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Floral Biology, Floral Resource Constraints and Pollination Limitation in *Jatropha curcas* L.

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Abstract: The present study deals with the floral biology, floral visitors and pollination of *Jatropha curcas* L. (Euphorbiaceae). Forenoon pattern of anthesis with subsequent pollen release was noticed. Each male flower produced 1617±100 pollen with P:O ratio as 539:1. The stigmas become receptive 2 h after anthesis with morphological differentiation. Nectar secretion coincided with pollen presentation schedule and stigma receptive duration. Each female flower produced higher amount (4.54±0.82 μL) of nectar than male flower (1.92±0.44 μL) in 1200 h. Fifty percent of female flowers set fruit with 53% fecundity rate, 32% apomixis rate and 2:3 seed-ovule ratio. The flowers attracted the insect visitors of Hymenoptera (*Apis dorsata, A. florea, A. mellifera, Eumenes conica* and *Vespa* sp.) and Coleoptera (Beetles). Among the different insect visitors *Apis* spp. were most frequent. It has been observed that the different grades of sucrose have an influence on insect behaviour, flower visits duration, pollen removal and deposition on stigmas by honeybees (*Apis* spp.). Highest number of pollen grains were transported and deposited on stigmas by *A. dorsata* when flowers were treated with 0.9 M sucrose, whereas, it was lowest by *A. florea* in 1.5 M sucrose treated flowers.

Key words: Floral biology, forage behaviour, sucrose treatment, pollen, stigma

INTRODUCTION

Availability of floral resources (nectar and pollen) for opportunistic floral visitors in animal pollinated plants can regulate flower visitation, maintain visitors population, rewards successful pollination and finally lead to seed/fruit harvest. Floral visitors are dependent upon nectar (intra-or extra-floral) and pollen for their nutrition. Among different groups of flower visitors insects are predominant. They visit to million of flowers and have been considered as potential pollinators following the rules governing co-evolution, generalized or specialized pollination systems and syndromes between conspecific plants and their pollinators. Lack of adequate floral resources may lead to weak interaction between plants and their appropriate pollinators, which in turn causes lower limits of flower visits and pollination success.

Several investigations have been done earlier in making the correlation between floral resource availability, flower visits by insects and pollination success on species by species basis. Cruden[1] reported on ecotypic adaptation based on the findings of intraspecific variation in pollen-ovule ratios and nectar secretion. Faegri and van der Pijl[2] provided a number of examples on floral visitors, floral resources and its dependence upon pollination and fruit set. Bino and Dafni[3] correlated between nectar secretion and entomophilous nature of diocious plants. Waser and Price[4] proved the effect of nectar guides on pollinator preference. Howell *et al.*[5] contemplated the importance of pollen availability on pollinating potential. Role of foraging dynamics and floral sex allocation on fruit set of certain plants were documented by Willis and Kevan[6], Brunet and Charlesworth[7], respectively. Dafni and Kevan[8] reported about functional significance of plant-pollinator interactions in relation to floral symmetry and nectar guides. Lughadha and Proenca[9] showed increased pollinators population in presence of nectar and pollen abundance. Agren[10] reported that population size and pollinator limitation affect seed set in *Lythrum salicaria*. Strauss[11] showed the relation between floral characters, pollinators and plant fitness; while Dafni and Giurta[12] enriched the literature by focusing the functional ecology of floral guides in relation to insect behaviour and vision.

The present investigation has been done with aim to find out the floral visitors and pollination of a cultivated small population of *Jatropha curcas* L. (Euphorbiaceae), an economically important tree of Central America and also to determine the relationship between floral resources (nectar, pollen), flower visitors' abundance, duration of

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visits and pollen deposition on stigmas by conducting direct experiment in field.

MATERIALS AND METHODS

Study site and plant material: Ten plants of the same age (10 years) were selected for present study. The investigation was carried out in National Botanic Garden, Lucknow, India (26.55°N, 80.59°E) where the germplasm (41 trees) of *J. curcas* is being maintained. The trees attain a height 3-5 m showing non-synchronous flowering. The growth and flowering of trees become high at sun-exposed area of germplasm site. The trees are monoecious showing male and female flowers in same and/or different inflorescences. The tree is valued for obtaining good amount of petro-fuel/biodiesel from its seeds.

