Original Article

POLLINATION STUDIES ON BOTH FLORET AND CAPITULUM LEVELS IN AN OIL SEED CROP, *GUIZOTIA ABYSSINICA* (L.F.) CASS.

Veereshkumar^{1,2,5*}K. M. Kumaranag^{1,3}Dibyendu Deb⁴Vasuki V. Belavadi^{*1}¹Department of Entomology, UAS, GKV, Bangalore, Karnataka, India²ICAR-Central Agroforestry Research Institute, Jhansi, Uttar Pradesh, India³Division of Entomology, Indian Agricultural Research Institute (IARI), New Delhi, India⁴ ICAR- Indian Grassland and Fodder Research Institute, Jhansi, Uttar Pradesh, India⁵Present address: ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, Karnataka, India

*corresponding e-mail: veeresh4279@gmail.com, vvbeldadi@gmail.com

Received: 17 December 2019; accepted: 09 November 2020

Veereshkumar, the lead author of the paper expired on 1st March 2021, when the paper was being processed for publication

Abstract

Niger, *Guizotia abyssinica* (L.f.), is an important oilseed crop widely grown in Ethiopia and India, but poor pollination is one of the major constraints in achieving the yield potential of the crop. Our aim was to understand better the pollination biology and role of flower insect visitors in reproduction success. Results indicated that full anthesis occurred in the morning between 06.00 and 08.00 h and pollen dehiscence eight to eleven hours after anthesis. Stigma receptivity commenced nine hours after anthesis and lasted for the next 24 h. A total of eighteen insect species representing six families visited Niger flowers, and among these, *Apis florea* and *A. cerana* were dominant. The flowers were self-incompatible as there was no seed set upon the selfing of floret and selfing of capitulum. The maximum seed set ($78.33 \pm 0.14\%$) was noticed when flowers were allowed for open pollination and the lowest was recorded when the whole plant was caged to exclude pollinator visits (0.33 ± 0.67). The time spent by *A. cerana* and *A. florea* was 1.35 ± 0.48 and 1.83 ± 0.12 seconds per floret, respectively and 9.63 ± 0.69 and 14.9 ± 0.94 seconds per capitulum, respectively. *A. cerana* and *A. florea* were found to be more efficient pollinators of *G. abyssinica*. Introducing bee colonies might greatly improve the yield, and it is also important to conserve bees in the vicinity of *G. abyssinica* fields during the flowering period.

Keywords: bees, foraging behaviour, florets, Niger, pollination, seed set

INTRODUCTION

Pollination is one of the most fascinating aspects of insect-plant interactions. The extent of interdependence is regulated by phenology and floral characters (Ram & Mathur, 1984) and their selection can shape the evolution of floral characters (Andersson, 2008). However, pollen dispersal patterns often reflect pollinator foraging behaviour and may not optimize the quality or quantity of mating in plant species

(Campbell & Dooley, 1992). Hence, the foraging behaviour of the pollinator has implications for a plant's fitness (Randall et al., 2009). Even in self-compatible species, the tendency of pollinators to visit several flowers of a single plant in a sequence increases the opportunity for self-pollination among flowers and results in increased selfing rate (Harder & Barrett, 1996; Snow et al., 1996; Karron et al., 2009). In self-incompatible species, the foraging behaviour of pollinators plays a vital role in seed set. Pol-

len-pistil interaction is a fundamental process leading to self-incompatibility (SI) (Edlund et al., 2004; Takayama & Isogai, 2005; Hiscock & Allen, 2008), which has been widely studied in several plant families including Asteraceae (Takayama & Isogai, 2005). It is estimated that 63% of species are SI, 10% are pseudo-self-incompatible and 27% self-compatible in Asteraceae (Ferrer & Good-Avila, 2007). However, pollinator foraging behaviour is influenced by floral resources and rewards that determine pollinator visitation rate and pollen dispersal (Murawski, 1987).

Niger, *Guizotia abyssinica* (L.f.) Cass. (Asteraceae) is an important edible oil seed crop and constitutes about 50% of Ethiopian and 3% of Indian oilseed production (Robbelen et al., 1989; Getinet & Sharma, 1996; Weiss, 2000). It is valued because of its high linoleic acid content in oil (55-80%) (Getinet & Teklewold, 1995) and it reduces the risk of coronary heart disease (Farvid, 2014). Niger with its complex floral biology is believed to exhibit variation in floral reward to modulate flower visitors. Insect pollinators and their foraging behaviour in relation to flower phenology of *G. abyssinica* need a thorough investigation for planning effectively managed bee pollination in order to optimize yields.

