



Research Article

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**POLLINATION EVENTS IN *NIGELLA SATIVA* L. (BLACK CUMIN)**Suchetana Mukherjee¹, Aninda Mandal¹, Sudha Gupta² and Animesh K. Datta^{1*}¹Department of Botany, Cytogenetics, Genetics and Plant Breeding Section, University of Kalyani, Kalyani 741235, West Bengal, India²Department of Botany, Pteridology-Palaeobotany Section, University of Kalyani, Kalyani 741235, West Bengal, India

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ABSTRACT

The pollination events in *Nigella sativa* Family: Ranunculaceae; common name: black cumin; significant plant species for therapeutic uses and possessing spice yielding property) has been studied in relation to floral morphology, consequences of pollination types on reproductive parameters, types of visitors (*Apis dorsata* Fabr. – honeybees; *Coccinella transversalis* Fabr. - lady bug, *Danaus plexippus* L. - butterfly and *Colletes succinctus* L. - wasp) and flower color changes from bud to blooming stage. Honeybees are the predominant pollinators. Pollination is of 'generalized type'. The study has provided insight on the effective mode of pollination in the species maximizing seed yield.

Keywords: *N. sativa*; insect visitors; pollination

INTRODUCTION

Nigella sativa L. (Family: Ranunculaceae; common name: black cumin) is an annual herb with immense therapeutic uses notwithstanding its significance as a spice yielding plant of commerce¹. Black cumin seeds are most revered in Middle East as it can heal every disease except death². Being a significant plant species undergoing sexual reproduction, pollination events are important for effective fertilization to develop fruits and seeds. The present study describes floral morphology of the species, different modes of pollination and its consequences on reproductive parameters, insect visitors during flowering phase and changes in flower color from bud to blooming stage with an objective to identify the best mechanism for pollination and subsequently enhancing seed yield.

MATERIAL AND METHODS

Selfed lines of *Nigella sativa* L. (Ranunculaceae) has been maintained in the experimental field plots of University of Kalyani (West Bengal plains, Nadia,

latitude 22°50' to 24°11' N, longitude 88°09' to 88°48' E; soil- 9.9% sand, 2.9% silt and 87.2% clay; organic carbon- 3.25%; soil pH 6.87) for last 10 years. Herbarium specimen (voucher no. NS-1/2004) has been deposited in the museum of Department of Botany, University of Kalyani. The plants are grown at 25×15 cm row to plant distance. The flowering phase in the plant species is from mid January to late February. Floral morphology, reproductive parameters namely capsule length, seeds/capsule, seed size and 100-seed weight was studied in relation to 3 pollination events (Table 1). The insects were found to visit plantation throughout the day during the flowering phase and they were collected using butterfly net. The collected insects were identified from Entomology section, Department of Zoology, University of Kalyani.

Spectral analysis of pale green petaloid sepals (bud condition) and pigeon blue petaloid sepals (blooming stage) was performed as per Tatsuzawa et al.⁷. Thin layer chromatography (TLC) was also performed with both types of petaloid sepal extracts.

Table 1: Reproductive characters at different pollination modes

Pollination types	Flower size		Capsule length (cm)	Seeds/capsule	Seed sizes		100-seed weight (gm)
	Length (cm)	Breadth (cm)			Length (mm)	Breadth (mm)	
Controlled pollination	3.04±0.03	3.02±0.02	0.81±0.06	41.20±4.02	2.00±0.04	1.09±0.03	0.18±0.003
Open pollination	3.05±0.01	3.02±0.03	1.13±0.08	77.40±3.49	2.55±0.02	1.42±0.02	0.21±0.003
Removal of petaloid sepals and open pollination	3.03±0.03	3.02±0.03	0.57±0.03	24.40±1.41	1.56±0.04	1.01±0.04	0.13±0.004
χ^2 value	0.00065	0.00	1.88	307.85	2.41	0.81	0.19
P value of χ^2 test of heterogeneity at 2 DF	>0.99	1.00	0.30-0.50	<0.001	0.20-0.30	0.50-0.60	>0.90