Flower visitors and floral biology: The flower visitors observed to visit the flowers of *J. curcas* were caught and identified on self-expertise basis consulting with reference materials. Flower anthesis was noticed by tagging ten flowering shoots from ten different plants, which were observed at 1 h intervals for 24 h. To study the pollen anthesis almost mature anthers were sectioned and immediately covered with cover glass before observation. Pollen production, pollen-ovule ratio, floral resource measurements and other floral characters were determined following Dafni[13] and Kearns and Inouye[14]. Pollen morphology was studied following acetolysis[15]. The fruit-set, fecundity rate and apomixis were estimated as per the process of Kearns and Inouye[14]. A total of 450 censuses (3 min each at intervals of 10 min) were made to record the mean durations of visits and mean abundance (%) of different flower visitors. Foraging behaviours of flower visitors were observed visually. Three inflorescences having both male and female flowers of each tree of ten different trees were targeted for determinations of durations of visits, mean abundance and forage behaviours.

Floral resource manipulation: Direct field experiment was conducted to know the *in vitro* effect of sucrose on the duration of visits by honeybees; their forage behaviours and pollen transfer by single visit. Sucrose (reagent grade, SRL) solutions (0.3, 0.6, 0.9, 1.2 and 1.5 M) were prepared and 2-3 μL sucrose was applied in each flower (male and female separately) of an inflorescence. Thus five male and five female flowers per tree of ten trees, adjacent to each other were treated with *in vitro* sucrose. Before sucrose application, the existing nectar of each manipulated flowers was sucked using minute filter paper strips. Sucrose treatment was done at peak visiting period of foragers. After that the duration of visits by honeybees on each manipulated flowers were counted. Mean values for male and female flowers separately were considered. To know the mean number of pollen grains deposited on stigmas by single visit, the virgin flowers that were already covered with paper caps were exposed to honeybees. After a single visit the stigmas were excised and mounted with glycerol-safranin (1:1) for scoring grains under light microscope.

RESULTS AND DISCUSSION

Jatropha curcas L. (Euphorbiaceae), a bio-fuel producing plant grows abundantly in Central America. It flowers during July-September. The unisexual, regular, greenish white flowers (17-105 male and 2-19 female per inflorescence) show forenoon (800-1200 h) pattern of anthesis with subsequent pollen release. Each flower produced 1617±100-pollen grains with low P:O ratio (539:1). The apolar-radiosymmetric, inaperturate pollen grains are spheroidal in shape having 94.5 μm diameter and 6.2 μm thick exine. The stigmas become receptive 1-2 h. after flower opening. Morphological differentiation of stigmas before and during their receptive period was noticed. Before receptivity, the stigma lobes remain unopened. During receptive period the stigma lobes open and increase surface area showing 6-furcated stigma tips, at the junction and in between of each furcating pollen grains were found to be adhered. Nectar secretion coincides with pollen presentation time and stigma receptive period. Female flower produced higher amount (4.54±0.82 μL) of nectar than male flower (1.92±0.44 μL) in 1200 h. The plant shows flower-fruit ratio (10:1) and 50% of female flowers set fruit (53% fecundity rate). As fruit abortion was noticed, so the apomixis rate was 32% (Table 1). Floral rewards (pollen, nectar) presentation starts after flower opening. At this time insects of Hymenoptera and Coleoptera pay visits with different modes to flowers (Table 2). With regard to floral structure and floral visitors adaptive features, it is stated that honeybees (*Apis dorsata*, *A. florea* and *A. mellifera*) are effective pollinators. *Eumenes*, *Vespa* and beetles are not considered as effective pollinators as the pollination syndromes of these visitors do not match with *Jatropha curcas* flower. Rather these visitors are considered to be nectar robbers. To know the effect of floral rewards, especially nectar, the field trial was performed using different grades of sucrose syrup. It has been seen that the different grades of sucrose syrup have an influence on behaviour and flower visits by honeybees, the effective pollinators. Differences of durations of visits paid by different honeybee species are obtained followed

Table 1: Floral characters of *Jatropha curcas*

Floral characters	Observations
Flowering period	July-September
Flower type	Unisexual, regular
Flower colour	Greenish white
Mean No. of male flowers per inflorescence	44±1.7, range 17-105
Mean No. of female flowers per inflorescence	10.2±0.64, range 2-19
Mean No. of inflorescence/branch	3.25, range 2-7
Flower odour	Slight
Nectar voloume (μL)/male flower	1.92±0.44
Nectar volume (μL)/female flower	4.54±0.82
Flower anthesis time (h)	800-1200
Pollen anthesis time (h)	830-1230
Mean No. of anthers/male flower	10
Mean No. of pollens/anther	162, range 70-275
Mean No. of pollens/flower	1617±100, range 725-2729
P:O ratio	539:1
Pollen type	Inaperturate, crotonoid
Pollen shape	Spheroidal
Pollen size	94.5 μ diam., exine 6.2 μ thick
Stigma type	6-furcated, receptive period 2 h after Anthesis
Mean fruit-set/inflorescence	5.4±0.73
Flower-fruit ratio/inflorescence	10: 1
Mean fecundity (%)	53, range 43-61
Apomixis (%)	32
S:O ratio	2:3