The publications from India have focused on the effect of bee pollination on seed yield in Niger (Gebremedhn & Tadesse, 2014; Painkra & Shrivastava, 2015; Sandipan et al., 2017; Kachhela & Pastgia, 2018; Rojeet et al., 2018). Studies on floral biology are limited to the determination of pollen germination (Veera Kumar, Gangappa, & Mahadevu, 2006). Self-incompatibility mechanism in Niger was reported by Chavan (1961), Mohanty (1964), Sujatha (1993), Veera Kumaret al. (2006), Patil & Duhoon, (2006), Geleta & Bryngelsson (2010). In the present study, for the first time we have attempted to examine detailed floral biology and interaction with pollinators at capitulum and floret levels in *G. abyssinica*. Our aim was to fully understand pollination biology and the role of flower insect visitors in the reproduction success of this plant. We hypothesized that self-incompatibility operates at the floret level but not at the

capitulum level, and that cross-pollination by bees enhances seed set.

MATERIAL AND METHODS

The study was conducted at the University of Agricultural Sciences (UAS), Gandhi Krishi Vignana Kendra (GKVK), Bangalore (13°N & 77°35'E; 930m above MSL), Karnataka, India. The study area was located in the Eastern Dry Zone of Karnataka State in south India. The climate of the area is typically semi-arid with a hot summer season followed by monsoon rains of low intensity and volume with a mild winter season. The experimental details are as follows: plot size- 3 acre; variety- KBN1; seed rate- 6 kg; spacing- 30 cm x 10 cm; plant density- 400,000; fertilizer- 35 kg N, 25 kg P₂O₅, 12 kg K₂O and 25 kg S per 3 acre.

The meteorological parameters viz., maximum and minimum air temperature (°C), relative humidity (%), and rainfall (mm) were recorded from the Automatic Weather Station located within 500 m radius of the experimental area in the UAS, Bangalore, India. The study was carried out from October to February. The mean maximum and minimum air temperature was 29.4 and 25°C, respectively. The morning relative humidity ranged between 82 and 98%, whereas the mean precipitation was 2.25 mm during the study period.

Floral biology

A total of thirty capitula were tagged and bagged with fine transparent muslin cloth bags to prevent insect visitation. The bags were removed carefully and the different phenological events were observed. While recording the observations bee visits to the tagged capitula were restrained considering the possibility of pollen deposition on the unopened anthers by foraging activity that may have resulted in wrong interpretation of anther dehiscence data. Each capitulum consisted of five or six rows of disc florets which followed centripetal succession for anthesis. Every day seven to ten florets per capitulum were opening, and these were used for the observing of the time

of anthesis, anther dehiscence, stigmatic lobes opening and floral longevity ($n=30$ capitula).

To record the stigma receptivity, a set of disc florets ($n=130$ florets; 5 florets/capitulum) were emasculated early in the morning before 06.00 h. Hand pollination was done at hourly intervals with a camel hair brush from 6 am to 6 pm using pollen from a neighbouring *G. abyssinica* plant. Similarly, stigma receptivity of ray florets (female flowers) were studied. Based on the preliminary results, the procedure was repeated at hourly intervals to record the time of peak stigma receptivity. Hand-pollinated pistils were fixed in Carnoy's fixative (absolute ethanol: chloroform: acetic acid; 6:3:1) twenty four hours after pollination. Pistils were cleared and stained according to methods proposed by Huang et al. (2004) and Veereshkumar et al. (2017). The percentage of germinated pollen grain was scored for receptivity. Pollen viability was estimated using 1% acetocarmine (Stanley & Linskens, 1974).

Quantity of nectar produced in the flowers, was estimated using calibrated capillary tubes (1mm diameter with 1 microliter volume). Nectar volume was measured at two-hour intervals ($n=10$ florets) from morning 6 am to 6 pm with a method adopted from Shrishail et al. (2011). Every time, nectar was estimated from the same florets.

Fully opened florets opened on the same day ($n=10$) were brought to the laboratory and examined for detailed floral structure (Veereshkumar et al., 2021). The floral parts were measured and photographed using a stereo binocular microscope (Leica M205C with auto-mountage and Leica DFC450 camera).

Flower visitors

Pollinator abundance was recorded during 25, 50, 75, and >90 % of floral density in the field. Each sampling day was divided into three time intervals of four hours each from 06.00 to 18.00 h. Samplings were done through visual counting of flower visitors for 15 minutes per predetermined transect of 1m^2 area, and this was repeated thrice in an hour with a five- minute gap between two subsequent

transects. Thus, we had three samples/hour and a total of thirty-six samples/sampling day. This was repeated twice during 25, 50, 75 and >90% of floral abundance in the field (Laroca & Orth, 2002; Belavadi & Ganeshaiah, 2013; Revanasidda & Belavadi, 2019; Veereshkumar et al., 2019). The percentage of flower abundance was calculated by number of plants produced flowers/ m^2 area (Belavadi & Ganeshaiah, 2013). The most frequent flower visitors of Niger were recognized during the sampling and further observed for their foraging behavior as described by Mattu et al. (2012), Belavadi & Ganeshaiah (2013), Sushil et al. (2013). Time spent by an individual bee per floret and capitulum ($n=30$ bees) and the number of capitula and florets visited by a single bee ($n=30$ bees) per five minute were recorded. The total number of visits received per floret were also recorded and observed, and capitulum for whole day and the average time lag between two subsequent bee visits were recorded before and after the peak stigma receptivity ($n=30$).

