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Pollination by Honeybee (*Apis mellifera*) Increases Seed Setting and Yield in Black Seed (*Nigella sativa*)

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ABSTRACT

In order to determine the effect of honeybee pollination on annual dicotyledonous Black seed (*Nigella sativa* L.) yield, an experiment was conducted with three treatments: (i) open visits of bees and other pollinators, (ii) caged plants with bees and (iii) plants caged without bees (control). Number of seeds set, seed weight and yield per plot were measured. Number of seed set and yield differed significantly among the treatments but non-significant difference in the weight of seeds was observed. It was concluded that visits of honeybees at the time of 5% flowers initiation are helpful in seed set and seed yield in black seed.

Key Words: Honeybee; Pollination; Black seed; Seed set; Yield

INTRODUCTION

Flowers of Black seed *Nigella sativa* L. (Ranunculaceae) annual herbaceous plant called as “Kalongi” produce abundant pollen, which attract bees and is good source of nectar (Amin, 1991; Dantuono *et al*., 2002). *N. sativa* a traditional medicine extensively used for healing various respiratory disorders from Morocco to Pakistan and in Southern Europe (Amin, 1991). Amazingly black seeds chemical composition is very rich and diverse. Aside from its primary ingredients, crystalline nigellone, black seed contains 15 amino acids, proteins, carbohydrates, both fixed oils (84% fatty acids, including linolenic & oleic) and volatile oils alkaloids, saponin and crude fibre, as well as minerals such as calcium, iron, sodium and potassium. The seeds have been widely added as a spice to a variety of foods such as bread, yoghurt, pickles, sauces and salads for flavouring. They are also used in traditional medicines for some respiratory, gastrointestinal, rheumatic and inflammatory disorder (Nafisy, 1989; Zargari, 1990; Amin, 1991). The seeds have been reported to contain essential oil, fixed oil, flavonoids, saponins, alkaloids and proteins (Zargari, 1990; Burits & Bucar, 2000; Al–Ghamdi, 2001). Because the traditional and folkloric uses of *N. sativa* seed are supported by a long history of human experience, this plant may be an important source for the isolation of potential drugs. All registered effects make *N. sativa* an ideal candidate for use in cancer prevention and cure (Nafisy, 1989; Zargari, 1990; Amin, 1991).

Yield instability is a common problem in *N. sativa* and little attention has been paid for crop pollination in Pakistan and the plant was never evaluated for pollination requirement under our weather conditions. Lloyd (1979) showed that *N. sativa* is self pollinated without mention of the mechanism; Zohary (1983) showed that *N. sativa* is capable of setting seed without being cross pollinated. The flowers of *N. sativa* are visited by honeybees (Ricciardelli & Oddo, 1981).

Pollination studies on *N. sativa* are very limited. Despite its great importance; little attention has been paid to improve the production so it remained as minor or under utilized crop. The honeybee (*Apis mellifera*) is of great economic importance in terms of increased yield and quality of commercially grown insect pollinated crops and also in assisting self pollinated crops in the world (Free, 1993; Hoehn *et al*., 2008). This study was conducted to determine the effect of honeybee on black seed production with respect to quantity and quality but also to raise the awareness about the pollination services among the black seed growers.

MATERIALS AND METHODS

Experimental details. A piece of land in experimental area of National Agricultural Research Centre Islamabad during 2006 was selected for this study. The experiment was arranged in a randomized complete block design (RCBD) with three treatments and four replications each. The plot size for each treatment was (2 x 2 m^2), which consisted of six rows with row to row distance of 30 cm. Treatments applied were: (i) open plots allowing free visits of bees + other pollinators (T_1), (ii) plots caged with bees (T_2) and (iii) plots caged without bees (control - T_3). Black seeds were sown in the field on 3 November, 2006 by hand sprinkling technique. Agronomic practices like hoeing, weeding,

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application of fertilizer (NPK) and farmyard manure were similar in all the treatments.

