



Pollination Ecology and Fruiting Behaviour in *Strychnos nux-vomical*. (Loganiaceae)

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Authors' contributions

This work was carried out in collaboration among all authors. Author MCB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AKM conducted the field work and managed the analyses of the study. Author TLM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Strychnos nux-vomica* is a valuable drug plant. Knowledge on its breeding system and pollination ecology will assist future research on genetic improvement for higher yield attributes.

Place and Duration of Study: The study was conducted in the Silvicultural Research Station, Bhubaneswar, Odisha, India between 2016-2018.

Methodology: Pollination experiment was conducted for autogamy, geitonogamy, xenogamy, apomixis and open pollination by allocating three hundred flowers for each experiment. Phenological events, pollinator visit and their behaviour were recorded at regular intervals.

Results: Pollination studies in *Strychnos nux-vomica* revealed mixed mating system in its flowers. Flowers are small, greenish-white, bisexual, nectariferous and emit mild sweet odour. They open during evening hours and offer pollen and nectar to the visitors. Bees, flies, wasps, butterflies, beetles and birds are the pollinators. *Apis dorsata* and *Euploea core* are the principal one and makes diurnal visit between 7.00-17.00 hours. The anther dehiscence starts at 16.00-16.15 hours. The capitata stigma receptive to pollen germination at 17.30-18.00 hours and remain in receptive

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state one day after complete anther dehiscence. Flowers favour cross-pollination (29.78-60.26%) however selfing is also possible (17.94-39.74%).

Conclusion: Information on breeding system and pollination ecology will be helpful in developing a breeding strategy for higher fruit set and seed yield.

Keywords: Reproductive biology; breeding system; medicinal plant; strychnine; brucine.

1. INTRODUCTION

Strychnos nux-vomica L. is a source of poisonous alkaloids strychnine, brucine and their derivatives [1]. Seeds after proper detoxification used as medicine in Unani, Ayurveda, Tibetan, Chinese, Homeopathy and modern treatise [2]. Detoxified seeds are used as an aphrodisiac, appetizer, anti-periodic, digestive, purgative and stimulant [3, 4]. Other parts of the plant viz. fruit pulp, root, bark, leaves are also advocated for the ailment of human diseases and disorders after proper processing [5]. Apart from medicinal uses, seed and leaves are valued for insecticide, nematicide, rodenticide, avicide, and piscicide [6]. The ripe fruit pulp is food for many birds (fruit bats, rhinoceros hornbills, parakeets) and animal (*Macaca radiata*) [7, 8].

Though not included in the IUCN red list of threatened species, it is believed to be vulnerable locally in some places of Eastern Ghats [9]. It is a potential species for commercial cultivation in rain-fed farming situations of south and central India.

Information regarding phenology and reproductive ecology is very much essential for undertaking any conservation program [10]. Detailed information relating to phenological features in Odisha condition and reproductive ecology as a whole in *S. nux-vomica* is rudimentary. Most of the research works have been concentrated on phyto-chemical screening, therapeutic uses and pharmaceutical ability. There is no commercial cultivation owing to lack of early fruiting variety and a standard agro-technique package. The present work is intended to develop base line information relating to morpho-phenological attributes, breeding systems and pollinator diversity. This will be very much helpful in carrying out breeding programmes on this species for successful commercial cultivation as well as conservation in the wild.

2. MATERIALS AND METHODS

The experiment was conducted during the period 2016-2018, in *S. nux-vomica* population distributed in the Silvicultural Research Station,

Bhubaneswar situated at 20°16'43" N latitude, 85°47'00" E longitude and 25.9 AMSL altitudes. The climate of the experimental site was tropical, with an annual rainfall of 1273.9 mm. The maximum temperature ranged from 29.4 to 38.0°C and the minimum temperature from 15.4 to 26.6°C. Phenological events leaf fall, leaf flushing, flowering, fruiting were observed at plant level regarding monthly changes and observation was based on twenty randomly selected trees having DBH (diameter at breast height) 45.0±5.0 cm. One hundred inflorescences from twenty conspicuous trees were tagged randomly to study the day-to-day change in flowering events anthesis, nectar production, withering of the corolla. Inflorescences were observed daily till they ceased flowering and care was taken to avoid repetition of the same flower. The flowering synchrony was quantified by determining the mean of flowering synchrony of all individual plants in the population (n=20) as per the mathematical equation given by Marquis [11].

$$\text{Synchrony Index} = \sum_{t=0}^N \left(\frac{x_i}{\sum_{t=0}^N x_t} \right) \times p_t$$

Where the synchrony in flowering of a plant "i" is a function of the proportions of flowers at time "t". p_t is the fraction of individual plants in flower at time "t" of individuals in flower at time t; x_i is the total flowers on individual "i"; and $\sum x_t$ is the total number of flowers during the season. Value of X varies from 1 (perfect overlap) to 0 (no overlap).