Number of pollen deposited: A set of capitulum were tagged and bagged early in the morning at 06.00 h to prevent bee visitation ($n=30$ capitulum). Each capitulum was unbagged and observed until an insect contacted a stigma. Insect species was recorded and then the stigma was carefully removed from the top of the style using fine-pointed forceps (Dafni, 1992). The collected stigma was transferred to 2 ml plastic vials and brought to the laboratory. Stigmatic surface was observed under stereo binocular microscope to count the number of pollens deposited by bees per visit.

Role of pollination in seed set

A field experiment was performed with seven pollination treatments (T1-T7) to determine the mating system of Niger and the role of flower visitors in seed set. T1) Florets compatibility: hollow polythene tube (7 mm diameter, 2 cm length) (Supplementary Fig.1) was placed on individual florets in a capitulum before the anther dehiscence. Pollen from the respective floret was dusted on stigmatic surface with the help of a camel hair brush ($n=30$). T2) Capitulum

compatibility: florets within a capitulum were randomly pollinated using pollen from other florets of the same capitulum and were covered with butter paper bags (n=30 capitula). T3) Compatibility of capitula from same branch: capitula from the same branch were tagged and hand pollinated with the pollen from other capitula of the same branch (n=30). T4) Compatibility between capitula from different branches: capitula were hand pollinated with the pollen from a capitulum of different branch of the same plant (n=30). T5) Compatibility between capitula from different plants: capitula were hand pollinated with pollen of another plant (n=30). T6) Caged plants: Plants were caged with a nylon mesh net to prevent pollinator visits (n=30) T7) Open pollination: capitula were allowed for open pollination (n=30). Flowers treated with different sources of pollen were in the same stage of development. We recorded the percentage of seed set in each pollination treatment.

In the experiment of controlled bee visits, *Apis cerana* and *A. florea* (1, 2, 3, 4 and 5 bee visits per capitulum) (n=30 each) were allowed to perform a fixed number of visits on tagged capitula and the percentage of seed set was recorded. A set of capitula were bagged randomly and then were later exposed individually to a single natural visit of *Apis cerana*. After the visitation, they were bagged again with nylon mesh to prevent further bee visitations and allowed for seed set. Similarly, for another set of capitulum, *A. cerana* was allowed to visit two times the same capitulum. Likewise, three, four and five visits were allowed to a set of capitulum and recorded percentage of seed set (n=30 capitula each). During the process, other flower visitors were chased away with the help of a sweeping net. This experiment was performed similarly with *A. florea*.

Statistical analysis

The data are presented as Mean $\pm$ SE in the results. The abundance of flower visitors was further subjected to a simple correlation coefficient analysis with prevailing weather conditions and the capitula abundance (Thriveni, 2019).

The percentage of seed set was calculated as described by Revanasidda & Belavadi (2019). Before ANOVA was performed, arcsine transformation (percent data) was used to normalize the data. One-way ANOVA was used for the analysis of data on the percentage of seed set influenced by pollination treatments. An analysis of the transformed data was conducted, and the untransformed means $\pm$ standard error are presented (supplementary). Two-way ANOVA was used to analyse the number of bee visits, bee species and their interactive effects on percent seed set using PROCGLM (SAS version 9.3, 2011; SAS Institute, Cary, NC, USA). When ANOVA indicated a significant F-value ($\alpha < 0.05$), the pollination treatment effects were further separated using Tukey's post hoc test.

RESULTS

Flower structure and floral biology

Niger is an annual herb and produced 22 ± 0.14 (Mean $\pm$ SE) primary branches (n=30 plants) with an average of 25 ± 0.18 capitula per branch (n=100 branches) (inflorescence of Asteraceae containing set of florets). Each plant produced 336 to 729 capitula, each capitulum is 1.5 to 2.2 cm in diameter (Fig. 1A), yellowish in color and with 5-6 rows of disc florets which followed centripetal succession for anthesis. There were 41-58 disc florets in a capitulum surrounded by a row of ray florets (7-12) and five green bracts. The disc florets were hermaphrodites with five yellowish orange united petals, five anthers and a centrally located style originating from the ovary, and the stigma was densely hairy. Each floret measured 11.90 ± 0.08 mm (n=30) in length, each disc floret was surrounded by a green ligule and ray florets were pistillate, measuring about 16.61 ± 0.43 mm (n=30) in length. The longevity of each capitulum, ray floret, and disc floret was 8.66 ± 0.11 , 6.63 ± 0.10 , and 2.47 ± 0.09 days (n=30), respectively. Capitulum, disk floret and floral phases are represented in Fig. 1A-E.