Table-2: Flower visitors of *Jatropha curcas*

Floral visitors	Visit duration (sec)	Mean abundance (%)	Forage type
Apis dorsata	8-15 (10.6)	31	P, N
A. florea	11-31 (19.7)	27	P, N
A. mellifera	12-25 (18)	13	P, N
Eumenes conica	3-7 (4.9)	18	N
Vespa sp.	2-6 (2.9)	5	N
Beetle	36-51 (44)	6	P, N

P=Pollen; N=Nectar

by the *in vitro* treatment of flowers with different concentration of sucrose syrup. In case of male flower the mean duration of visit was highest (35 sec) in *A. florea* at 1.2 M sucrose followed in degree of prevalence by *A. mellifera* (30 sec) in 1.2 M sucrose, *A. florea* (24 sec) in 0.9 M sucrose, *A. dorsata* (21 sec) in 1.2 M sucrose. The duration of visit was found to be lowest (5 sec) by *A. dorsata* in 0.3 M sucrose (Fig. 1). In case of female flower duration of bee visits were also different among different sucrose concentration and between different bee species. Visiting period on sucrose manipulated female flower was highest (37 sec) in 1.2 M sucrose by *A. dorsata* followed in degree of prevalence by 34 and 30 sec in 1.2 and 0.9 M sucrose, respectively by *A. mellifera*, whereas, 28 sec in 1.2 M sucrose by *A. florea* (Fig. 2). The numbers of pollen grains transported by honeybees and deposited on stigmas get affected with sucrose treatment. Highest number of pollen grains was transported by *A. dorsata* and deposited on stigmas when flowers were treated with 0.9 M sucrose, whereas, pollen transportation was lowest by *A. florea* in 1.5 M sucrose treated flower (Fig. 3).

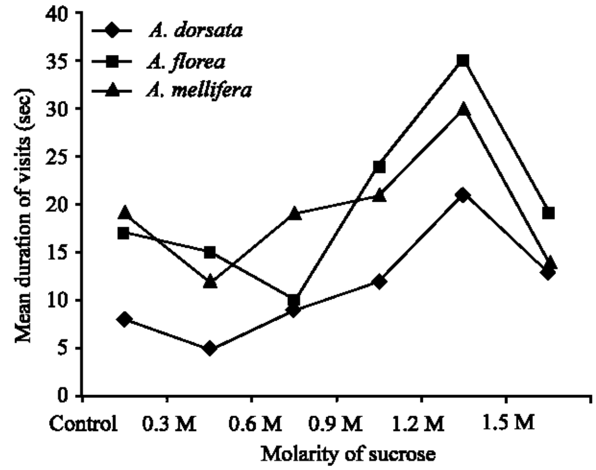

Fig. 1: Effect of sucrose on honey bees' foraging on single male flower

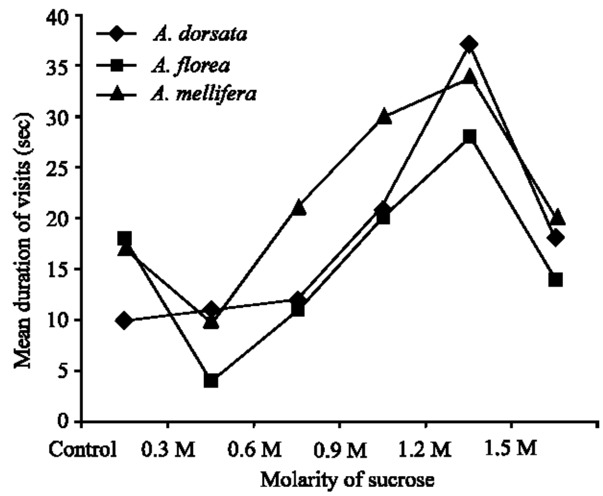

Fig. 2: Effect of sucrose on honey bees' foraging on single female flower

This study demonstrated that pollination in a small population of introduced *Jatropha curcas* was potentially influenced by both pollen deposition on stigmas and nectar availability. The factors may constrain the floral visitors' duration and pollen deposition on stigma during different periods of the reproductive season. Both male and female flowers showed forenoon pattern of anthesis and attracted different insect visitors. Among these *Apis dorsata, A. florea* and *A. mellifera* were most common and effective pollinators with regard to the floral structure and pollination syndrome. Anthesis pattern, visitors' attraction and pollinators' discrimination are related to floral features and availability of maternal resources[2,11,16,17]. Male-female flower ratio was approximately 4:1 with higher amount of nectar per flower

