

Studies on pollination ecology and seed development in black cumin (*Nigella sativa*)

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ABSTRACT Black cumin (*Nigella sativa*) is an annual flowering plant and its seeds are commonly known as black cumin and kalonji. The flower of this crop basically shows a unique mechanism to ensure self pollination, although abundant pollinators also visit flowers. Seed development is an important event which determines the quality of seed being harvested. The aim of this study was to study the pollination ecology and seed development and maturation under Delhi conditions. It was observed that many pollinators visited flowers and the most common visitor was *Apis florea fabricius*, followed by *Apis cerana cerana fabricius* and *A. dorsata dorsata fabricius*. The present study revealed that black cumin was able to produce seeds under open condition (T_0), under selfing (T_1) and by hand pollination of emasculated flowers (T_2). The seed yield of 8.46, 8.10 and 7.83 g/plant was obtained in the treatments T_0 , T_1 and T_2 , respectively. Seed development and maturation, onset of germination, desiccation tolerance and period required for seed maturation was also studied. The onset of germination took place after 35-40 days of pollination, while acquisition of desiccation tolerance took place 40-45 days after pollination when the seeds attained harvestable maturity.

Keywords: Black cumin, medicinal crop, pollination, seed development, desiccation tolerance

Nigella (Ranunculaceae) includes about 20 species, but most commonly cultivated species are *Nigella sativa* L., *Nigella damascena* L. and *Nigella arvensis* L. [1-2]. It is native to south and southwest Asia [3]. It is a multipurpose *rabi* crop and the plant is cultivated worldwide for medicinal and culinary purposes. Under north and central Indian conditions, it is sown during October-November and harvested during March-April. The crop duration is about 5 months under Delhi conditions. It is an annual herbaceous plant and produce abundant pollen, which attract bees as it is a good source of nectar due to presence of nectaries on reduced corolla [4-5]. Despite its great importance; little attention has been paid to improve the production of this seed spice crop [6]. Pollination ecology is the scientific study of relationships between pollinators and their flower hosts. Pollination ecology is concerned with the life histories, distribution, and behaviour of individual species as well as the structure and function of natural

systems at the level of populations, communities and ecosystems. The famous findings on the interactions between flowers and insects that transport pollen grains to the stigmas of plants, demonstrating that they are essential for the production of fruit and seeds [7]. Since then, the knowledge of pollination as an essential process of ecosystem has vastly increased. A large majority of flowering plant species (more than 87%) interact with pollinators [8]. It has been suggested that over 300,000 animal species are directly dependent upon floral resources [9]. Many species have evolved specialised floral structures, which promote out-crossing. Despite this, self-fertilisation is possible, frequent and even promoted in some flowers (within and among individuals, populations and species [10-11]). However, it is not clear what drives plants to adopt autonomous pollination strategies, although possibilities include reproductive assurance in the face of pollen limitation, maintenance of locally-adapted genotypes and lack of requirement for a mate during colonisation of new habitats [12-13].

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The evolution of self-pollinating taxa from outcrossing progenitors is well documented in angiosperms. In *N. sativa*, the male phase is initiated a few days before the stigmas become receptive; however, the maturing styles often become twisted around the last dehiscing anthers, which results in self-pollination [12]. *N. sativa* is capable of setting seed without being cross pollinated [3]; its flowers are visited by honeybees [14]. Therefore, the present study was conducted to determine the pollination behaviour of plants and to determine seed yield under different conditions. The seed development process from ovule fertilization to physiological maturity can be divided into four phases; phase I and II comprise cell division and expansion, while reserve accumulation occurs in Phase III as seed dry mass increases. At the end of this phase, seed moisture loss is intensified (Phase IV). Seed maturation refers to morphological, physiological and functional changes that occur from the time of fertilization until the seeds are ready for harvest [15-16]. The seed is said to be physiologically mature when it attains maximum dry weight, germinability and vigour. Hence, seed maturation is an important event which determines the quality of seed being harvested. There were no studies available on seed maturation of *N. sativa*. Hence, study on seed development and maturation was undertaken.