Anthesis occurred between 06.00 and 08.00 h (peak between 06.30 and 07.30 h), afterwards, the anther lobe projected outside and extended up to 2.89 ± 0.28 mm (n=30) within three to four

Fig. 1. Capitulum and floret phenology of *Guizotia abyssinica*. A) Capitulum: a-ray floret, b-opened disc florets, c-unopened disc florets; B) Floret bud of disc floret; C) Opened disc floret with anther dehiscence (arrow); D) Stigmatic lobes opening (arrow); E) Elongation of stigmatic surface (arrow).

hours and then dehiscence (Fig. 1C) started eight to eleven hours after anthesis (between 14.00 to 17.00 h with peak dehiscence between 15.00 to 15.30 h). The style extended beyond the anther lobe up to 2.41 ± 0.20 mm ($n=30$) within two hours of anther dehiscence and then the stigmatic lobes split open to form a bilobed surface (Fig. 1D). It was also observed that if a floret was not pollinated, the lobes of stigma curved further downwards to expose a greater surface area for pollinators (Fig. 1E).

Stigma became receptive nine hours after anthesis and lasted for 24-25 hours. The peak receptivity with 68.2% pollen germination was observed twenty-eight hours after anthesis between 10.00 and 14.00 h on the following day. Pollen grains remained viable for up to twenty-eight hours after anther dehiscence from 14.00 h (day one) to 18.00 h (day 2). Nectar cavity was located at the base of the style (Fig. 2A), and the peak nectar secretion (0.28 ± 0.08 μ l per floret ($n=10$)) coincided with peak stigma recep-

Fig. 2. Pollen germination, nectar cavity and seed set in *Guizotia abyssinica* A) Nectar cavity (arrow) inside the tube; B) No seed set in capitulum; C) Seed set (arrow) in ray floret

Fig. 3. Floral biology of Niger, *Guizotia abyssinica* (* indicates peak).

tivity (Fig.7). Ray florets did not produce nectar. Illustrations of floral biology are given in Fig. 3.

Flower visitors

A total of eighteen insect species representing six families visited Niger flowers (Supplementary Tab. 1). Hymenopterans were the most abundant (93.05%) followed by Diptera (6.16%) and Lepidoptera (0.79%). Among flower visitors, *Apis cerana* and *A. florea* constituted 90.34%, and so only these two species were observed for foraging behavior. These bees commenced their activity around 07.30 h; *A. cerana* had

two peaks of activity, one between 10.00-11.00 h and the other between 16.00-17.00 h, while *A. florea* had a single peak of activity between 11.00-14.00 h (Fig. 4). The abundance of bees was positively correlated with capitulum abundance ($r=0.99$) and maximum air temperature ($r=0.89$), while it was negatively correlated with relative humidity ($r=-0.47$) and rainfall ($r=-0.92$) (Supplementary Fig. 2).

The mean time spent per floret and capitulum by *A. cerana* was 1.35 ± 0.04 and 9.63 ± 0.69 seconds ($n=30$) and that by *A. florea* was 1.83 ± 0.12 and 14.9 ± 0.94 seconds ($n=30$), respectively (Fig. 5).

Table 1.

Effect of different pollination treatments on seed set of *Guizotia abyssinica*

Sl. No.	Pollination treatments	Seed set (%)
T1	Selfing of floret	00.00 ^e
T2	Selfing of capitulum	00.00 ^e
T3	Pollination between capitula of same branch	60.53 $\pm$ 1.14 ^d
T4	Pollination between capitula of different branch	65.66 $\pm$ 0.84 ^c
T5	Pollination between capitula of different plant	73.27 $\pm$ 0.84 ^b
T6	Plant caged	0.33 $\pm$ 0.67 ^e
T7	Open pollination	78.33 $\pm$ 0.14 ^a
	<i>F</i> value	4285.12
	<i>P</i> value	<0.001

Mean $\pm$ SE followed by same letter do not differ statistically at $P>0.05$ (post hoc Tukey's HSD test following One-way ANOVA)

Fig. 4. Abundance of *Apis cerana* and *A. florea* on *Guizotia abyssinica* throughout the sampling day.

Fig. 5. Time spent on *Guizotia abyssinica* by *Apis cerana* and *A. florea*: A) per floret; B) per capitulum.

On average, 23.33 ± 0.37 and 47.50 ± 0.83 visits were received for a single floret and capitulum, respectively, in a day. A single *A. cerana* forager visited 151 ± 1.89 florets and 29.00 ± 0.77 capitula per five minutes, whereas *A. florea* visited 135.66 ± 8.62 florets and 26.75 ± 1.03 capitula per five minutes. An *A. cerana* forager deposited a slightly greater number of pollen grains per visit (8.15 ± 1.05 (n=10 florets)) on the stigmatic surface compared to an *A. florea* forager (6.90 ± 0.72 (n=10 florets)).

The time lag differed between two subsequent bee visits recorded before and after peak stigma receptivity. Bee visitation was more frequent during the peak stigma receptivity. A floret was visited by bee once in every 3.13 ± 1.04 minutes at peak stigma receptivity and once every 8.58 ± 1.26 minutes before and after the peak stigma receptivity. Similarly, the rate of nectar secretion was more (0.28 ± 0.08 μ l nectar/ disc floret) at the time of peak stigma receptivity compared to before and after the

Fig. 6. Nectar and bee visits variation in *Guizotia abyssinica* before and after stigma receptivity and at the time of stigma receptivity.