The quantitative attributes relating to flower morphology and reproductive organs were based on thirty randomly tagged inflorescences. The anther dehiscence and pollen dispersal mode was determined by adopting procedures of Dafini [12]. The ovule was counted with a stereoscopic microscope and pollen was counted with a hemocytometer following Kaul et al. [13]. The pollen to ovule ratio (P/O) was determined by dividing the number of pollen grains with the number of ovules per flower. Pollen viability was observed through the hanging-drop method using 10% sucrose solution. Pollen germination on stigma surface was studied following the

procedures of Cruden [14]. Pollination experiment for autogamy, geitonogamy, xenogamy, apomixis and open pollination was conducted to know the breeding behaviour. Each treatment was allocated three hundred flowers [15]. For autogamy three hundred matured buds (ten each from thirty inflorescences on five trees) were bagged a day before anthesis and left undisturbed. Controlled pollination was done at the stigma receptive stage (viscid, reflexes and white colour stigmatic surface) between 7.00-9.00 hours, using fresh pollen. For geitonogamy, matured buds were emasculated, bagged a day before anthesis and very next day pollinated with fresh pollens from the flowers of the same tree and re-bagged. In the case of manual cross-pollination, emasculated bagged mature buds were brushed with fresh pollens collected from flowers of another tree. The process of brushing stigma surface with pollens in geitonogamy, Controlled pollination and manual cross-pollination was repeated continuously for five days. For knowing fruit set through apomixis mature flower buds were emasculated, bagged and left as such. Three hundred flowers (30 inflorescences in five trees) were tagged and left undisturbed to know natural open pollination [16]. Pollinators visiting to flowers and their activity were recorded manually using binoculars & digital camera.

3. RESULTS AND DISCUSSION

3.1 Flowering Phenology and Synchrony

S. nux-vomica exhibits en masse winter blooming. Like other winter-blooming tropical trees of India, flowering in *nux-vomica* takes place with concurrent leaf flush during the dry cool season (February-March). Leaf shedding and flushing in *S. nux-vomica* occur during winter season. Leaf fall occur from the last week of December and continued until the second week of January. Plants remain completely leafless for two weeks. Leaf budburst was observed in the second week of February and it took 10.0 ± 2.1 days for complete leaf flush. Newly emerged leaves were crimson red and turned light green after 4.0 ± 1.6 days. Flower bud initiation started with new leaf flush and inflorescence appeared during 3rd week of February. Blooming started during the 1st week of March (4th March) and continued until the 4th week of April (28th April) with peak in between 15th March to 18th April. Anthesis took place during evening hours (15.30 to 15.45 hours). Flower in a particular inflorescence mature and open in succession for

6.0 ± 1.85 days. Corolla withered and detached from the whorl after 48-52 hours of anthesis (Fig. 1). An important phenological feature observed in this species was the alternating blooming behaviour in some individuals. Out of the population size of fifty-five plants growing at the study site, four plants did not bloom and eight plants bloom alternatively and this trend was repeatedly observed for three years (2016-18).

The index for flowering synchrony of conspicuous individuals ranged from 0.46 to 0.92. The flowering index for the population was 0.77, indicating good overlapping of flowering time among individuals. Among the twenty trees under study, 85% of individual trees were having flowering synchrony index >0.75 , indicating mass blooming and winter leaf fall might be a cue for this (Fig. 2) [17]. Flowers of *S. nux-vomica* are not colourful and showy but highly synchronized flowering and aroma attracts pollinators. Fruiting commenced in April-May and maturation continued till December (10th), colour change from green to orange indicated fruit maturity. Indehiscent berries started falling in January (18th January) and continued till mid- February (17th February).