The crop was visited five days a week from the date when flowers began to open i.e., 16 March, 2007, cages covered with muslin cloth were placed over T2 and T3 plots. Approximately 200 honeybees worker with mated queens were introduced in imported thermopore nucs (small boxes with 3-4 frames & approximately 300 bees) with mated queens, which proved good insulator against heat. Nucs were placed in caged as well as open plots 2-3 days after 5% flowering initiation has taken place. These nucs were placed on wooden boards nailed with strong pegs two feet above the ground level to save the nucs from rain, water and termites attack. Some wooden pieces were placed in plastic bowls for easy access of bees to water source. It eliminated the chance of dipping of bees, while sucking water. Water bowls were replaced twice a week to avoid water contamination with fungus etc. As some weeds also attract bees so complete eradication of weeds was done in experimental plots for maximizing exposure of black seed flowers to honeybees.

Number of foraging bees and other pollinators. The number of bees and other pollinators foraging in the open treatment were observed in one m^2 area for five minutes five days a week during the whole flowering period. The data was recorded at 9.00, 11.00, 13.00 and 15.00 hours a day.

Seed yield and yield components. At the time of maturity 10 mature pods were selected randomly from each replication and the number of seeds produced was counted manually. Harvesting was done from each plot when seeds were mature. The seeds were separated manually from the pods and yield was calculated per plot for all the treatments. Two hundred and fifty seeds from each replication of each treatment were randomly collected after harvesting their weights were determined and expressed as 1000 seed weight.

Statistical analysis. To analyze our data we used SPSS statistical programme version fourteen in which the data may appear to be in the same spread sheet form as a package such as excel but the approach is rather different as the statistics are not displayed on the spread sheet but in separate windows. Comparisons between means were made using the least significant difference (LSD) at 0.05 probabilities (SPSS). For statistical data, standard descriptive statistics were performed for each of the quantitative parameters.

RESULTS AND DISCUSSION

Pollination studies of *N. sativa* are scarce globally and none available from Pakistan. The number of bees and other pollinators visiting the open trial were observed and it was found that honeybees were the most frequent visitor with maximum activity at 11:00 am (Fig. 1). This may be due to the bee's activity being limited by environmental factors such as the radiation rate and daily temperatures. Visitations were estimated by counting the number of visiting tours and those with anther or stigma contact. Counts were made for 5 min, when the flowers were open. Pollination may accidentally take place without any relationship between blossom and agent. Even with the concept of a definite relationship, it is difficult to draw a line between pollinators and accidental visitors. The quantity of pollen transferred from anthers to stigmas, visit frequency to flower, pollinator forage pattern during anthesis and floral rewards availability are parameters that can adequately explain the pollination efficiency of floral visitors (Herrera, 1987 & 1989). It is generally thought the more visits made, the more efficient is the pollinator, though this also depends on the per visit pollen contribution to the pistillate flower part (Herrera, 1989).

We observed that the flower of black seed comprises five to ten petals and characterized by the presence of nectaries and a good source of pollen, as the pollen baskets of bees foraging on the flowers are mostly full of pollen. That might be the reason, why we found honey bees as the most or might be the only pollinator visiting the flowers. It is worth mentioning that even we have other crops like linseed and sarson producing flowers at the same time but bees preferred black seed instead of other crops, which is also confirmed by Ricciardelli and Oddo (1981), who mentioned that flowers of *N. sativa* are visited mostly by honeybees.

The flowers of *N. sativa* were un-attractive to wild bees visitors as the other recorded pollinators, which were very few in numbers were butterflies, syrphid flies, beetles and blow flies. An important aspect used in many pollination studies is the number of visits made by a pollinator (Proctor *et al*., 1996). The un-attractiveness of *N. sativa* flowers to wild bees may be attributed to several factors such as the presence of other floral resources. The number of seeds set/pod for the first treatment i.e., open visits of honeybees and other pollinators ranged (the most basic measure of dispersion) from 25 to 125 with the mean value 92.45, while the range and mean number of seeds produced for the caged plants with bees were 45 to 130 and 68.72 and the said values for the caged plants without honeybees were 45 to 130 and 87.47, respectively (Fig. 2).