Fig. 7. Number of bee visits per capitulum (one, two, three, four, and five bee visits) in *Guizotia abyssinica* and corresponding seed set (%). A) *Apis cerana* visits; B) *A. florea* visits.

peak stigmatic receptivity period ($0.09 \pm 0.02 \mu$ l nectar/disc floret) (Fig. 6).

Role of pollination in seed set

The percentage of seed set significantly varied among the pollination treatments ($F=4285.12$, $df=6$, 203 ; $P<0.001$) (Tab. 1). The maximum (78.33 ± 0.14) was recorded in T7 ($P<0.05$) when flowers were allowed for open pollination, and the lowest was recorded in T6 (0.33 ± 0.67) when the whole plant was caged to exclude pollinator visits. However, no seed set was observed during either floret selfing or capitulum selfing (Fig. 2B).

The role of *A. cerana* and *A. florea* visits on pollination was studied with controlled bee visits (1-5 visits per capitulum). The seed set significantly varied with a corresponding increase in the number of bee visits (Fig. 7) ($F=78.17$, $df=4$, 90 ; $P<0.001$) and also with the bee species ($F=12.69$, $df=1$, 90 ; $P=0.0006$). There was no significant difference between the number of bee visits and bee species ($F=0.64$, $df=4,90$; $p=0.63$).

DISCUSSION

Flower morphology often exhibits high phenotypic stability within and among plants of the same species or population (Stebbins, 1974) and drives pollination success (Grant, 1949). The capitulum of *G. abyssinica* has five to six rows of hermaphrodite disc florets surrounded by a row of large female ray florets (Getinet & Sharma, 1996). The ray florets enhance a plant's visual display to their mutual partners and a positive association has been observed between pollination success and the possession of rays (Stuessy et al., 1986). Unlike in the sunflower, ray florets are fertile and set seeds in *G. abyssinica* (Fig. 2C). To prevent selfing, disc florets exhibited adaptations such as anther lobe projection and extension, style elongation beyond the anther lobe, and split opening of stigmatic lobes. Stigmatic lobes opening after anther dehiscence could be an evolutionary strategy of the plant species to prevent selfing. In self-incompatible species, geitonogamous pollination can reduce

seed production because self-pollen interferes with outcrossed pollen-tube growth and increases seed abortion (Snow et al., 1996). In *G. abyssinica*, geitonogamy operates between capitulum of the same plant but not at the floret level. Floret is self-incompatible and bees must be there to transfer the pollens from one capitulum to another capitulum. To prevent self-pollination, Niger plant has evolved homomorphic pretenders of self-compatibility mechanism (Patil & Duhoon, 2006). The system of incompatibility of the Asteraceae family was confirmed by the dominant relationship of incompatibility genes in this family (Crowe, 1954). Nemomissa et al. (1999) studied the self-incompatibility system in the Ethiopian Niger population. The sporophytic self-incompatibility was believed to be controlled by a single 'S' locus which causes the inhibition of self-pollen germination (Prasad, 1990).

Bees are the main pollinators of Niger (Ramachandran & Menon, 1979; Veera Kumaret al., 2006; Gebremedhn & Tadesse, 2014; Sandipan et al., 2017; Kachhela & Pastgia, 2018; Rojeet et al., 2018). Pollinator visitation frequency depends on the quantity and quality of the floral rewards (Herrera, 1989). The activity of bees was more frequent during peak stigma receptivity due to higher nectar secretion compared to before and after peak stigma receptivity. The reproductive success of any bee-pollinated plant depends on the number of bee visits and foraging pattern. On average, seed set increased by 8.82 per cent per bee visit.

A single visit by *Apis cerana* or *A. florea* contributed for 26 per cent seed set and with each additional visit to the capitulum the average incremental increase in seed set was 4.48 per cent. This might be due to the initial huge deposition of pollen. Among the floral visitors, *Apis* species were effective pollinators (Sandipan et al., 2017; Kachhela & Pastgia, 2018; Rojeet et al., 2018). Seed set was observed when pollen was donated from flowers of the same branch, different branches of the same plant or from different plants. Maximum seed set was recorded during open pollination indicating that each capitulum requires a biotic vector and a

larger number of visits. Bee pollination has been reported to results in a 22-33% increase in the Niger yield (Panda et al., 1988). Sandipan et al. (2017) reported that the seed yield (401 kg/ha) was highest in open pollinated crop supplemented with *Apis mellifera* hive compared to without bee hives (287 kg/ha). Interestingly, a very low seed set (1.22 to 2.65%) was recorded when the whole plant was caged without pollinator visitation. This could be due to pseudo-self-incompatibility (Nemomissa et al., 1999), which is dictated by segregating polygenic modifiers of S-gene action (Levin, 1996) or due to a lack of a biotic pollen vector.