MATERIALS AND METHODS

The field experiment was conducted at Division of Seed Science and Technology, Indian Agricultural Research Institute, New Delhi. The crop was sown in October, 2011 and harvested on April, 2012. The experimental material was NRCSS AN 1, a variety of *N. sativa* L. obtained from NRC Seed Spices (Ajmer, Rajasthan).

In order to study the pollination behaviour of annual dicotyledonous black cumin, an experiment was conducted

comprising of three treatments (Table 1). The plants were tagged randomly and exposed to open pollination which may be selfed or crossed (T_0). Alternatively, the plants were covered with net and only selfing was allowed (T_1) (Fig. 1a), while in T_2 , the plants were caged and flowers were emasculated before anthesis and pollinated from pollen of other plant. To investigate behaviour of pollinators, observations were taken to record the number of visits of pollinators per flower per hour continuously for two days. The duration of visit and time spent on each flower was also observed by randomly selecting five flowers in five plants. The observations on plant height (cm), number of primary and secondary branches, number of capsules per plant, seed yield per plant and seed quality as reflected by seed weight, size and colour were taken.

About 50 flowers were tagged and monitored for 45 days till seed reaches to maturity to study seed development and maturation. The capsules were collected at 5 days interval and seeds were obtained after the first week of head emergence and observations were taken up to 45 days on capsule length and width, seed length and width, fresh and dry weight of capsules and seeds till the seed maturity (Fig. 1b-f). The dry weight of capsules was determined by hot air oven method; 12 capsules in batches of 4×3 replicates were weighed after drying at 103°C for 17±1 h in oven. To study maturation stage, the fresh and dry weight of seeds at different developmental stages was taken. Moisture content was determined during seed development until maturation by low temperature hot air oven method at 103°C for 17±1 h. The onset of germination was analysed by placing seeds on Whatman No. 1 filter paper at 25°C. Acquisition of desiccation tolerance and imposition of dormancy were studied in seeds by drying in ambient condition for 4 h and placed for germination in Whatman No. 1 filter paper.

Table 1. Treatments under field experiment for study of pollination ecology

Treatments	Methodology
T ₀ Control	5 plants were tagged and left for open pollination which may be self or cross (Control)
T ₁ Geitonogamy	5 plants were covered by cloth net cages to ensure self pollination (Only caged)
T ₂ Xenogamy	5 plants were covered by cloth net cages and each flower was emasculated before the start of male phase and pollen grains collected from other plants (outside the cage) were used for hand pollination (Caged and emasculated)

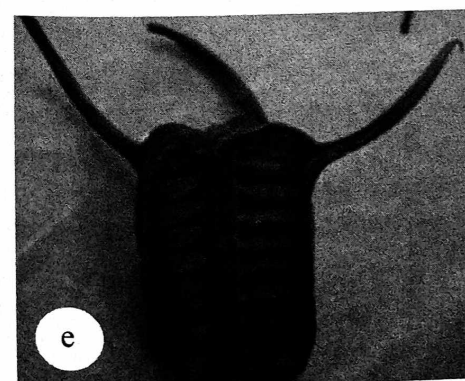
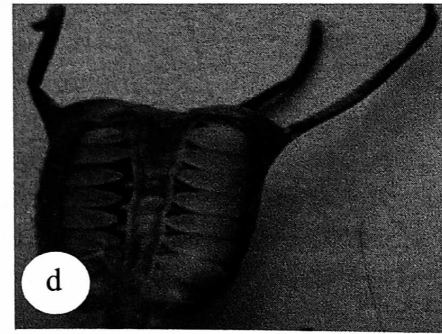
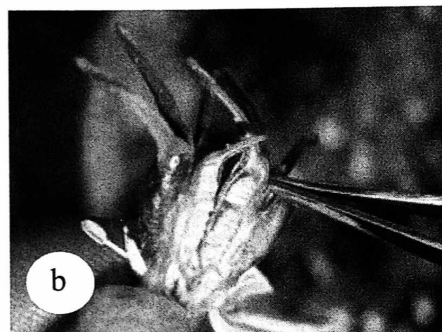