3.2 Flower Morphology

Flower bud appeared in 38.46 ± 3.35 cm long terminal cymes with divaricating branches. Each inflorescence consisted of 59.18 ± 5.314 numbers of flowers (R38-89N) and opened sequentially for ten days. Flowers were complete, hermaphrodite, nectariferous, tubular, odoriferous and small in size (9.84 ± 0.87 mm long and 6.87 ± 0.60 mm broad at mouth (Fig. 1). Corolla was tubular with abrupt widening at the throat, outside glabrous, having pubescence at the base inside, lobes ovate and greenish-white in colour. Stamens were epipetalous, haplostamenous, alternate with petal lobes and seem to be inserted at the mouth of the corolla tube. Anthers were dithecal, dorsifixed, elliptical-spherical and protrude out. They grouped around stigma head in bud condition and radiates out at full bloom (Fig. 1). The pollen grains dehisced few minutes after anthesis. Pollen grains were fertile, cream-white, tricolpate, oblate-spheroidal and $41.51 \pm 3.62 \mu\text{m}$ in size (Fig. 4D). The mean number of pollen grains per anther was 1340 ± 56.19 and per flower was 6700 ± 85.60 . The gynoecium was superior with bicarpellary syncarpous ovary having a bilocular, ovoid, chamber which possesses 20.00 ± 1.75 numbers of anatropous ovule exhibiting axile

placentation (Fig. 4C). The style was filiform, glabrous; stigma capitate, papillate, cream-white and $1.38 \pm 0.12 \times 0.39 \pm 0.034$ mm across (Fig. 1C & D). The ratio of pollen grain to ovule was 335:1.

3.3 Floral Biology

The flower opened during evening hours (15.30-16.00 hours) with maximum blooming during 20.00 hours. As the flower opened, anthers positioned little above the corolla mouth and dehisces pollens by longitudinal slits. Dehiscence of anther started 30-45 minutes after anthesis. During the initial hours of dehiscence, 99.36%

pollens were viable and it reduced gradually up to 2.27% after 84 hours (Figs. 3 & 4F). During initial hour of anthesis, stigma was fresh, turgid, viscid, papillate and cream-yellow coloured.

The anther dehiscence started after 30-45 minutes after anthesis and continued for the next 16 to 17 hours. The stigma became receptive 2.0-2.30 hours after anthesis and continued for next 24.0-25.30 hours. There was an overlap in stigma receptivity and pollen shedding. This overlapping of pollen shedding and stigma receptivity indicated the possibility of self pollination.

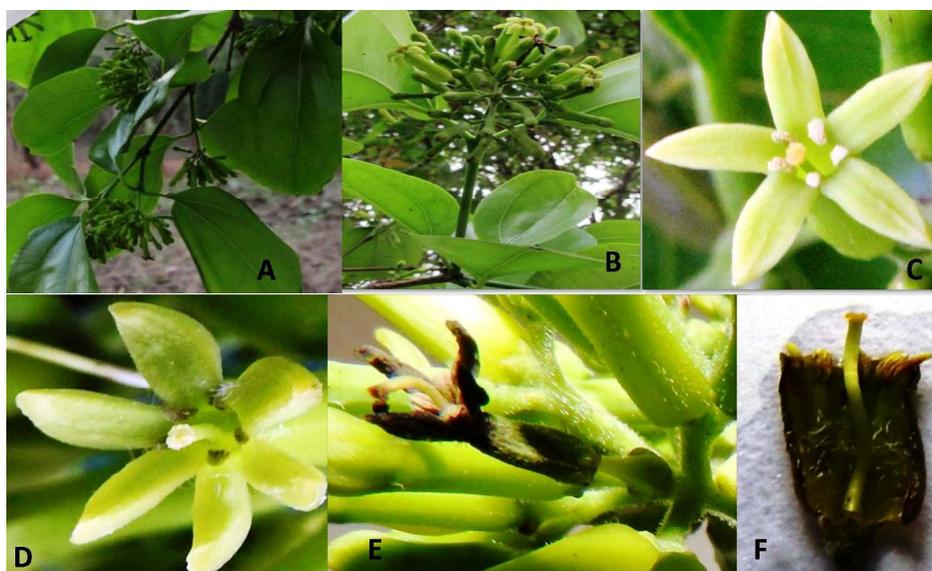


Fig. 1. *S. nux-vomica* flower A & B-Inflorescence, C-On set of anther dehiscence, D- Anthers after dehiscence, E- Withering of corolla, F-Position of anther & stigma head at bud stage