The number of seeds set among the three treatments was analyzed and it was found that the treatments had significant ($P<0.001$) effect on the number of seeds produced. The effect of replications on the number of seed setting showed no significant ($P>0.001$) effects. Comparison of treatments showed that open visit of honey bees and other pollinators was significantly ($P<0.001$) different from the caged plants without honeybees as well as from caged plants with honeybees ($P<0.05$). The second treatment (caged plants with honeybees) was also found to be significantly different from the third treatment (caged plants without honeybees, $P<0.001$). Similar information has been documented for cucumber (Sajjanar *et al*., 2004), while Collision and Martin (1979) found a positive correlation

Fig. 1. The average number of honeybees visiting open plot treatment for 5 min

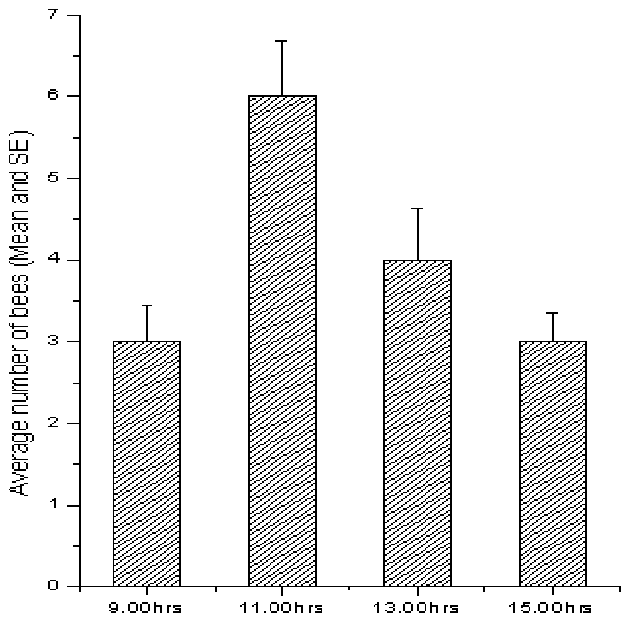


Fig. 3. Seed yield/plot (g) produced from plots under different treatments

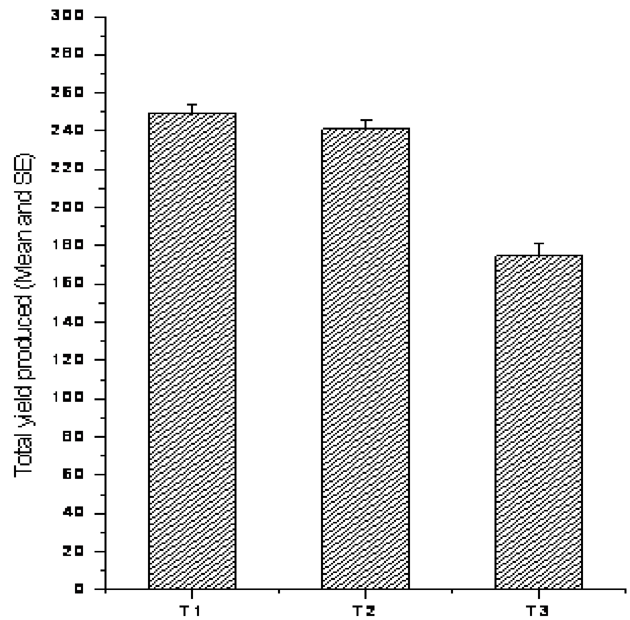


Fig. 2. The number of seeds produced/pod for different treatments

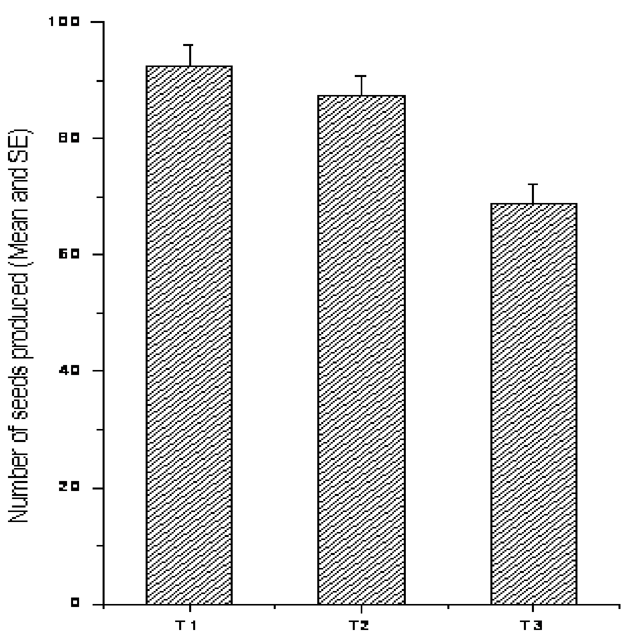
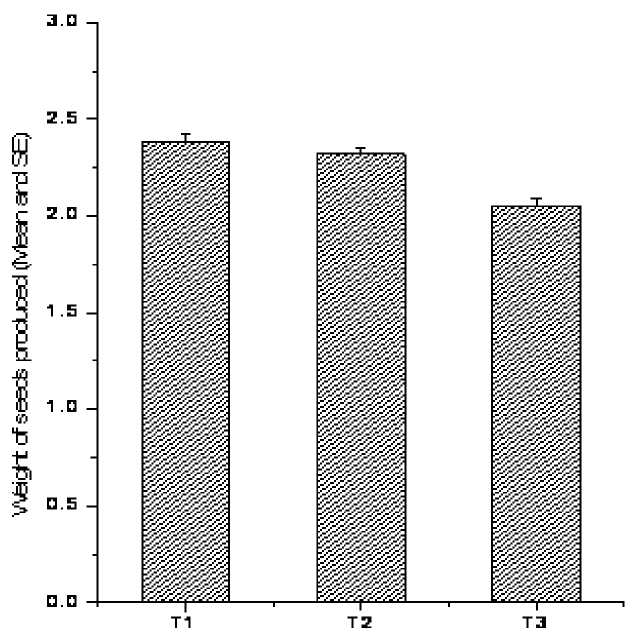


Fig. 4. The weight of 1000 seeds produced from pods harvested from different treatments



between daily fruit set and the amount of pollen being distributed with each bee visit.

The total yield of plots under different treatments, were compared and significant (P<0.001) differences were found. The yield did not differ for the treatment open visits of honeybees and other pollinators (249.7) and the treatment caged plants with honey bees (241.0) but both the treatments were statistically different from the treatment caged plant without bees (175.0) (Fig. 3). These results are in general agreement with many previous studies (Kochetov, 2004; Sajjanar *et al.*, 2004). Jyoti and Brewer (1999) showed that in the presence of honeybees, sunflower seed set increased by 7%, 1000 seed weight by 7 g and seed oil percentage was boosted by 3%.

Weight of 1000 seeds for treatment were 2.38 for open visits allowed, 2.32 caged plants with bees and 2.05 for caged plants without bees (Fig. 4). However, we found no significant difference among the treatments (P>0.05), which corroborates the findings of Garcia *et al*. (1998) for melon and Nizar and Khairala (2004) for Black seed pollination. Results revealed a significant increase (28%) in the *N. sativa* total yield (878.7 kg ha^{-1}) in the bee-pollinated plot, while the yield of the self-pollinated plot was 632.3 kg ha^{-1}. No difference was found in 1000 seed weight, which was same for both pollination conditions (3.35 g).

