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Effect of Indigenous Bee Attractants on Qualitative and Quantitative Parameters of Egyptian Clover, *Trifolium alexandrinum* L.

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ABSTRACT

Background: Egyptian clover (*Trifolium alexandrinum* L.) is one of the most important annual, winter, multi-cut fodder legumes grown in northern India. Many a times, poor activity of pollinators cited for lower seed production in berseem. Therefore, an appropriate local food-based bee attractants need to be identified and their effectiveness in enhancing berseem seed yield needs to be assessed.

Methods: The experiment was conducted to evaluate the significance of bee visit on yield parameters of berseem. In addition, the effect of attractant (jaggery and sugar at 10%) in enticing honey bees towards the berseem flower was evaluated at ICAR-IGFRI, Jhansi during 2017 and 2018.

Result: Both the attractants (jaggery and sugar at 10%) are equally effective in drawing the *Apis dorsata* towards berseem and higher bee visits (8.75 and 8.54/m^2/5 min) were recorded one hour after application. Maximum seed setting percentage was recorded in plots sprayed with jaggery (83.53) followed by sugar solution (82.58), which is significantly superior over farmers practice *i.e.* crop left for open pollination (74.86). Maximum seed yield was obtained from plots sprayed with jaggery solution (8.15 q/ ha). The bee visit had significant positive effect on seed yield (0.685*) and 1000 seed weight (0.671*).

Key words: Attractants, Jaggery, Seed setting, Seed yield, *Trifolium alexandrinum*.

INTRODUCTION

Honey bees are regarded as an efficient and ideal pollinators for increasing the productivity of cross pollinated crops (Abrol, 2012). The effectiveness of bee pollination depends on the plant's incentive offering to pollinators (pollen, nectar or both) in exchange for the value of pollination (Simpson and Neff, 1981). The bees use both visual cues and flower volatile to locate the flowers; hence the floral scent plays a commercial role in attracting the pollinators (Moyroud and Glover, 2017). Several substances were tested as attractants to honey bees (Ambrose *et al.,* 1995). However greater part of the studies in the past focused mainly on crops of commercial importance, neglecting other crops such as forage legumes. Clovers are one such fodder legume, considered important for many reasons *viz.,* having high fodder value to livestock, green manures and soil fertility maintenance.

Egyptian clover (*Trifolium alexandrinum* L.) vernacularly known as berseem, is one of the most important annual, winter, multi-cut fodder legume native to western Asia (Muhammad *et al.,* 2014). Following its introduction to India during 1904, the crop has been widely adopted and grown in irrigated cropping systems of northern India (Singh *et al.* 2019). Several studies in India proposed that the populations of berseem were largely self-pollinated, but requires bee pollination for better seed production (Beri *et al.,* 1985; Dixit *et al.,* 1989; Roy *et al.,* 2005). The presence of honey bee colonies in the berseem seed producing field has also been reported to boost seed setting percentage and yield (Dhaliwal and Atwal, 1976; Bakheit, 1989). Hence, any material that increases the bee visits to berseem crop would be of great value in harnessing the benefits of cross-pollination to increase the quality and quantity of the seed. In this context, attractants play a crucial role in attracting bees towards the plants. The attractants are of three type's *viz*., food based, pheromones based and plant origin. Given the prohibitive cost and availability of commercial bee attractants, the best alternative under Indian conditions is the use of indigenous bee attractants. Therefore, the appropriate local food-based bee attractants need to be identified and their effectiveness in attracting and enhancing berseem seed yield needs to be assessed. Consequently, the present study was conducted to evaluate the effects of

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certain food based attractants on foraging activity of native bee pollinators and their impact on qualitative and quantitative aspects of berseem seed yield and the findings are reported here.

MATERIALS AND METHODS

Location

The current studies were undertaken during the winter season of two consecutive years 2016-17 and 2017-18 at central research farm, ICAR-Indian Grassland and Fodder Research Institute, Jhansi (UP) India (24° 11′N altitude, 78° 17′ E longitude and 271m above MSL).