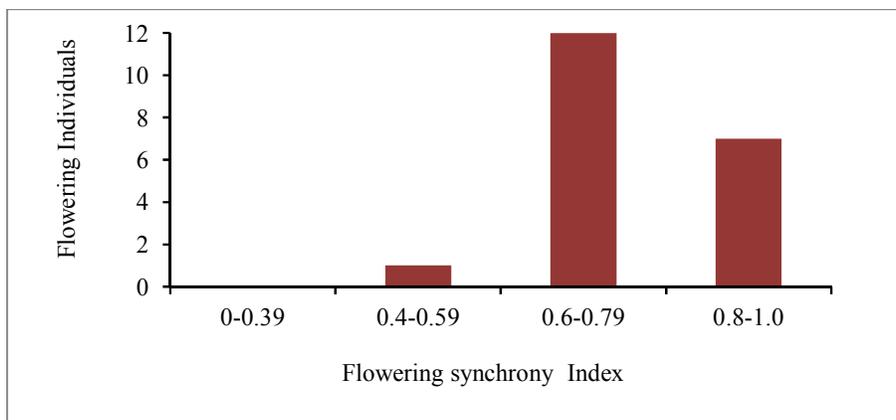


Fig. 2. Frequency distribution for values of flowering synchrony (N = 20)

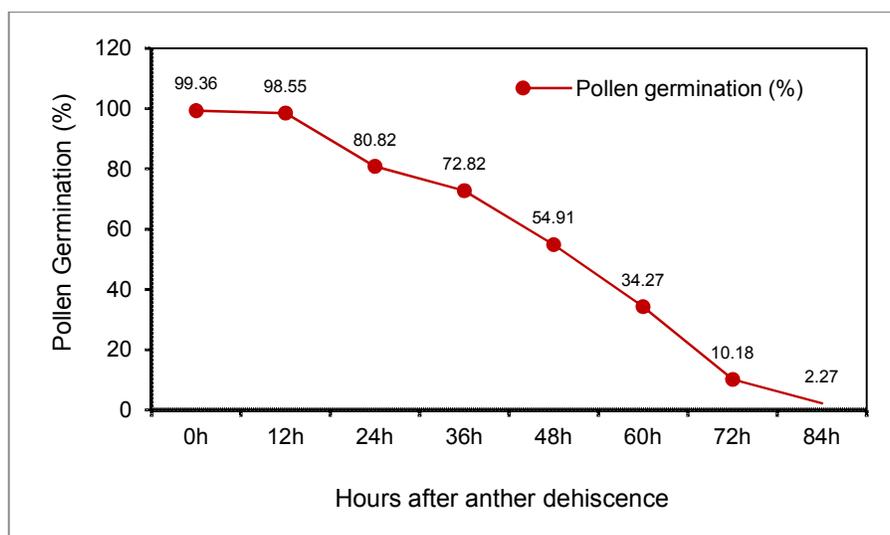


Fig. 3. Pollen germinability at different time intervals after anther dehiscence (by hanging-drop method)

The papilla on stigma surface remained turgid and fresh for another 8.0-8.30 hours still after complete withering of anthers (Fig. 1D). The freshness and turgidity of papilla coupled with yellowish-creamy colour of stigma indicated its receptive state. Its receptivity was confirmed with the formation of bobbles with 3% hydrogen peroxide (Fig. 5B). After that, the colour of stigma surface was changed to pinkish black indicating loss of its receptivity (Fig. 5D). The temporal gap in between prolonged receptive stage of stigma after complete anther dehiscence might create a chance for the pollens from other plants to pollinate stigma. This indicated possibility of dichogamy i.e. *protandry*. Temporal dichogamy particularly *protandry* is a common phenomenon in bisexual flowers. It reduces interference in pollen export and receipt. *Protandry* increases the competitive ability of pollens to produce vigorous progeny and favours cross-pollination [18].