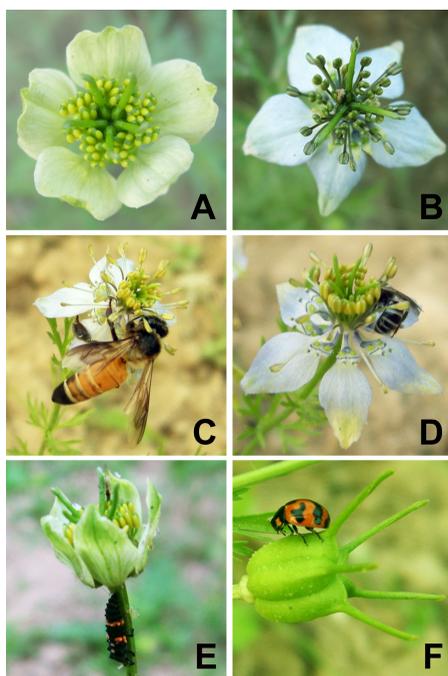


Figure 1: *Nigella sativa*. A- Flower showing pale green coloration of petaloid sepals; B- Pigeon blue flower at full bloom; C- *Apis dorsata* as visitor to flower; D- *Colletes succinctus* as visitor to flower; E- Larva of *Coccinella transversalis* in the peduncle of flower; F- *C. transversalis* in fruit

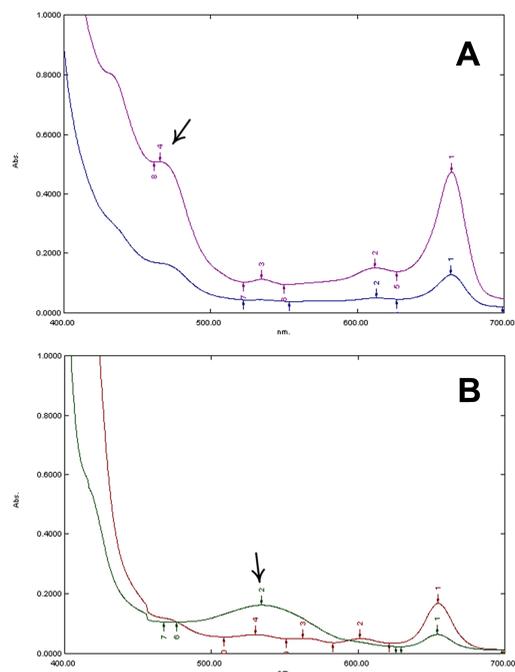


Figure 2: Absorbance peak (A- methanol acetone extraction; B- acidified methanol). A- Prominent carotenoid (→) and chlorophyll peaks in pink line; B- Pigeon blue sepal extraction showing anthocyanin peak (→) in green line, red line indicates no anthocyanin in pale green petaloid sepals

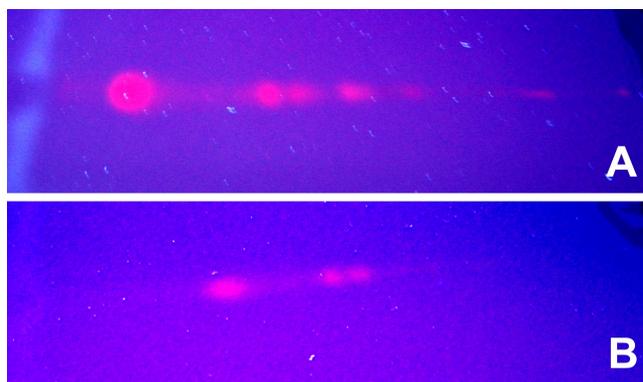


Figure 3: TLC profiles. A- Extraction from pale green sepals; B- Pigeon blue sepals

RESULTS AND DISCUSSION

For obtaining precise knowledge about the pollination events it is a prerequisite to attain information about floral morphology. Flower hermaphrodite, solitary on the main axis; size $3.04 \text{ cm} \pm 0.03 \times 3.02 \text{ cm} \pm 0.03$; color pigeon blue (RAL 5014); flower without any involucres of bracts, pedunculate, peduncle long, erect; petaloid sepals broad, ovate in a single whorl, 4 to 6, mostly 5 and characterized by the presence of nectaries; flower fertility on an average 89.89%; stamens in 3 to 4 whorls, numerous (32 to 66; mean 49.6 ± 2.7) and shed their pollen grains (anthesis: 7 am to 10 am) as the filament bent outward during male phase and pollination is effected on synchronization of both male and female phases; gynoecium 5, completely united follicles, each with a long indehiscent style and composed of variable number of multi ovule carpel, developing into a follicle after

pollination; fruit capsular. Abu-Hammour³ reported that anther receptivity occurred between 8.00 am to 1.00 pm for one day only and synchronization of male and female stages occur on the last day of flowering. Postweiler et al.⁴ suggested that high temperature effect fertilization success by affecting stigma receptivity and accelerating ovule degeneration.