In angiosperms, the stigma is the first female structure the pollen grains and pollen tubes have to face on their way to stigma. The stigma provides an adequate environment for pollen grain germination (Knox, 1984; Helsop-Harrison & Shivanna 1997). An important features of stigma is its receptivity, defined as the ability of the stigma to support pollen germination (or the effective pollination period). This is a decisive stage in fertilization success (Helsop-Harrison, 2000). In this study, the stigma began to be active from 9:00 am, maximum at 11.00 am and then started to decline. This was also confirmed from the bees foraging on the plant, as they have enough pollen pellets in their pollen baskets at 11:00 am, which declined as the day progressed. Interestingly, the stigma of *N. sativa* is receptive only for few hours. This is due to the fact that the stigma has direct exposure to the sun, which may increase the exposed area. In addition, the receptivity of the stigma occurred after the stigma lost most of the anthers surrounding it, thereby exposing entire stigma to the sun further exposing it to sun. That implies that high temperatures affect stigma receptivity and reduces receptivity intervals. There is evidence that ensures the stigma responds to high temperatures (Hall, 1992; Stephenson *et al.*, 1992).

High temperature damages stigma, reduces the period of stigmatic receptivity and accelerates ovule degeneration (Postweiler *et al*., 1985). The duration of stigma receptivity shows inter-specific variation, which is usually greater in wind-pollinated than in insect-pollinated species (Khadari *et al*., 1995). Thus, the stigma can be receptive for not more than few hours (e.g. in *Avena* or *Dactylis*) to as long as a week (e.g., in *Pennisetum*, *Zea* and *Eucalyptu*) particularly in hostile environments (Helsop–Harrison, 2000). From an agricultural perspective, stigmatic receptivity also has practical value as it limits floral receptivity (Guerrero-Prieto *et al.*, 1985) and hence fruit set (Sanzol & Herrero, 2001). Moreover, in an ecologist context, by altering stigmatic receptivity, flowering plants may influence the likelihood of fertilization by indirectly controlling the number and the quality of mating by controlling the number of pollen grains deposited and their time of germination (Cruden *et al*., 1984; Primack, 1985; Galen *et al*., 1986).

CONCLUSION

Visits of honeybees in black seed increased the number of seeds set and yield produced but had no effect on the weight of seed produced. Thus strategies to promote pollination by honeybee may be helpful in enhancing seed yield in *N. sativa* and possibly in other related species.

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REFERENCES

Al-Ghamdi, M.S., 2001. The anti-inflammatory, analgesic and anti-pyretic activity of *Nigella sativa*. *J. Ethnopharmacol*., 76: 45–48

Amin, G.R., 1991. *Popular Medicinal Plants of Iran*, Vol. 1, pp: 118–119. Ministry of Health Publications, Tehran, Iran

Burits, M. and F. Bucar, 2000. Antioxidant activity of *Nigella sativa* essential oil. *Phytother. Res*., 14: 323–328

Collision, C.H. and E.C. Martin, 1979. The relationship of foraging activity of fruit set and shape in the pollination of pickling cucumber, *Cucumis sativus* L. *Proc. 4th Int. Sym. on Pollination. Agric. Exp. Sta. Spec. Misc. Pable*., 1: 183–188

Cruden, R.W., L. Hermanutz and J. Shuttleworth, 1984. The pollination biology and breeding system of *Monarada fistuloba* (Labiatae). *Oecologia*, 64: 104–110

Dantuono, L.F., A. Moretti and F.S.A. Lovato, 2002. Seed yield, yield component, oil content and essential oil content and composition of *Nigella sativa* L. and *Nigella damascena* L. *Indus. Crops Prod*., 15: 59–69

Free, J.B., 1993. *Insect Pollination of Crops*. Academic Press, London

Galen, C., J.A. Shykoff and R.C. Plowright, 1986. Consequences of stigma receptivity schedules for sexual selection in flowering plants. *American Nat*., 127: 462–476

Garcia, R.C., R.V. Resende, A.A. Marcos, D.P. Toloi, J.A.J. Faleiros, R.P. Turquino, R.S. De Souza and S. De Souza, 1998. Study of melon (*Cucumis melo*) pollination by *Apis mellifera* in the greenhouse. UNIMAR. *Ciencias*, 7: 123–131

Guerrero-Prieto, V.M., M.D. Vasilakakis and P.B. Lombard, 1985. Factors controlling fruit set of Napoleon sweet cherry in western Oregon. *Hort. Sci*., 20: 913–914