During these periods, spatial change in position of stigma and anthers was also noticed. At the onset of flower opening non-receptive stigma was at par with the curvature of anthers. As anthesis advances and corolla fully opened up, receptive stigma slowly protrudes out of anther curvature due to stylar growth. This creates a gap and makes easy access for a pollinator to get the reward (nectar) at the base of the corolla tube (Fig 1D-F). Floral trait "pollen-ovule ratio (P/O)" has some impact on the breeding system in angiosperms. Generally, lower P/O value

corresponds to obligate autogamy condition where as higher value correlates with obligate xenogamous state [15]. The observed 335:1 P/O ratio in this species did not inferred towards complete self pollination or complete cross-pollination.

3.4 Flower Visitors and Pollination

A set of morphological traits (colour, odour, shape, etc.) and functional attributes (reward, anthesis, etc.) can be associated with pollination syndromes [19]. These syndromes are generally common or specific to plant species adapted to a certain type of pollinator. Pollination syndromes take into account these set of floral characters, which would allow determining the likeliest group of pollinators [20]. The morphological traits in *S. nux-vomica* flower attracting pollinators include colour (greenish-white corolla), odour (mild sweet) and reward (pollen & nectar). The flowers are not large enough and colourful to attract the attention of pollinators but synchronized blooming and their arrangement in inflorescence rachis makes them showy. The deep green foliage enhances flower visibility from distance. Simple & generalist blossom features like tubular corolla with rim curvature at the mouth, greenish-whitish colour, and mild scent attract a group of insects and birds towards *S. nux-vomica* flower. Flowers were visited by different type of insects and bird species from 7.00-17.00 hours. A total of 10 species consisting of bees, flies, wasp, butterflies, and birds were recorded (Table 1).

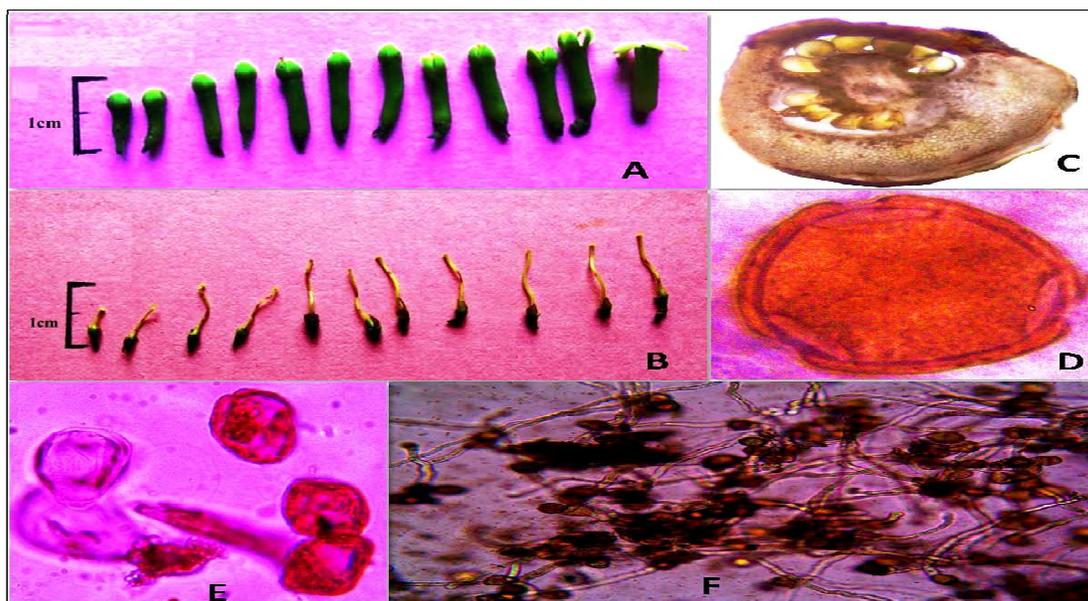


Fig. 4. Development of floral parts A -Stages of flower development, B- Stages of gynoecium development, C-Cross section of ovary, D- Tricolpate pollen, E- Oval shaped pollen, F- Pollen germination on 10% sucrose