Fig. 1. (a) Caged plants in the field, Seed development stages of *Nigella sativa* (b) Before fertilisation (c) 5 DAF* (d) 10 DAF (e) 15 DAF (f) 20 DAF, *DAF: Days after fertilization

The embryo-seed ratio and level of embryo differentiation was determined by section cutting and staining. The seeds were soaked in distilled water for overnight at room temperature (20-22°C) prior to staining. The soaked seeds were cut with sharp knives or razor along its length so that the linear and basal embryo becomes clearly visible. The cut section of seeds was stained in tetrazolium solution (1 %) for 4 hours at room temperature. Finally, stained sections were examined under stereobinocular microscope to see the staining pattern of the embryo and endosperm.

RESULTS AND DISCUSSION

The pollination studies on *N. sativa* are very limited. It was observed that flowers of *N. sativa* are visited by bees [14], while seven kinds of bees / visitors were recorded [17] and highest seed yield was obtained under open conditions. Further, it was observed that pollination by honeybees (*Apis mellifera*) increased seed setting and seed yield [6]. Perusal of data in Table 2 revealed that seed yield of 8.46 g per plant was obtained under open conditions, suggesting that *N. sativa* was able to produce seeds under open conditions (un-covered and tagged plants, T₀), 8.10 g per plant under selfing (plants covered by net cloth, T₁) and 7.83 g per plant in case of hand pollination of emasculated flowers (plants covered by net cloth and flowers emasculated periodically, T₂); though the seed yield per plant obtained in three treatments was not significantly different from each other. However, 1000 seed weight was more *i.e.* 1.78 g in case of seed obtained from plants that were allowed only selfing, compared to open and cross pollinated conditions. The experimental findings clearly suggest that *N. sativa* was able to produce seeds by selfing as well as by cross pollination, irrespective of seed weight (Table 2).

It possesses characteristic feature of both entomophilous and anemophilous flowers. As entomophilous flower, it shows

petaloid sepals, large flower size, presence of nectaries in corolla, attractive flowers, sticky pollen grains and insect activity. On the other hand, it shows self-pollination mechanism, reduced corolla, large number of stamens and large number of pollen grains as anemophilous flower. Although many pollinators visited flowers during day time, but the frequency was maximum in case of *Apis florea fabricius*, followed by *Apis cerana cerana fabricius*, other pollinators like *Apis dorsata dorsata fabricius*, *didea* species recorded a very low activity (Fig. 2). The bee activity started from 9.30 am onwards and maximum activity was observed from 10.30 am to 11.30 am. The number of visits for *Apis florea fabricius*, *Apis cerana cerana fabricius*, *Apis dorsata dorsata fabricius* and other species were 20-25, 10-15, 5-10 and 1-2 per flower per hour respectively during peak period *i.e.* 10.30-11.30 am (Fig. 3). The highest bee activity was recorded during third week of February, 2012.

Most of research work usually involved seed sampling at pre defined intervals, followed by the determination and identification of specific morphological plant and characteristic seed parameters associated with the maturation process. Physiological maturity and time of harvesting are the major considerations in the production of quality seeds. Many species acquire the ability to germinate only when the seed switches from a developmental to a germinative mode during maturation drying. It was revealed that the capsule and fertilized ovule are small structures with respect to final size of capsule and seed (Fig. 1b-1f). The phases of embryogenesis involve intense cellular division and expansion, up to 15 days of pollination. These lead to progressive increase in size of capsule and seed, which attained maximum size at 20 days of pollination. At this stage, the maximum capsule length and width were 13.98 mm and 9.61 mm, respectively (Fig. 4a & 4b), and the maximum seed length and width were 2.91 mm and 1.53

Table 2. Effect of pollination treatments on plant height, number branches, capsules, seed yield and test weight in *Nigella sativa*