Treatment

The berseem cv. wardan was raised by following recommended cultivation practices and care has been taken to avoid or minimize pesticide application in the field. The field experiments were laid out with five treatments in randomized block design and replicated four times. The berseem fodder was harvested for three times before imposing the treatment. The treatments involved the spraying of food-based bee attractants such as sugar (T1; refined sugar extracted from sugarcane) and jaggery solution (T2; unrefined sugar extracted from cane juice) at 10% in each plot four times at three-day intervals during peak flowering stage. The completely caged (T3, without insect pollination: WIP) and caged with hand pollination (T4) plots were taken to know the impact of bee visits on seed setting (Fig 1). Around 50 berseem flower buds were randomly picked and tagged in the T4 plots. The plot was entirely encased in net before flower opening to avoid pollination similar to T3. As these flowers opened and become receptive, they were hand pollinated (11:00 AM to 13:00 PM) and further enclosed. An untreated control plot (open pollination, OP; T5), were not caged and kept open giving a free access to any mode of pollination maintained for comparison. It was observed that *Apis dorsata* was the most dominant and frequent visitor of berseem flower and peak activity was recorded between 10:00 to 11:00 AM (Kumar *et al.,* 2021).

Data recording

Observations were recorded on *A. dorsata* population/m^2 area for five minute during its peak active hour. Three samples of five minutes each were taken with a break of ten minutes between two successive counts. The activity observation was also recorded 1, 2, 3, 4 and 5 hours after each spray. A total of 50 flower head randomly selected in each replication of different treatments are tagged. The flower head were manually harvested at maturity and the quantitative parameters like number of florets/head, seed setting percentage, seed yield, dry weight and 1000 seed weight as well as qualitative parameter such as vigour index, germination percent and seedling length were recorded in laboratory.

1. Number of florets per head

The numbers of florets in randomly selected ten racemes were counted and their mean was calculated.

2. Seed setting percentage and seed yield

At harvesting stage, the number of seeds in each raceme was counted (n= 50) and weighed. The seed setting percentage was counted using the formulae:

$$\text{Seed setting (\%)} = \frac{\text{Number of seed per raceme}}{\text{Number of florets per raceme}} \times 100$$

The seed yield/ha was calculated by extrapolating the average yield data of plot area (5m × 5m) into quintal per hectare (q/ha).

3. Germination percentage

A total of fifty seeds from each treatment were taken and placed on the wet circular filter paper in a Petri dish (90 mm), kept in a germinator at constant temperature of 21±10°C and RH of 75 per cent was maintained throughout the experimentation. After six days, the number of seeds germinated were counted and expressed in percentage.

4. Seedling length

Ten normal seedlings were randomly selected from germination test and length between tips of the primary shoot



Fig 1: Experimental setup in the research plots representing caged condition.

to tip of primary root was measured and expressed in centimeter.

5. Seedling dry weight (mg)

Ten normal seedlings were dried in hot air oven at 80±10°C for 10 hours and their weight measured using analytic balance.

6. 1000 seed weight (g)

Observation was made by weighing 1000 dried seeds from each treatment (replicated thrice) with the help of electronic weighing machine.

7. Vigour index

It was calculated by using the procedure suggested by Abdul-Baki and Anderson (1973) and expressed in whole number. The vigour index was calculated by using

Vigour index = Germination (%) × Total seeding length (cm)

Statistical analysis

A. dorsata activity at different intervals after bee attractant spray and the data on different qualitative/quantitative parameters were statistically analyzed by SPSS through univariate analysis of variance with Tukey's honest significant difference (HSD) test. The bee visit was correlated with the yield attributes of berseem and the relationship was worked out.

RESULTS AND DISCUSSION

Bee visitation

Observation recorded on honey bee, *A. dorsata* visitation on berseem flower treated with attractants during peak flowering stage (spanning 15 days duration) during 2017 and 2018 was pooled and presented (Fig 2). One hour before each spray (10:00 AM), the number of bees visiting berseem flower ranged from 6.33 to 7.66 bees/m^2/5 minute and did not differ significantly among the treatments except in caged treatment (*F value*= 5.943; *df* = 17). However, one hour after the spray (11:00 AM) jaggery and sugar solution (10%) attracted higher number of bees (8.75 and 8.54/m^2/5 min, respectively) and significantly superior over the plot left for open-pollination (T5). Further, two hours after spraying (12:00 PM), the visitation of the bee was comparatively lesser than 1 hour after spray but higher than visited before attractant application. Thereafter the bee visit started declining in all the imposed treatments. However, the plot treated with sugar and jaggery attracted more bees even after 3rd, 4th and 5th hour after spray. The open-pollination plot sprayed with water was least effective in attracting bees towards berseem flowers. The similar trend of visitation was recorded during remaining sprays applied during peak flowering stage of both the years. In the current study, both the attractants are equally effective in attracting pollinator and bee visit play a significant role in improving the seed yield. Several researchers cited that spraying attractants such as jaggery and sugar solution was effective in attracting and boosting pollinator activity in crops like onion (Kumari and Rana, 2018) and Alfalfa (Sreedhara *et al.,* 2012). There were many reports citing beneficial effect of attractants on other crops, but literature on berseem is lacking, hence result on other crops have been used for comparisons.