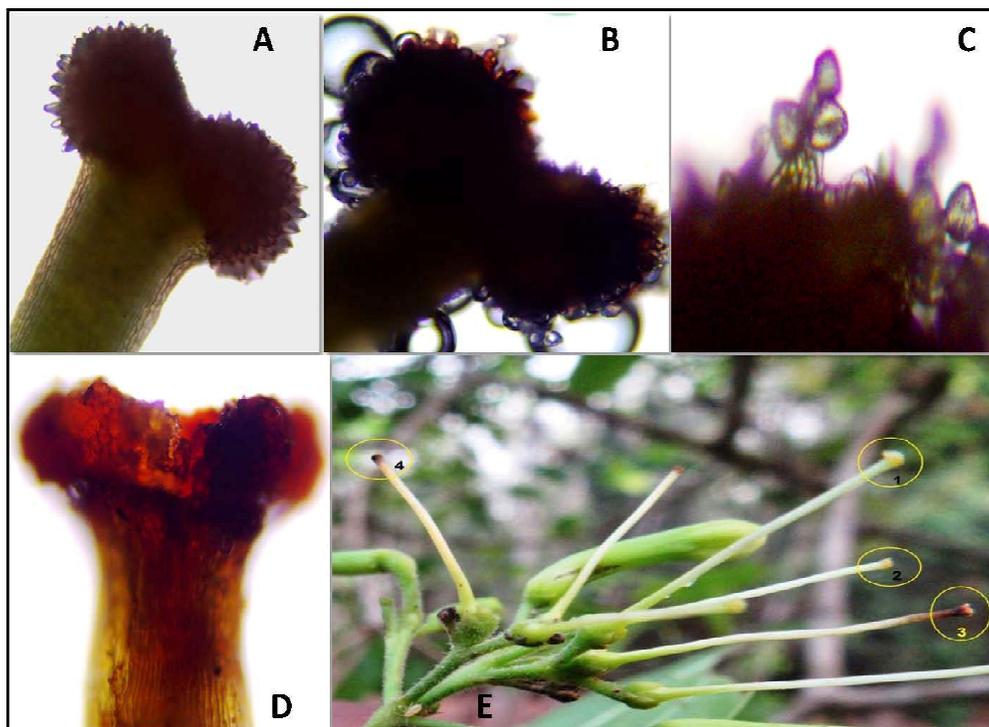


Fig. 5. Stigma receptivity A. Viscid stigma head with papillae, B. Bobble formation with 3% hydrogen peroxide, C- Pollen germination on stigma surface, D- Post receptive stigma head, E- Visual observation of stigma receptive stages in field

The bees included *Apis dorsata*, *Apis mellifera* and *Trigona species*. Of these, *Trigona species* visits occasionally and the other two are regular. They foraged both for honey and pollen. The activity of *Apis dorsata* was at peak during 9.00-12.00 hours. Four species of butterflies *Euploea core*, *Junonia iphita*, *Danaus chrysippus*, and *Catopsilia pyranthe* visited flowers between 7.00-17.00 hours. Among the butterfly visitors, *Euploea core* and *Junonia iphita* visited regularly. One fly species *Episyrphus balteatus* visited regularly for honey. One species of bird (*Leptocoma zeylonica*) visited occasionally for honey. Among the visitors *Apis dorsata* (bee) constituted 32.10%, *Euploea core* (butterfly) 13.23% and *Episyrphus balteatus* (12.65%) of the total visits. The foraging activity of *A. dorsata* was peak between 10.00-12.15 hours, *E. core* between 10.00-11.00 hours and *E. balteatus* between 9.00-10.00 hours.

Tubular corolla allows bees and butterflies to land on the top of the flower. The bees probed the flowers in an upright position where the stigma head gets easily contacted by its position and thus the transfer of pollen load. The early withering of stamens and enhanced position of stigma above corolla brim creates a small gap and provides sufficient space for pollinators to collect nectar present at the base. Small length of corolla tube assists flower visitors in getting relatively copious amount of nectar. Specialised morphological traits, apart from attracting diversified pollinators, insures their rate of visit adequate for successful pollination thus increasing the chances of out-crossing [20].