Treatments	Plant Height(cm)		No. of Priming Branches/plant		No. of Secondary Branches/plant		No. of Capsules		Seed yield plant (g)		1000 seed Wt. (g)
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean
(T ₀)	79.75	4.13	8.25	0.48	25.75	1.11	50.25	1.55	8.46	0.24	1.72
(T ₁)	81.00	3.34	7.75	0.25	25.75	0.48	27.50	2.84	8.10	0.29	1.78
(T ₂)	78.25	1.18	8.00	0.41	26.25	0.85	20.25	1.93	7.83	0.21	1.24
CD											
(p=0.05)	NS		NS		NS		7.06		NS		

*NS (Not Significant)



Fig. 2. Pollinating agents of *Nigella sativa* (a) *A. dorsata dorsata fabricius* (b) *A. florea fabricius* (c) *Didea* species (d) Unidentified (e) *A. dorsata dorsata fabricius* (f) *A. cerana cerana*

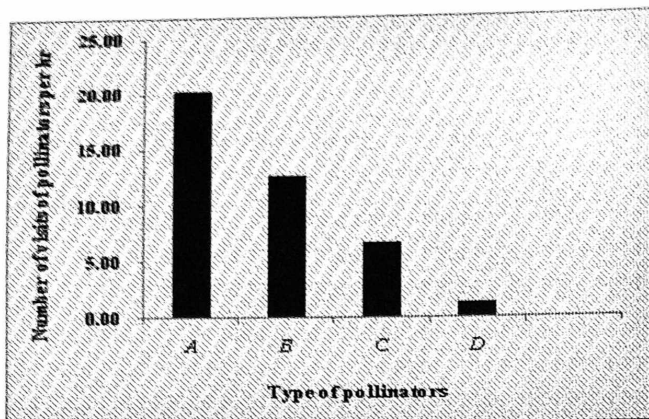


Fig. 3. Number of visits of pollinators per flower per hour, *A (*Apis florea fabricius*), B (*Apis cerana cerana fabricius*), C (*Apis dorsata dorsata fabricius*), D (Others)

mm, respectively (Fig. 4c & 4d). Thereafter, there was a marginal reduction in size of capsule and seed.

After fertilization, the developing capsule begins to increase in fresh weight as well as dry weight, resulting in nutrient accumulation and water uptake. The fresh weight of capsule is high due to high water content; subsequently, the fresh weight decreases and dry weight increases as maturation progresses. The point at which capsules reach maximum dry weight varies according to species. The capsule showed maximum dry weight *i.e.* 1.15 g at 35 days of pollination (Fig. 4e) and thereafter, no increase in dry weight was observed. Seed moisture content was very high at the time of fertilization, which decreases during subsequent development and maturation; although it remains relatively high throughout the maturation period because water is the vehicle for transferring nutrients from the parent plant to the developing fruits and seeds [18]. Seed filling (dry weight increase) was initially slow. The initial phase of dehydration was slow, which accelerated from the time they reach maximum dry weight. The seeds of *Nigella* reached maximum dry weight at 35-40 days after pollination (DAP) (Fig. 4f), which may be

considered physiologically independent of parent plant. The decrease in moisture content proceeds until hygroscopic equilibrium is attained *i.e.* after 40 days of pollination. From this point onwards, changes in moisture content are associated with variations in relative humidity [19].

Seeds were able to germinate at about 35-40 days after ovule fertilization. In this case, germination refers to the protrusion of the primary root [19], rather than formation of a normal seedling because his to differentiation has not been completed and reserve accumulation is still incipient at this phase. Therefore, seed germination does not lead to the production of vigorous seedlings. Theoretically, it is possible to consider that the percentage of germinable seeds increases during maturation, reaching a maximum around the time when seeds attain maximum dry weight of 0.146 g (Fig. 4f). This is only found in species where dormancy does not occur, because the imbalance in the germination promoters/inhibitors induced during the reserve accumulation period may directly affect seed germinability [19]. The seeds are not tolerant of drying at every stage during their development and will also undergo a transition from desiccation sensitive to desiccation tolerant stage [20]. The present study suggested that acquisition of desiccation tolerance in *N. sativa* occurs 40-45 DAP.

