (parrot). These belong to different orders viz. Hymenoptera, Lepidoptera, Coleoptera, Hemiptera, Trochiliformes and Psittaciformes. It is interesting to note that all of them are not pollen carrier. Among 9 vectors, only four are pollen carrier as evident by the presence of pollen on their body parts. On the basis of their visitation rate, pollen load on their body parts, *Apis mellifera*, *Apis cerana* and Mormon butterfly are found to be the most efficient pollinators. Beside, wasp (*Polistis orientalis*) also shows a remarkable amount of pollen load on their body parts.

The insects visiting the flower were Chinch bug, Bamboo carpenter bee and Hawk moth. The visitation rate of these insect are low and pollen load on their body parts of these insect are more or less negligible. Therefore, it is revealed that these insect are not effective pollinators of the flowers of *B. variegata*.

**Time of Visitation and Visitation Rate of Pollinators**

The visitation time of insect and their rate has been described in table 11. It is evident from Table that Honey bee, Mormon butterfly, Bamboo carpenter bee, Wasps

Hawk moth, Chinch bug and Humming bird visit the flower. Most of Pollinators visit the flower in the morning and some pollinator visit the flower from evening to night. However, their visitation time varies from species to species. The honey bees visit the flower between 7.00-10.00 am. The Mormon butterfly also visits the flower between 12.00 am 5.00 pm. in the evening. However, Bamboo carpenter bee visits the flower at night between 6.00 pm 10.00 pm. The wasp visits the flower between 6.00pm 9.00pm and Hawk moth visits the flower in the morning between 8.00 9.00am. Chinch bug also visit the flower in the morning between 8.00 8.45am and humming bird also visit the flower in the morning between 9.00 11.00 am. The pollinators observed in flower of *Bauhinia variegata* were both pollen carrier and nectar robber. Honey bees Mormon butterfly and Bamboo carpenter bee act as a pollen carrier. However, Wasps, Chinch bug, Hawk moth and parrot act as a nectar robber.

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