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QUANTITATIVE AND QUALITATIVE ENHANCEMENT IN *TRIFOLIUM ALEXANDRINUM* SEED PRODUCTION THROUGH POLLINATION BY *APIS MELLIFERA* LINN.

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ABSTRACT

Studies on the contribution of *Apis mellifera* Linn. and other pollinators in seed production of *Trifolium alexandrinum* L. variety BL 42 revealed that significantly highest yield increase over control (exclusion of pollinators) was recorded in open pollination (125.34 %) whereas *Apis mellifera* Linn. pollination resulted in 115.98 per cent increase in yield over control. Seed germination was also highest in seeds obtained from open pollinated plots (90.67 %) followed by in plots caged with *A. mellifera* (88.67%) and plots caged without bees (80.00%). Seed vigour was also maximum in open pollinated plots (289.12). *A. mellifera* pollination resulted in significantly better seed vigour (278.17) than in seeds from plots caged without bees (221.59). So, pollination by *Apis mellifera* resulted in higher seed yield with better seed germination and vigour than control. Pollinators other than *A. mellifera* also collectively resulted in improvement of these parameters.

Key words: *Apis mellifera*, crop pollination, Egyptian clover; seed germination, seed production, seed vigour, *Trifolium alexandrinum*

The genus *Trifolium* belongs to the family Leguminosae and includes many species, of which Egyptian clover (*Trifolium alexandrinum* Linn.) is widely used as high quality forage. Egyptian clover, also known as berseem, is a winter crop widely grown in the Punjab on 0.21 million hactares (Anonymous, 2010) and other countries. *T. alexandrinum*, a predominantly self–pollinated legume is an important fodder crop. For better seed setting, tripping of flowers by any pollinating agent is needed in *T. alexandrinum.* The crop is in dilemma with regard to its self–fertility and infertility. Some authors regarded berseem as cross–fertilized (Latif *et al*., 1956; Roy *et al*., 2005) and others as self–compatible (self–fertile) (Beri *et al*., 1985). The present studies were planned with an objective of determining the role of *Apis mellifera* and other major pollinators visiting *berseem* on qualitative and quantitative parameters of the seed produced.

MATERIAL AND METHODS

Studies were conducted on BL 42 variety of *T. alexandrinum* at Punjab Agricultural University Seed Farm, Nabha (Distt. Patiala), Punjab during May–June, 2011.

To study the contribution of *Apis mellifera* Linn. and other pollinators in enhancing seed production of *T. alexandrinum*, three treatments namely, intensive pollination, open pollination and pollinators' exclusion were planned. Under intensive pollination, an *A. mellifera* colony of four bee–frames strength was enclosed with the crop under nylon cage of the size 6x3x3m. The honey bee colonies were placed inside the cages (one in each) at the start of flowering. These colonies were provided 1,000 ml sugar solution prepared by mixing sugar and water in equal quantities (w/w) to avoid any dearth of feed inside the enclosure. Stored pollen was removed from the colonies at the beginning of the experiment to encourage foraging of honey bees for pollen in order to get higher number of bee visitation on the flowers whereas in treatment of open pollination, the crop was not caged and was accessible to all the pollinators available in the area. These two treatments were compared with pollinators' exclusion where the crop was caged with nylon net of size 6x3x3m making it inaccessible to pollinating fauna. All the treatments were replicated six times.

Yield parameters were recorded to evaluate the impact of insect pollination on quantity and quality of the seed produced. The quantitative evaluation was done on the basis of per cent seed set, number of seeds per inflorescence and seed yield per hectare. The qualitative parameters recorded includes seed germination and seed vigour index.

Further, to determine the major insect pollinators on *T. alexandrinum* flowers, intensity of insect pollinators was recorded / m^2 / minute at randomly distributed five spots in each of the experimental plots at 0900, 1200 and 1500h at weekly interval. Since, pollen foragers are the major contributors in seed setting, diurnal population of nectar and pollen foragers of all the major pollinators was

recorded separately by observing twenty honey bees per replication and their percentages were worked out.

RESULTS AND DISCUSSION

A. Foraging Intensity: Number of individuals of different *Apis* spp., visiting / m^2 / minute were counted at randomly distributed five spots in each of the experimental plot. Intensity of *A. mellifera* was the highest (3.80 bees/m^2/ minute) followed by that of *Apis dorsata* Fabricius (1.13 bees/m^2/minute) irrespective of the time of the day (Fig. 1). Significantly higher mean foraging intensity of *A. mellifera* was recorded at 1500 h (5.40 bees/m^2/min) and at 1200 h (4.50 bees/m^2/min) than at 0900 h (1.50 bees/m^2/ min) (Table 2). In the case of *A. dorsata*, mean foraging intensity was also maximum at 1500 h (1.90 bees/m^2/min) followed by at 1200 h (1.40 bees/m^2/min) and minimum at 0900 h (0.10 bees/m^2/min). Mean foraging intensity did not differ either across dates or times in the case of *Apis cerana* Fabricius.