In the present investigation 3 different types of pollination have been conducted to assess significant variations, if any, among the reproductive parameters (Table 1). Apart from seeds per capsule, none of the studied traits are affected due to different modes of pollinations. Flower size seems to possess non-significant outcome on the seed production. Maximum yield of seeds per capsule has been observed on open pollination (selfed/crossed) and it decreased markedly on the removal of petaloid sepals prior to pollination. Controlled pollination (selfing

following bagging) provided moderate seed yield per capsule. Due to bagging in control pollination and due to removal of petaloid sepals containing nectaries, the visitors may have been restricted. Maximization of seeds/capsule through open pollination possibly suggested the role of the visitors in pollination.

The visitors (Figure 1:C-D, F) noted during flowering phase of the species are namely, *Apis dorsata* Fabr. (Family: Apidae; honeybee – Figure 1:C), *Coccinella transversalis* Fabr. (Family: Coccinellidae; lady bug – Figure 1:F), *Danaux plexippus* L. (Family: Nymphalidae; butterfly) and *Colletes succinctus* L. (Family: Colletidae; wasp – Figure 1:D). The insects are found to visit plantation throughout the day (7.00h to 17.30h) during the flowering phase. The guts of honeybees, wasps, lady bugs and butterflies (as well as wing) have been dissected out, washed in sterile water, centrifuged (5000 rpm for 5 minutes, in each case), residue suspended in glycerol and observed under light microscope. Microscopic observations of each sample revealed pollen grains of *N. sativa* along with pollen grains from other sources. This indicates possible mutualistic interaction between the species (undergoing pollination) and insect visitors (nectar present in petaloid sepals). Pollinators are the key component to healthy ecosystems. Pollinators associated to black cumin pollination are found to carry pollen grains from other sources as well, which may be used for ecological benefit. As multiple pollinators are observed in a species, the type may be referred to 'generalized pollination'. Honeybees are found to be the predominant visitor. Lady bug has been found to lay eggs and produce larva (Figure 1:E) on the peduncle of the flower. Asian honeybees are reported to visit mostly pale colored flowers in deforested areas and crops⁵ as well as tropical and subtropical herbs, grasses and plantation trees⁶.

Significant in the species is that the flower color (color of the petaloid sepals) changes from pale green (RAL 6021) to pigeon blue (RAL 5014) at full bloom (Figure 1:A-B). Possibly color changes attracts the visitors for pollination events. Removal of all the anthers followed by bagging of floral buds also showed similar change of color in flowers which indicated that color change is associated with maturity of plants. Spectral analysis⁷ where sample A (extract of pale green petaloid sepals) showed carotenoid (460 nm absorbance) and chlorophyll (650 nm absorbance) peaks with acetone methanol (1:1) extraction as compared to prominent anthocyanin (530 nm) peak in

B (extract of pigeon blue petaloid sepals) sample (Figure 2:A-B) following extraction in acidic methanol (0.1% HCl). Therefore, there exist distinct variations in pigmentation in the samples. TLC (Thin layer chromatography) profile of A and B samples (extraction: acetone 22 ml : petrol ether 3 ml : spatula tip-full of CaCO₃; mobile phase – petroleum ether 100 ml : isopropanol 11 ml : distilled water 5 drops) also showed variation in spots (6 spots in A sample and 3 spots in B sample; R_f: 0.131 to 0.693; only 1 spot with 0.389 is common). The spots are pink under ultraviolet B (Figure 3:A-B). Further analysis is required to identify the anthocyanin produced in blooming flowers.

CONCLUSION

Pollination in *N. sativa* is found to be influenced by insect visitors and it is of 'generalized pollination' type. Honeybees are the predominant pollinators and maximization of seed yield occurs with open pollination event.

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