Some flower abnormalities were observed in *Nigella* during course of field experiment (Fig. 5). Both gynoeocious and androeocious flowers were present on same plant, along with normal hermaphrodite flowers. The abnormality of flowers affected reproductive parts of flower only, whereas no abnormality was seen in development of accessory parts of flower. In gynoeocious flowers, where well developed and normal fertile ovary was seen; while anther development was arrested in early stage and

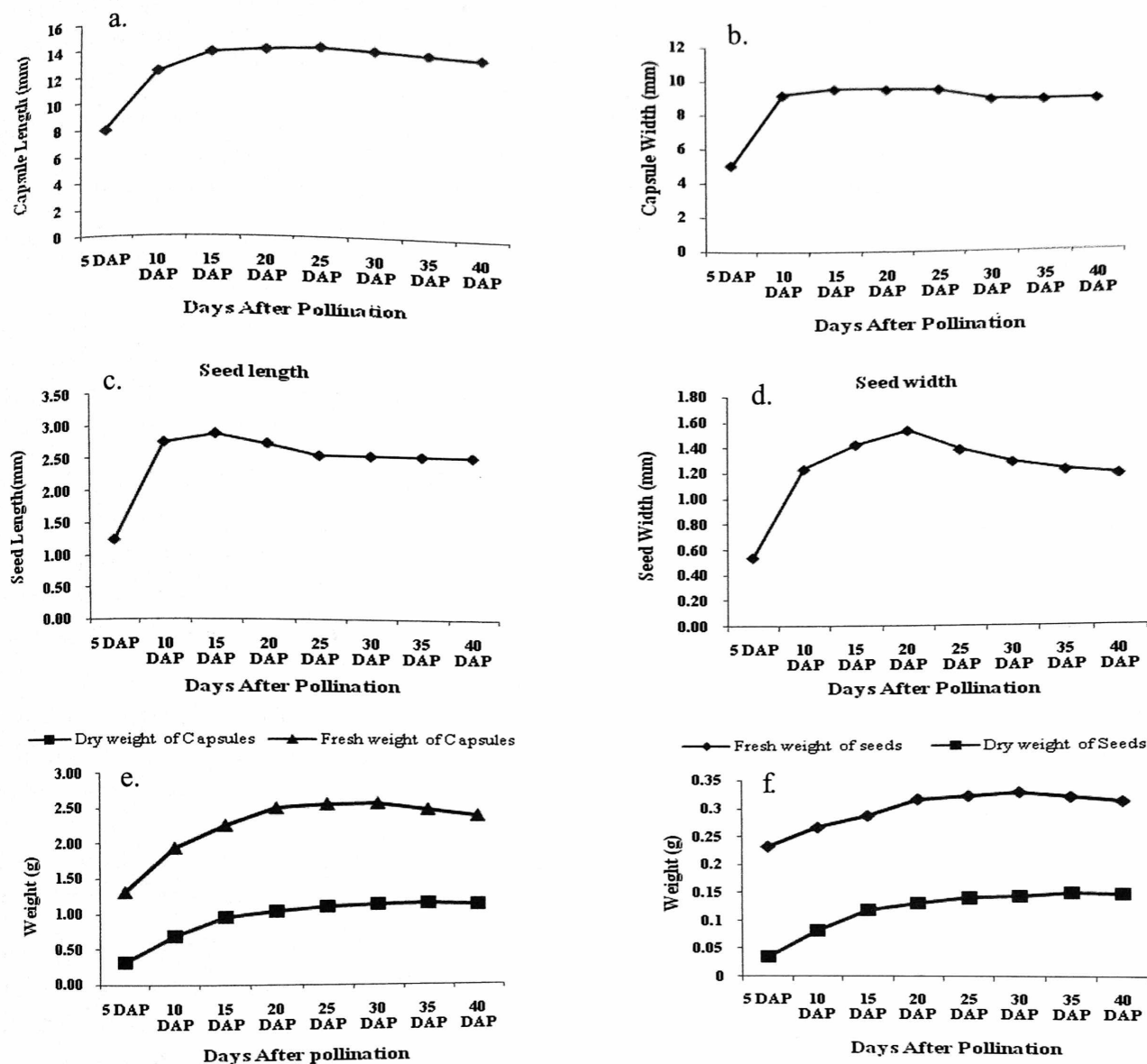


Fig. 4. Capsule/Seed growth parameters at different stages of seed development in *Nigella sativa*

short filament was produced which possessed withered, discoloured and infertile anthers (Fig. 5a). On the contrary, normal fertile anthers with long filament were produced in androecious flowers (Fig. 5b); ovary formation was arrested and no ovule formation was seen. In some emasculated flowers, more curved stigma was formed where initial development of ovary was normal; but no seed formation took place in the later stage (Fig. 5c). In this crop, the flower abnormalities are observed for the first time, as there were no reports in literature regarding this aspect.