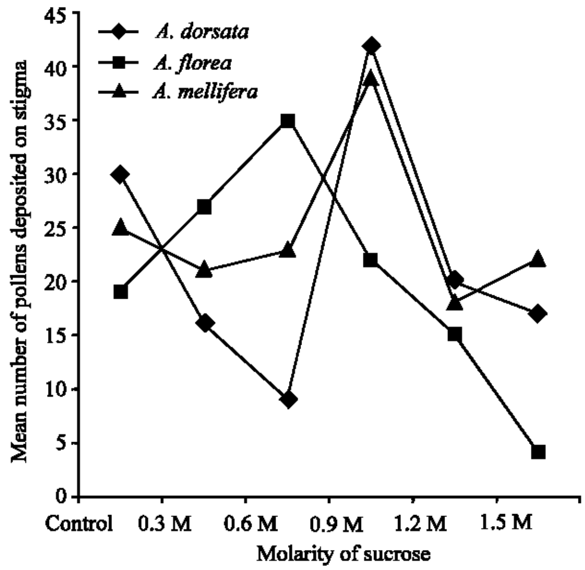

Fig. 3: Effect of sucrose on number of pollens deposited over stigma by a single visit

in female than male. A total of 1617±100-pollen grains are produced per flower with P:O ratio of 539:1. The ratio of male and female flowers per inflorescence, even per tree of any dioecious or monoecious plant may not be considered as conservative indices, because the floral bud initiation, flower maturation and production, although genetically regulated and should have constancy, yet it may have dependence upon nutrient contents, various physical and physiological factors and possibly by other adaptive features of a plant acclimatized in a specific micro-environment. According to Niesenbaum[18], Brunet and Charlesworth[7] the sex ratio and floral sex allocation are not conservative, rather sequentially variable. The production of high amount nectar in female flower may be attributed to a selective force operating in plant systems offering more nectar to flower visitors. Male flowers, on the other hand, produced low amount of nectar but offered both pollen and nectar to visitors, it could be a reproductive trade off among male individuals. Moreover, a male-female competition may generate to attract flower visitors and finally it may affect the adaptive radiation of pollinating system and success in this plant. If the primary floral reward (nectar, pollen) competition continues in a plant during whole reproductive season, then flower visitors may get preferences of choosing the flowers, extend their visit durations, increase foraging efficiency, extract more rewards, gain additional energy, increase pollen transfer ability and deposit greater amount of pollen leading to increased pollination success. We predict that in such cases improper pollen deposition may affect true pollination success, because of the fact, flower visitors may keep themselves busy to gain rewards and energy without rigorous movements upon floral parts; as a result male-female interaction might be disrupted. According to Bino and Dafni[3] secretion and amount of nectar is associated with adaptive and evolutionary significance of pollination types. Pollinators' preferences are also affected by nectar guides[4]. Forage behaviour of honeybees is in accordance with Dafni and Giurta[12]. Even, the functional ecology of male-female competition with regard to floral rewards offering to visitors and adaptability to pollination and plant fitness may attain a prominent role in flower evolution and plant-insect interaction in this plant. Dafni and Kevan[8] postulated the evolutionary significance of floral symmetry and nectar guides reporting various ontogenic constraints from floral development and colour pattern rules have a paramount importance in flower visitors' attraction. *Jatropha curcas* is predominantly out breeding and this is clearly shown in many of the reproductive traits: high abortion rate and apomixis, relatively low seed-ovule ratio, low fecundity percentage, absence of auto deposition of pollen due to unisexual nature, high attractiveness to pollinators, delayed stigma receptive period and morphological differentiation of receptive stigma for proper pollen reception. Molau[19] stated that typical traits of P:O ratio, low S:O ratio and floral features preclude selfing in the absence of legitimate pollen vectors are connected with an out breeding mating system. The present study found variation among honeybees in the duration of visits and pollen deposition on stigmas in sucrose treated flowers. There was no relation between the quantities of sucrose syrup residue left after visiting the treated flowers by honeybees. Moreover, it is predicted that much of the variation among honeybees in the mean duration of visits to sucrose treated flowers arose from differential thoroughness in applying sucrose syrup. It is true that honeybees spent longer at sucrose treated flowers and eventually deposited more number of pollen grains on stigmas, possibly due to greater amount of sucrose accessibility to their capability of ingestion. Therefore, it is concluded that variation among honeybees in the duration of visits to sucrose treated flowers resulted from differences in foraging speed, although the exact reason of this variation is unknown. As the *in vitro* sucrose treatment showed positive influence on honeybees' visit duration and pollen deposition on stigmas, on the whole, the floral resource (nectar) availability might constrain the pollination system of this plant in natural population.

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