Hall, A.E., 1992. Breeding for heat tolerance. *Plant Breed. Rev*., 10: 129–168

Helsop-Harrison, Y., 2000. Control gates micro-ecology: the pollen-stigma interaction in perspective. *Annl. Bot*., 85: 5–13

Helsop-Harrison, Y. and K.R. Shivanna, 1997. The receptive surface of the angiosperm stigma. *Annl. Bot*., 41: 1233–1258

Herrera, C.M., 1987. Components of pollinator "quality": comparative analysis of a diverse insect assemblage. *Oikos*, 50: 79–90

Herrera, C.M., 1989. Pollinator abundance, morphology and flower visitation rate: Analysis of the "quantity' component in a plant-pollinator system. *Oecologia*, 80: 241–248

Hoehn, P., T. Tscharntke, J.M. Tylianakis and I. Steffan-Dewenter, 2008. Functional group diversity of bee pollinators increases crop yield. *Proc. Royal Soc. (B)*, 275: 2283–2291

Jyoti, J. and G.J. Brewer, 1999. Effect of honeybee (*Hymenoptera; Apidae*) pollination on sunflower hybrid. *Proc. 21st Sunflower Research Workshop, National Sunflower Association*, pp: 103–107. January 14-15, 1999

Khadari, B., M. Gibernau, M.C. Ansett, F. Kjellberg and M. Hossartmckey, 1995. When fig wait for pollinators: the length of fig receptivity. *American J. Bot*., 82: 992–999

Knox, R.B., 1984. Pollen-pistil interactions. *In*: Linskens, H.F. and J. Helsop-Harrison (eds.), *Cellular Interactions. Encyclopedia of Plant Physiology, NS*, pp. 508–608. Springer-Verlag, Berlin, Germany

Kochetov, A., 2004. Use of Carpathian bees for cucumber pollination in film green houses. *Kartofel-I-Ovosbchi*., 6: 28

Lloyd, D.G., 1979. Some reproductive factors affecting the selection of self fertilization in plants. *American Nat*., 113: 67–79

Nafisy, A.T., 1989. *A Review of Traditional Medicine in Iran.* Isfahan University Publications No. 122: Isfahan, Iran

Nizar, J.H. and K. Khairala, 2004. *The Role of Honey Bees (Apis mellifera Syriaca) in the Pollination of Black Cumin (Nigella sativa)*. First European Conference of Apidology, Udine, 19-23 Sep, 2004

Postweiler, K., R. Stosser and S.F. Anvari, 1985. The effect of different temperatures on the viability of ovules in cherries. *Sci. Hort*., 25: 235–239

Primack, R.B., 1985. Longevity of individual flowers. *Annl. Rev. Ecol. Syst*., 16: 15–37

Proctor, M., P. Yeo and A. Lack, 1996. *The Natural History of Pollination*. Harper Collins Publishers, London

Ricciardellid, D. and P. Oddo, 1981. *Flora Apistica Italiana*. Institution sperimentale zoologia agraria and Federazione italiana apicoltori, Interstampa, Rome

Sajjanar, S.M., G.C. Kuberappa and H.P. Prabhuswamy, 2004. Insect visitors of cucumber (*Cucumis sativus* L.) and the role of honeybee (*Aips cerana* F.) in its pollination. *Pest Manag. Eco. Zool*., 12: 23–31

Sanzol, J. and M. Herrero, 2001. The effective pollination period in fruit trees. *Sci. Hort*., 90: 1–17

Stephenson, A.G., T.C. Lau, M. Quesada and J.A. Winsor, 1992. Factors that affect pollen performance. *In*: Wyatt, R. (ed.), *Ecology and Evolution of Plant Reproduction*, pp: 119–134. Chapman and Hall, New York

Zargari, A., 1990. *Medicinal Plants*, Vol. 1, 5th edition, pp: 43–44. Tehran University Publications, Tehran, Iran

Zohary, M., 1983. The genus *Nigella* (Ranunculaceae) - a taxonomic revision. *Plant Syst. Evol*., 142: 71–107

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