G. abyssinica is a highly cross pollinated crop and its reproductive success depends on pollinators. The general yield level of the crop is about 300 kg/ha while the potential yield could be four times more than this (Krishna, 2013). The low yield levels in both India and Ethiopia could be because pollinators are neglected. Hence, introducing bee colonies and conservation of native bees in the vicinity of *G. abyssinica* fields during its flowering period might greatly improve the yield of Niger. It may be necessary to work out the number of colonies required per ha in the future research.

ACKNOWLEDGMENTS

VK would like to thank Jagadeesh Patil, National Bureau of Agricultural Insect Resources for the statistical analysis; Tharini, B. K. and F. R. Pattan, University of Agricultural Sciences, Bangalore and Revanasidda, Indian Institute of Pulse Research, Kanpur, Uttar Pradesh for their help in taking observations and useful discussions during the course of the study.

REFERENCES

- Andersson, S. (2008). Pollinator and non-pollinator selection on ray morphology in *Leucanthemum vulgare* (Oxeye Daisy, Asteraceae). *American Journal of Botany*, 95(9), 1072-1078. <https://doi.org/10.3732/ajb.0800087>
- Belavadi, V.V., & Ganeshaiah, K.N. (2013). Insect Pollination Manual. Indian Council of Agricultural Research, New Delhi.
- Campbell, D.R., & Dooley, J.L. (1992). The spatial scale of genetic differentiation in a hummingbird-pollinated plant: comparison with models of isolation by distance. *The American Naturalist*, 139(4), 735-748. <https://doi.org/10.1086/285355>
- Chavan, V.M. (1961). Niger and Safflower. Indian Central Oilseeds Committee, Hyderabad.
- Crowe, L.K. (1954). Incompatibility in *Cosmos bipinnatus*. *Heredity*, 8(1), 1-11. <https://doi.org/10.1038/hdy.1954.1>
- Dafni, A. (1992). *Pollination Ecology: A practical approach*. Oxford University Press, New York, pp.254.
- Edlund, A.F., Swanson, R., Preuss, D. (2004). Pollen and stigma structure and function: the role of diversity in pollination. *Plant Cell*, 16(suppl), S84-S97. <https://doi.org/10.1105/tpc.015800>
- Farvid, M.S., Ding, M., Pan, A., Sun, Q., Chiuve, S.E., Steffen, L.M., ... Hu, F.B. (2014). Dietary Linoleic Acid and Risk of Coronary Heart Disease: A Systematic Review and Meta-Analysis of Prospective Cohort Studies. *Circulation*, 130(18), 1568-78. DOI: 10.1161/CIRCULATIONAHA.114.010236
- Ferrer, M.M., & Good-Avila, S.V. (2007). Macrophylogenetic analyses of the gain and loss of self-incompatibility in the Asteraceae. *New Phytologist*, 173(2), 401-414. <https://doi.org/10.1111/j.1469-8137.2006.01905.x>
- Gebremedhn, H., & Tadesse, A. (2014). Effect of honey bee (*Apis mellifera*) pollination on seed yield and yield parameters of *Guizotia abyssinica* (L.F.). *African Journal of Agricultural Research*, 9(51), 3687-3691.
- Geleta, M., & Bryngelsson, T. (2010). Population genetics of self-incompatibility and developing self-compatible genotypes in Niger (*Guizotia abyssinica*). *Euphytica*, 176(6), 417-430. DOI: 10.1007/s10681-010-0184-1