B. Foraging Mode: The percentage of bees engaged in nectar foraging was much higher than pollen foragers and nectar + pollen foragers. In the case of *A mellifera*, 58.15 per cent bees were foraging for nectar, 37.76 per cent for nectar + pollen and only 4.09 per cent for pollen (Fig. 2). In the case of *A. dorsata,* bees 82.50 per cent bees foraged for nectar, 17.50 per cent bees for both nectar and pollen (Fig. 3) whereas in case of *A. cerana,* 91.61 per cent bees were nectar foragers while 8.39 per cent were dual foragers (Fig. 4). No pollen gatherers were recorded both in *A. dorsata* and *A. cerana*.

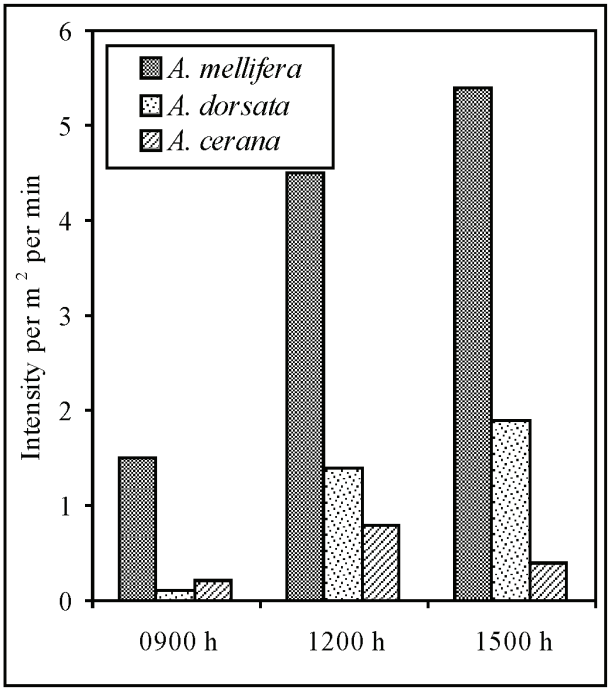

Figure 1. Diurnal intensity of different *Apis* spp.