3.5 Breeding and Fruiting Behaviour

Details of fruit and seed set are presented in Table 2. The response of *S. nux-vomica* to different pollination methods tested showed mixed mating type as was evident by fruit and seed set in autogamy, geitonogamy (manipulated selfing), open pollination and xenogamy (manipulated cross pollination) treatments. Fruit set under xenogamy was the best among all the treatments with 12% fruit set and an average of 4.38 seeds per fruit. None of the emasculated and bagged flowers sets fruits indicating the absence of apomixis (T₅) in this species. No significant difference was observed in open pollination (T₃) and xenogamy (T₄) for all the parameters. But, a significant difference was observed among autogamy (T₁) and manipulated selfing (T₂). Larger sized fruit (37.99 mm dia.) was recorded in xenogamy, which was statistically at par with open pollination (37.34 mm) and geitonogamy (36.00 mm) but no conclusion can be drawn about performance rate. The number of seeds/fruit and seed weight directly related to the size of fruit and ranged from 1-14. In treatment T₄ (xenogamy), weight of fruit (34.11g), number of seeds/fruit (4.38 nos.) and hundred seeds weight (164.3 gm) was significantly higher than open pollination, geitonogamy, and selfing.

Mixed mating in which a hermaphrodite flower reproduces seed both by self as well as cross fertilization is common in seed plants [21]. Mixed mating is a transition state of breeding system evolution in plants where plants develop some

Table 1. Foraging activity of insects and bird species on *S. nux-vomica*

Insect species	Foraging time (h)	Reward	No of foraging visits/day*	Percentage of total visits	Nature of visit
Bees					
<i>Apis dorsata</i>	7.00-17.00	P, N	165	32.10	} 45.72 Regular
<i>Apis mellifera</i>	7.00-17.00	P, N	46	8.95	
<i>Trigona Sp.</i>	9.00-11.00	P, N	24	4.67	
Flies					
<i>Episyrphus balteatus</i>	8.00-16.00	N	65	12.65	Regular
Wasp					
<i>Vespa Sp.</i>	10.00-16.00	N	30	5.84	Occasional
Butterflies					
<i>Euploea core</i>	8.00-16.00	N	68	13.23	} 33.46 Regular Regular Occasional Occasional
<i>Junonia iphita</i>	8.00-16.00	N	42	8.17	
<i>Danaus chrysippus</i>	8.00-16.00	N	34	6.61	
<i>Catopsilia pyranthe</i>	8.00-16.00	N	28	5.45	
Birds					
<i>Nectarinia zeylonica</i>	8.00-12.00	N	12	2.33	Occasional

* 500 anthesed inflorescences were selected to record foraging visits

** Ten observations were made for each insect species; N –Nectar P-Pollen

Table 2. Results of breeding system in *S. nux-vomica*

Treatment	Parameter				
	Fruit set (%)*	Fruit size (Dia. mm) *	Fresh fruit wt. (g) *	Seed / Fruit (No.) *	100 Seed wt. (g)*
Apomixis	0.00	0.00	0.00	0.00	0.00
Autogamy	5.89 (3.43)	30.49 (6.49)	26.49 (6.15)	2.15 (2.47)	131.70 (12.48)
Geitonogamy	7.15 (3.67)	36.00 (7.00)	32.53 (6.70)	3.75 (2.93)	155.05 (13.45)
Xenogamy	12.00 (4.61)	37.99 (7.16)	34.11 (6.69)	4.38 (3.09)	164.30 (13.82)
Open Pollination	9.775 (4.13)	37.34 (7.11)	32.34 (6.69)	4.25 (3.06)	161.13 (13.69)
CD _{0.05}	0.497 (0.082)	2.172 (0.518)	0.984 (0.087)	0.412 (0.107)	3.137 (0.124)

Values in parenthesis are transformed values ($\sqrt{x+1}$), * Significant at $p < 0.05$

phenomenon to cope with stochastic environmental limitations such as scarcity of potential mate or pollinators. The evolution of mixed mating system in *S. nux-vomica* is a reproductive strategy to assure reproduction and maximisation of seed production even in a pollinator scarce situation. It prefers out crossing, as evident from the pollination experiment there by maintaining diversity in the population.

4. CONCLUSION

S. nux-vomica is a valuable drug plant. Most of the industrial requirement meets from seeds collected from the wild. Unregulated heavy seed harvest reducing its population from forest. Thus there is a vast scope for its cultivation. Information pertaining to floral biology, reproductive phenology, mating system and pollinators will be much helpful in developing a breeding strategy for selection of elite genotypes as well as conservation in the wild.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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