Therefore, further research need to be done to confirm this phenomenon.

CONCLUSION

N. sativa was able to produce seeds under open (T_0), selfing (T_1) as well as under cross pollinated (T_2) conditions and seed yield of 8.46, 8.1 and 7.83 g/plant was obtained under T_0 , T_1 and T_2 , respectively. Therefore, it was able to produce seeds under complete selfing condition, where no pollinators are present. The onset of germination took place at about 35-40 DAP, while acquisition of desiccation

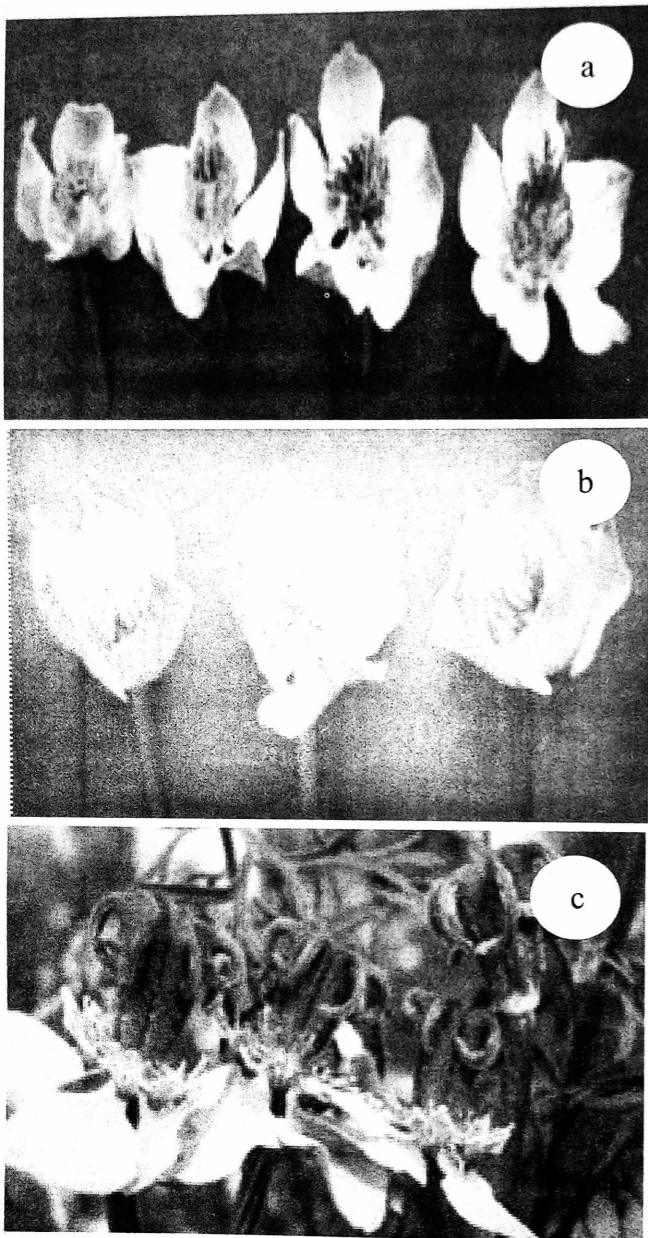


Fig. 5. Abnormalities in flowers and capsules of *Nigella sativa* (a) Aborted anther (b) Aborted female part (c) Abnormal capsule after emasculation

tolerance occurred after 40-45 DAP. The seeds of *Nigella* attained harvestable maturity at 45 DAP.

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