- Getinet, A., & Teklewold, A. (1995). An agronomic and seed quality evaluation of Niger (*Guizotia abyssinica* Cass.) germplasm grown in Ethiopia. *Plant Breeding*, 114(4), 375-376. <https://doi.org/10.1111/j.1439-0523.1995.tb01256.x>
- Getinet, A., & Sharma, S.M. (1996). Niger. *Guizotia abyssinica* (L. f.) Cass. Promoting the conservation and use of underutilized and neglected crops. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome. [https://www.biodiversityinternational.org/fileadmin/migrated/uploads/tx_news/Niger ___ Guizotia_abyssinica__L.f.__Cass._136.pdf](https://www.biodiversityinternational.org/fileadmin/migrated/uploads/tx_news/Niger___Guizotia_abyssinica__L.f.__Cass._136.pdf)
- Grant, V. (1949). Pollination system as isolating mechanisms in angiosperms. *Evolution*, 3(1), 82-97. <https://doi.org/10.2307/2405454>
- Harder, L.D., & Barrett, S.C.H. (1996). *Pollen dispersal and mating patterns in animal-pollinated plants*. In: Lloyd, D.G. & Barrett, S.C.H. (Eds.), *Floral biology: studies on floral evolution in animal-pollinated plants*. New York, Chapman and Hall, pp.140-190.
- Herrera, C.M. (1989). Pollinator Abundance, Morphology, and Flower Visitation Rate: Analysis of the "Quantity" Component in a Plant-Pollinator System. *Oecologia*, 80(2), 241-248. <https://doi.org/10.1007/BF00380158>
- Hiscock, S.J., & Allen, A.M. (2008). Diverse cell signalling pathways regulate pollen-stigma interactions: the search for consensus. *New Phytologist*, 179(2), 286-317. <https://doi.org/10.1111/j.1469-8137.2008.02457.x>
- Huang, Z.H., Zhu, J.M., Mu, X.J., Lin, J.X. (2004). Pollen Dispersion, Pollen Viability and Pistil Receptivity in *Leymus chinensis*. *Annals of Botany*, 93(3), 295-301. <https://doi.org/10.1093/aob/mch044>
- Kachhela, H.R., & Pastagia, J.J. (2018). Abundance of flower visitors and their foraging behaviour in Niger. *Journal of Entomology and Zoology Studies*, 6(6), 562-564.
- Karron, J.D., Holmquist, K.G., Flanagan, R.J., Mitchell, R.J. (2009). Pollinator visitation patterns strongly influence among-flower variation in selfing rate. *Annals of Botany*, 103(9), 1379-1383. <https://doi.org/10.1093/aob/mcp030>
- Krishna, K. R. (2013). Minor Oilseeds: Niger (*Guizotia abyssinica*) In: Agroecosystems: Soils, Climate, Crops, Nutrients Dynamics, and Productivity. Apple Academic Press, CRC Press, Taylor & Francis Group. Pp 208.
- Laroca, S., & Orth, I. (2002). *Melissocoenology: historical perspective, methods of sampling, and recommendations*. In: Kevan, P.G. & Imperatriz-Fonseca, V.L. (Eds.), *Pollinating Bees: The Conservation Link between Agriculture and Nature*. Ministry of Environment, Brasília, Brazil. pp. 217-225.
- Levin, D.A. (1996). The evolutionary significance of pseudo-self-fertility. *The American Naturalist*, 148(2), 321-332. <https://doi.org/10.1086/285927>
- Murawski, D.A. (1987). Floral resource variation, pollinator response, and potential pollen flow in *Psiguria warscewiczii*. *Ecology*, 68(5), 1273-1282. <https://doi.org/10.2307/1939212>
- Randall, M. J., Irwin, R.E., Flanagan, R. J., Karron, J.D. (2009). Ecology and evolution of plant-pollinator interactions. *Annals of Botany*, 103(9), 1355-1363. <https://doi.org/10.1093/aob/mcp122>
- Mohanty, R.N. (1964). Seed setting of Niger under controlled environmental conditions. *Indian Oilseeds Journal*, 8, 158.
- Mattu, V.K., Raj, H., Thakur, M.L. (2012). Foraging behavior of honeybees on apple crop and its variation with altitude in Shimla hills of western Himalaya. *International Journal of Science and Nature*, 3(2), 296-301.
- Nemomissa, S., Bekele, E., Dagne, K. (1999). Self-incompatibility system in the Ethiopians populations of *Guizotia abyssinica* (L.F.) Cass. (Niger). *SINET: Ethiopian Journal of Science*, 22(1), 67-88.
- Panda, P., Sontakke, B.K., Sarangi, P.K. (1988).