Effect of bee visitation on yield parameters of berseem

Influence on the quantitative parameters of berseem

The flower head of berseem comprises on an average of 103.14 to 108.56 florets/head and no significant difference could be observed between treatments. The lower seed set was recorded in the WIP plot (6.45%), while hand pollination was found to have a marginal benefit over WIP (8.65%). The plots exposed for open-pollination shown 74.86 per cent of seed setting, on the other hand the plot sprayed with jaggery (83.53) and sugar (82.58) spray shown 11.58 and 10.31 per cent increase over open-pollination, respectively (Table 1). The outcome of the current study clearly indicated the role of honey bees as a pollination agent and its effect on seed setting. Several workers reported similar result of higher bee visit increased the per cent seed set in berseem (Chaudhary, 1998; Jat *et al.,* 2014). Despite the fact that attractant-treated plots

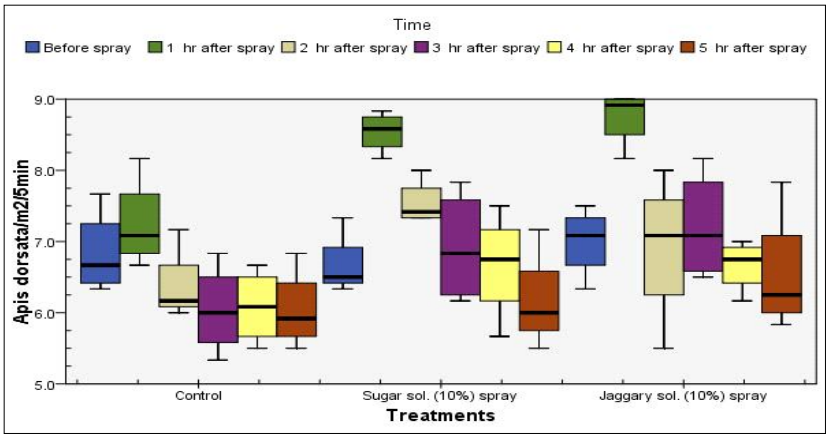


Fig 2: Effect of bee attractant on activity of Apis dorsata in berseem.

generated more seeds than open-pollinated plots, the differences are statistically insignificant (Table 1).

Among the different yield parameters, application of jaggery and sugar registered a significant positive effect on 1000 seed weight (3.17 and 3.16 g) respectively over other treatments. The findings demonstrate the significance of honey bee visits in boosting seed weight. Similarly, Free (1993) and Singh *et al.* (2012), also reported higher 1000 seed weight in bee pollinated plots compared to seeds harvested from WIP plots. The plots treated with jaggery spray (8.15 q/ha) produced the highest seed yield when compared to sugar spray (8.07 q/ha) and open-pollination (7.61 q/ha). The results are in close agreement with the report of Hosamani *et al.* (2020), who reported the significant increase in the seed yield of onion in plots treated with jaggery solution (10%). The seed yield is highly complex phenomenon which is greatly influenced by external application of plant growth regulators and insect attractants (Maynard *et al.,* 1992). The higher seed yield was recorded with application of jaggery and sugar solution when compared to open-pollination to the extent of 7.10 and 6.07%, respectively. The higher seed yield in attractant sprayed plot is due to higher seed setting per cent (Mazeed and Zidan, 2019).

Influence on the qualitative parameters of berseem

Among the seeds obtained from different treatments, seeds obtained from attractant treated plots recorded significantly more germination percent (sugar-92.67; jaggery- 92.33) over caged (79.33) and hand pollinated (82) plots, but not different from the seeds obtained from plots exposed to OP (Table 2). Similar observations were made by Singh *et al.* (2012) and Jat *et al.* (2014), who reported higher seed germination of seeds obtained from open pollination plots compared to WIP. Similar to the observation made in other parameters, no variation could be recorded for seedling length and vigour index of seeds harvested from plots visited by honey bee. However, lower seedling length (4.57 and 4.58 cm) and vigour index (375.27 and 363.31) was recorded in the plots with manual pollination and WIP plots, respectively (Table 2).

The plots left for open pollination yielded highest returns (3.54) for each rupee invested, compared to the plots received indigenous attractant sprays (Table 3). The plots that received attractants (jaggery and sugar) yielded the highest gross returns (1,91,700 and 1,90,260 rupees, respectively), but had a lower B:C ratio. Because of the high cost of application, the attractant-treated plot has the lowest B:C ratio. No variation was observed in the yield of the green fodder among the treatments (90 tonne). The bee visit was positively correlated with seed setting (0.176), seed yield (0.685*), 1000 seed weight (0.671*) and germination percentage (0.437) (Table 4). Long and Morandin (2011) found a substantial positive correlation between honey bee activity and onion seed set, as well as a link between lower bee visit and yield reduction.