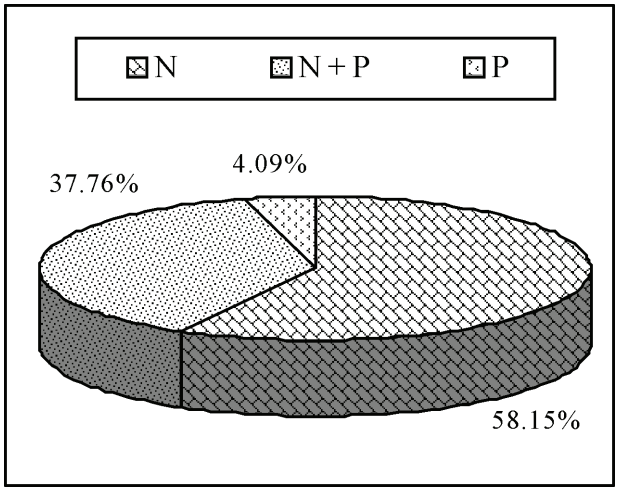

Figure 2. Foraging mode of *Apis mellifera* on *T. alexandrinum*

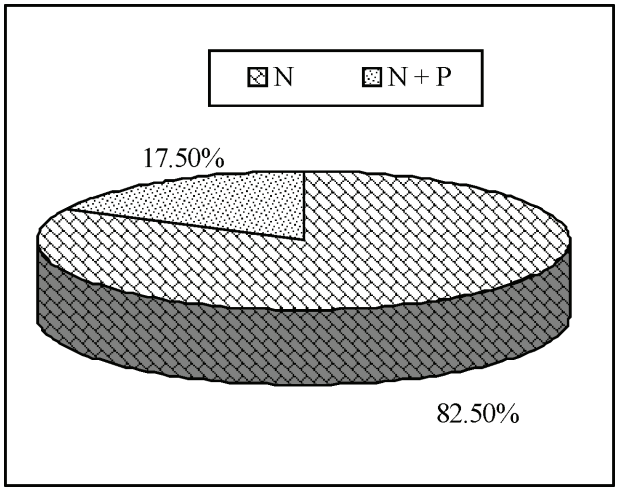

Figure 3. Foraging mode of *Apis dorsata* on *T. alexandrinum*

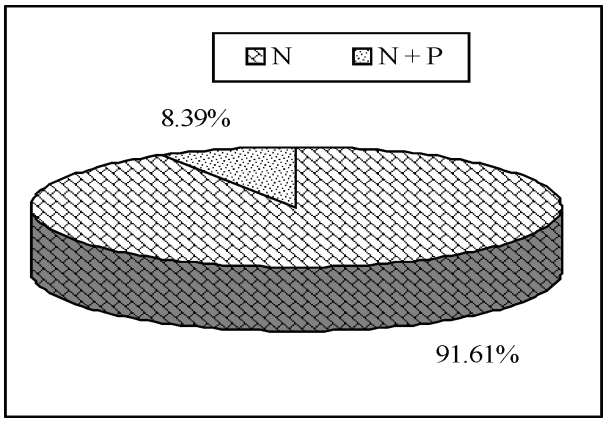

Figure 4. Foraging mode of *Apis cerana* on *T. alexandrinum*

Table 1. Effect of pollination by *Apis mellifera* on yield parameters of *Trifolium alexandrinum* cv. BL 42 at PAU Seed Farm, Nabha (District Patiala) during 2011

Treatment	Seed yield parameters*				
	Seed setting (%)	Thousand seed weight (g)	Germination (%)	Seed yield/plot (g)	Seed vigour
Intensive Bee Pollination	61.01 (51.42)	2.47	88.67 (70.83)	136.33	278.17
Open Pollination	62.46 (52.30)	2.51	90.67 (72.52)	130.67	289.12
Exclusion of Pollinators	40.33 (39.36)	2.22	80.00 (63.48)	60.50	221.59
L.S.D. (p=0.05)	(5.81)	0.18	(5.22)	11.32	

*Figures in parentheses are the means of arc sine $\sqrt{\text{percentage}}$ transformations.

C. Yield Parameters: Pollination of *T. alexandrinum* by *A. mellifera* and open pollination enhanced seed setting over control (exclusion of pollinators) by 51 and 55 per cent, respectively (Fig. 5). The seed setting was significantly highest in open pollinated plots (62.46 %) and was statistically at par with intensive pollinated plots (61.01 %). However, significantly lowest (40.33%) seed setting was recorded in control plots caged without bees. These findings corroborate the findings of Bhardwaj and Kumar (2005) who reported that seed setting increased in the presence of honey bees, which were responsible for increase in seed setting by tripping. They found that under open pollination and exclusion of pollinators, seed count was 5,106 and 305 per 100 flower heads. The present findings also are in line with the findings of Latif *et al*. (1956) who reported that number of seeds per head in *A. cerana* pollination and exclusion of pollinators was 17.0 and 0.5, respectively. These findings also corroborate the findings of Narayanan *et al*. (1961) who reported that in exclusion of pollinators and open pollination, number of seeds per 100 heads was 8.3 and 23.14, respectively. Later on, Beri *et al*. (1985) also reported that seed setting in open pollination, exclusion of pollinators and exclusion of pollinators plus hand tripping was 53, 26.3 and 52.3 per cent, respectively. Iannucci (2001) also reported that although *T. alexandrinum* is predominantly self–