- Preliminary studies on the effect of honeybees (*Apis cerana indica* F.) Pollination on yield of sesamum and Niger. *Indian Bee Journal*, 50(3), 63-64.
- Patil, H.S., & Duhoon, S.S. (2006). Self-incompatibility, male sterility and pollination mechanism in Niger, *Guizotia abyssinica* (L.F.) Cass.- a review. *Agricultural Reviews* 27(2), 113-121.
- Prasad, V. (1990). Pollen tube growth and site of incompatibility reactions in Niger (*Guizotia abyssinica* Cass.). *Current Science*, 59(9), 466-468.
- Painkra, G.P., & Shrivastava, S.K. (2015). Effect of pollination by Indian honey bee, *Apis cerana indica* on yield attributing characters and oil content of Niger, *Guizotia abyssinica* Cass. in Surguja of Chhattisgarh. *Journal of Entomology and Zoology Studies*, 3(4), 218-222.
- Ram, M.H.Y., & Mathur, G. (1984). Flower-insect interaction in pollination. *The Proceedings of the Indian Academy of Sciences*, 93(4), 359-363. <https://doi.org/10.1007/BF03186255>
- Ramachandran, T.K., & Menon, P. (1979). Pollination mechanisms and inbreeding depression in Niger (*Guizotia abyssinica* Cass.). *Madras Agricultural Journal*, 66, 449-454.
- Revanasidda, & Belavadi, V.V. (2019). Floral biology and pollination in *Cucumis melo* L., a tropical andromonoecious cucurbit. *Journal of Asia Pacific Entomology*, 22(1), 215-225. <https://doi.org/10.1016/j.aspen.2019.01.001>
- Robbelen, G., Downey, K.R., Ashri, A. (1989). *Oil Crops of the World*. New York: McGraw-Hill.
- Rojeet, T., Ramya, H.R., Deka, M.K., Borah, R.K., Singh H.R. (2018). Pollinator diversity and foraging behaviour of honey bee, *Apis cerana indica* on Niger. *Indian Journal of Entomology*, 80(3), 867-869.
- Sandipan, P.B., Sharma, S.R., Jagtap, P.K., Patel, M.C., Solanki, B.P., Rathod, N. (2017). Seed yield increase in Niger crop in to relation to honeybee and other pollinators. *Cercetări Agronomice în Moldova*, 2(170), 73-81. DOI: 10.1515/cerce-2017-0016
- Shrishail, K. Kulloli, Arun, N. Chandore, Makarand, M. Aitawade (2011). Nectar dynamics and pollination studies in three species of Lamiaceae. *Current Science*, 100(4), 509-516.
- Snow, A.A., Spira, T.P., Simpson, R., Klips, R.A. (1996). *The ecology of geitonogamous pollination*. In: Lloyd, D.G. & Barret, S.C.H. (Eds.), *Floral biology: studies on floral evolution in animal-pollinated plants*. New York: Chapman and Hall, pp.191-216.
- Stanley, R.G., & Linskens, H.F. (1974). *Pollen: Biology, Biochemistry, Management*, Springer, New York, NY, USA.
- Stebbins, G.L. (1974). Flowering plants: Evolution above the species level. Edward Arnold, London, UK.
- Stuessy, T.F., Spooner, D. M., Evans, K.A. (1986). Adaptive significance of ray corollas in *Helianthus grosseserratus* (Compositae). *American Midland Naturalist*, 115(1), 191-197.
- Sujatha, M. (1993). Pollen-pistil interactions and the control of self-incompatibility in Niger (*Guizotia abyssinica* Cass). *Journal of Oilseeds Research*, 10(2), 334-336.
- Sushil, S.N., Stanley, J., Hedau, N.K., Bhatt, J.C. (2013). Enhancing seed production of three Brassica vegetables by honey bee pollination in north-western Himalayas of India. *Universal Journal of Agricultural Research*, 1(3), 49-53.
- Takayama, S., & Isogai, A. (2005). Self-incompatibility in plants. *Annual Review of Plant Biology*, 56, 467-489. <https://doi.org/10.1146/annurev.arplant.56.032604.144249>
- Thriveni, K.P. (2019). Correlation of whitefly population with weather parameters and management of leaf curl of chilli. *Journal of Pharmacognosy and Phytochemistry*, 8(3), 4624-4628.

- Veera Kumar, G.N., Gangappa, E., Mahadevu, P. (2006). Studies on floral biology and autogamy in Niger [*Guizotia abyssinica* (L.f.) Cass]. *Indian Journal of Genetics and Plant Breeding*, 66(2), 131-133.
- Veereshkumar, Uthappa, A. R., Madhulika Srivastava, Vijay, D., Kumaranag, K.M., Manjunatha, N., ... Chaturvedi, O.P. (2017). Floral biology of *Grewia flavescens* Juss.: an underutilized crop. *Genetic Resources and Crop Evolution*, 64(7), 1789-1795. <https://doi.org/10.1007/s10722-017-0536-y>
- Veereshkumar, Belavadi, V.V., Revanasidda, Srinivasa, Y.B. (2019). Stamen elongation in sunn hemp appears to allow delayed self-pollination in the absence of pollinators - A case of bet-hedging? *South African Journal of Botany*, 127 (1), 110-116. <https://doi.org/10.1016/j.sajb.2019.08.052>
- Veereshkumar, Kaushik, S.K., Rajarajan, K., Kumaranag, K.M., Uthappa, A.R., Sridhar, K.B., Badre Alam, Handa, A.K. (2021). Pollination biology of *Pongamia pinnata* (L.) Pierre: a potential biodiesel plant. *Genetic Resources and Crop Evolution*. 68(1), 59-67. <https://doi.org/10.1007/s10722-020-01010-6>
- Weiss, E.A. (2000). *Oil seed crops*. Blackwell Science Ltd., London.

Supplementary Table 1.

List of flower visitors recorded in *G. abyssinica*

S.I.no	List of flower visitors	Family
1	<i>Apis dorsata</i>	Apidae
2	<i>A. cerana</i>	
3	<i>A. florea</i>	
4	<i>Haplonomia westwoodi</i>	
5	<i>Lasioglossum</i> sp1	
6	<i>Lasioglossum</i> sp2	
7	<i>Seladonia</i> sp	
8	<i>Tetragonula iridipennis</i>	
9	<i>Xylocopa latipes</i>	
10	<i>X. ruficornis</i>	
11	<i>Megachile lerma</i>	Megachilidae
12	<i>M. cephalotes</i>	
13	<i>Campsomeris</i> sp.	Scolidae
14	<i>Eristalis arvorum</i>	Syrphidae
15	<i>Ischiodon</i> sp.	
16	<i>Tirumala limniace</i>	Nymphalidae
17	<i>Chrysippus</i> sp.	
18	<i>Pieris</i> sp.	Pieridae

Supplementary Fig. 1. Hallow tubes placed on the capitulum for floret self-pollination experiment within a capitulum.

Supplementary Fig. 2. Biplot of pollinators abundance and environmental variables; Minimum & Maximum Air Temperature (°C); RF- rain fall (mm); RH- relative humidity (%).