Table 1: Influence of bee attractants on the quantitative parameters of berseem seeds.

Treatment	Floret/ head			Seeds/ head			% increase over OP	Seed setting (%)			% increase over OP	Seed yield (q/ ha)			% increase over OP	1000 seed weight (g)			% increase over OP
	2017	2018	Mean	2017	2018	Mean		2017	2018	Mean		2017	2018	Mean		2017	2018	Mean	
Open pollination	101.80	108.3	105.05a	73.47	82.37	77.92a		73.24	78.05	74.86b		7.52	7.701	7.61a		3.03	2.99	3.01ab	
Manual pollination	99.08	107.2	103.14a	9.67	9.67	9.67b		10.19	8.99	8.65c		1.28	1.24	1.26b		2.83	2.61	2.72bc	
Sugar (10 %)	104.83	103.7	104.27a	76.73	91.87	83.98a	7.78	73.23	88.87	82.58a	10.31	7.801	8.34	8.07a	6.07	3.15	3.16	3.16a	4.98
Jaggery (10 %)	103.40	107.97	105.68a	77.67	90.30	84.30a	8.19	76.43	84.80	83.53a	11.58	8.09	8.21	8.15a	7.10	3.13	3.21	3.17a	5.32
Caged (WIP)	102.71	114.4	108.56a	5.27	9.17	7.22b		5.07	7.97	6.45c		0.85	0.712	0.78b		2.78	2.37	2.57a	
CD (5%)			NS			4.96				5.57				6.19				0.26	

Table 2: Influence of bee attractants on the qualitative parameters of berseem seeds.

Treatment	Germination (%)			% increase	Seeding lenth (cm)			% increase	Vigour index-I			% increase
	2017	2018	Mean	over OP	2017	2018	Mean	over OP	2017	2018	Mean	over OP
Open pollination	91.33	91.00	91.17a		5.42	5.75	5.59a		495.03	523.25	509.14	
Manual pollination	81.85	82.15	82.00b		4.68	4.46	4.57b		384.03	366.93	375.48	
Sugar 10%	91.99	93.35	92.67a	1.65	5.66	5.71	5.69a	1.79	520.90	533.03	526.96	3.50
Jaggery 10%	91.85	92.81	92.33a	1.27	5.64	5.77	5.71a	2.15	518.20	535.10	526.64	3.44
Caged (WIP)	78.33	80.33	79.33b		4.51	4.65	4.58b		353.54	373.82	363.68	
CD (5%)			4.68				0.52				41.77	

Table 3: Economics of berseem seed production through application of bee attractant (Indian rupees).

Treatment	Production value (Rs/ha)	Green fodder value (Rupee)	Seed value (Rupee)	Gross return (Rupee)	*B/C ratio
Open pollination	40000	45000$	136980	181980	3.54
Sugar 10%	47600#	45000	145260	190260	2.99
Jaggery 10%	49200##	45000	146700	191700	2.89

*One liter Solution for 5m × 5m area (25 m^2 area), considering four time spray during flowering season.

Includes attractant application cost of 7600; ##Includes attractant application cost of 9200.

$ Green fodder value calculated at 0.5 rupees per kg of berseem.

Table 4: Correlation between bee visit and seed quality attributes.

	Apis dorsata (No./m^2/5 min.)	Seed setting (%)	Seed yield (g/m^2)	1000 seed weight (g)	Germination (%)
Apis dorsata (No/m^2/5 min.)	1	0.176	0.685*	0.671*	0.437
Seed setting %		1	0.112	0.571	0.842**
Seed yield (g/m^2)			1	0.381	0.037
1000 seed weight (g)				1	0.798**
Germination (%)					1

*Significant at the 0.05 level (2-tailed); **Significant at the 0.01 level (2-tailed).

CONCLUSION

The results of the current study concluded that, use of bee attractants has non-significant effect on berseem seed yield but has other advantages like improvement in qualitative parameters of berseem seeds. The plots sprayed with attractant resulted in higher berseem seed yield (7.10 and 6.04%), but not significant over farmer's practice of open-pollination. The application of local attractant has limited scope in improving the quantitative and qualitative parameters of berseem seeds. Keeping the unavailability of commercial bee attractant and subpar performance of local bee attractive, the application of bee attractant has little scope in berseem seed production in central Indian condition.

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Conflict of interest

All authors declared that there is no conflict of interest.

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