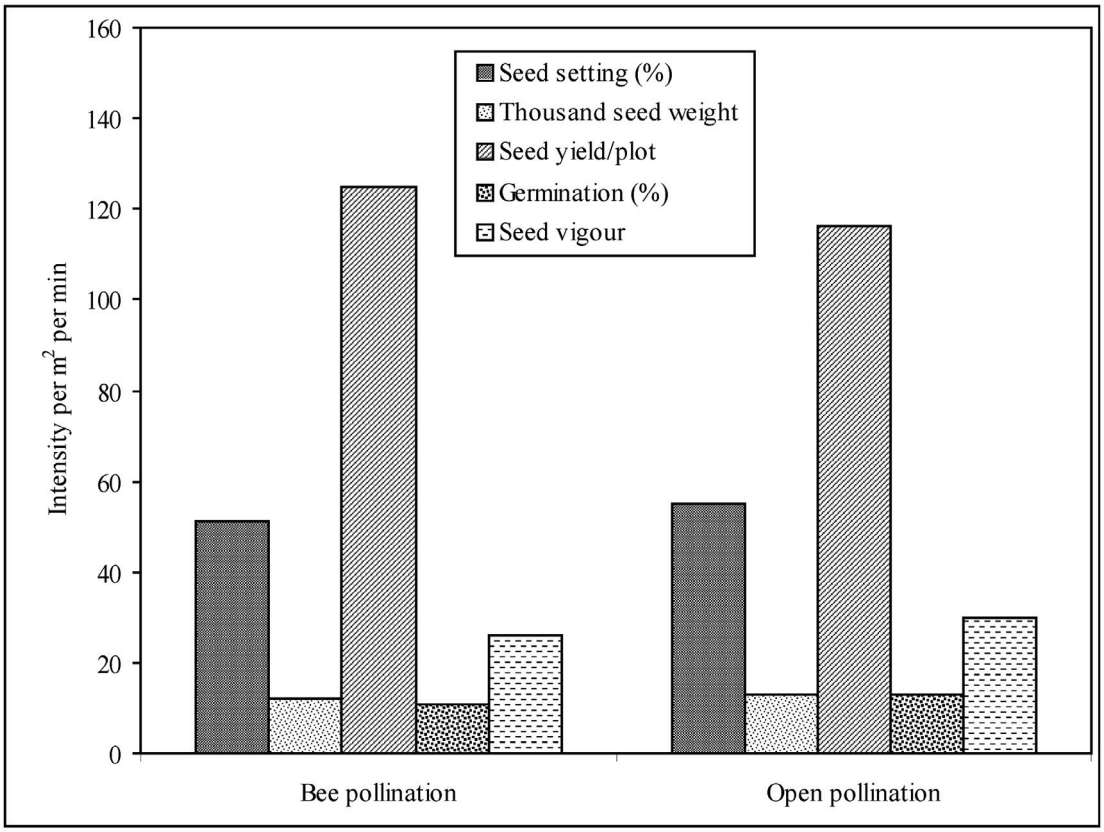

Figure 5. Influence of pollination on seed parameters of *T. alexandrinum*

pollinated but tripping of flowers is required, which is done by honey bees. Earlier, Dhaliwal and Atwal (1974) suggested keeping bee hives within one km of the crop for better seed setting. Roy *et al* (2005) also reported 12.3–99.2 per cent reduction in seed setting in various lines of *T. alexandrinum* under caged (exclusion) condition.

Improvement in thousand seeds weight over control was 12 and 13 per cent in *A. mellifera* pollination and open pollination, respectively. Thousand seed weight was also significantly high in open pollinated plots (2.51 g) which was statistically at par with plots caged with *A. mellifera* colony (2.47 g), whereas seed weight was significantly the lowest in control plots caged without bees (2.22 g). These results are in line with the findings of Free (1993) who reported that seed weight per 100 heads was 13.8, 0.2 and 6.1 in bee pollination, exclusion of pollinators and open pollination, respectively in 1958 and the corresponding values for 1959 were 13.0, 0.2 and 5.6, respectively.

Seed yield per plot (6×3 m^2) was 136.33 g in open pollination followed by 130.67 g in plots caged with *A. mellifera* and 60.50 g in plots caged without bees/ pollinators (Table 1). Crop accessible to all the pollinators and the crop caged with *A. mellifera* colony resulted in 125.34 and 115.98 per cent increase in seed yield over control where no pollinator was available to the *T. alexandrinum* crop bloom. The findings in the present study are in line with the findings of Dixit *et al*. (1989) who reported that seed setting efficiency and seed yield of berseem under natural tripping or open tripping condition was extremely high (22.38g/m^2) as compared with that under completely caged condition (0.52g/m^2), which clearly indicated the significance of pollinators. Earlier, Hassanein (1953) had also reported that the number of seeds per head, enclosed in screen cage with bees, without bees and not caged was 38.9, 1.9 and 23.5 at one location and 42.7, 1.3 and 22.5 seeds per head at another location.

Crop accessible to *A. mellifera* only or to all the available pollinators in the area improved both quality parameters (seed germination and seed vigour) of the seed produced. Placement of *A. mellifera* in *T. alexandrinum* and open pollination improved seed germination by 11 and 13 per cent, respectively over control. Seed germination was the highest 90.67 % in seeds obtained from open pollinated plots followed by 88.67% in plots caged with *A. mellifera* which were statistically at par with each other. However, germination of seed produced from the plots caged without bees was significantly poor i.e. 80.00 per cent. Seed vigour was also enhanced by *A. mellifera* pollination and open pollination over control by 26 and 30 per cent, respectively. Seed vigour was also found to be the maximum in seeds obtained from open pollinated plots (289.12) followed by 278.17 in plots caged with *A. mellifera* and the minimum 221.59 in seeds obtained from plots caged without bees. The findings of the present study conclude that *A. mellifera* colonies placed in *T. alexandrinum* crop significantly enhanced quantity and quality of the crop seed. However, a little better, although non–significant, yield and quality of seed obtained in open pollination indicated that pollinators other than *A. mellifera* also contribute in pollination of *T. alexandrinum.*

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