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Roles of Honeybee-Pollination on Major Agronomic Performances of Alfalfa (*Medicago sativa* L. var. Hunter River)

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Abstract: Provision of pollinators is the most important moments for alfalfa productivity. Honeybee, due to its abundance and mobility is a significant pollinator in the production of alfalfa seed. Problems that arise in pollination of alfalfa led many researchers to pay attention to the pollination of this crop. This study presents the results obtained by use of honeybee in alfalfa pollination in relation to yield, yield components and quality of seed. To this effect, the experimental plots (3×3 m) were arranged in a randomized complete block design with three replications. Three pollination treatments: caged with honeybee (T1), caged without honeybee (T2) and open-pollinated (T3) were applied. The effects of honeybee on yield and yield components were investigated. The seed yield was found to be significantly different between treatments ($p<0.05$). The mean yield obtained from the plots caged with honeybee pollination was highest (118.8±5.0 kg ha^{-1}) followed by plots left open to be pollinated under natural (98.5±7.5 kg ha^{-1}). The lowest mean yield (43.2±6.0 kg ha^{-1}) was obtained from the plots excluded from honeybees and other pollinators (self-pollinated treatment). With regard to 1000 seed weight, the caged without honeybees treatment had significantly lower seed weight as compared to caged treatment with honeybee and open plots treatment. Similarly, lower germination percentage was investigated in caged plots without honeybee while significantly the largest germination percentage was recorded for cage plots with honeybee.

Key words: Seed yield, seed quality, pollinators, cage, self-pollination

INTRODUCTION

Insects are essential for pollination of agricultural crops. As a result the need for insect pollination is becoming popular by agricultural community to increase the productivity of crops. Nevertheless, lack of sufficient pollinators is reported to be one of the limiting factors in agricultural crop productivity. According to Free (1970), honeybee pollination does not only improve the productivity but also the seed quality of agricultural crops. On the other hand, pollination deficiency in crops results in reduced yields due to lower fruit and seed setting, longer duration for germination and increased inbreeding depression within a crop population.

Medicago sativa (Lucerne) is a perennial herb cultivated throughout the world mainly as a forage crop. It is known as a highly productive and nutritious legume that is well adapted to a range of climates throughout the world. An extended taproot allows the plant access to water and nutrients deep in the soil profile which therefore, helps it be superior in drought tolerance in comparison to shallower grass pastures. Lucerne is grown for its seed, livestock pasture, hay and silage. For its low fiber and high calcium, phosphors and protein contents it is used as a supplemental feed to maximize milk production in dairy industries.

Despite its importance, it is not widely used by commercial growers or small scale dairy industries mainly due to its seed setting problems especially in the highland areas of Ethiopia.

Moreover, self-sterility is high in varieties of lucerne which imply that cross-pollination is necessary if payable seed yields are to be produced (Cecen *et al*., 2008). This problem can be solved by using insect pollination, primarily the honeybees for maximum productivity and seed quality of the crop. Cecen *et al.* (2008), mentioned that cross-pollination increases the percentage of flowers forming seed pods, the number of seeds per pod and the size of seeds. In Ethiopia, information on roles of honeybee pollination on alfalfa seed production and quality is limited. On the other hand, the value of lucerne as a quality animal feed and high profit earning opportunity from seed sale (1 kg costs for >600 Eth birr equivalent to 32 US dollar) in Ethiopia is demanding for large scale seed production. Therefore, this study was designed with the objective to investigate the role of local honeybee pollination in seed yield, quality and other agronomic performances of alfalfa.

MATERIALS AND METHODS

Location and climate of the study area: The present field study was carried out in Arsi zone, Arsi University Research Farm. The study area is located between 7°54'55"N-8°00'05"N, 39°06'10"E-39°10'00"E. The study area has an altitude of 2400 m above sea level. The average temperature of the area over the past 35 years is 14.7°C with an average annual rainfall of 1200 mm.

Experimental setup: The test crop was raised with the recommended agronomic package practices. The crop was planted with a seed rate of 10 kg ha^{-1} in rows with 30 cm spacing between the rows. The experiment was laid out in a complete randomized block design with three treatments and three replications with an experimental plot size of 3×3 m.

Treatments: In the first Treatment (T1) at about 20% of flowering of the alfalfa, honeybee colonies were enclosed with mesh wire for the intensive pollination. Prior to enclosing the colony in the cage, the colony was strengthened. In the second Treatment (T2), the plot was caged with mesh wire without inclusion of honeybee colonies and excluding all other insect pollinators. In the third Treatment (T3), the plant was left open for natural pollination as a control treatment. The comparative efficiency of each treatment was evaluated on the basis of seed yield, 1000 seed weight (TSW), number of pods per plant, number of seeds per pod and pollination efficiency.

Statistical analysis: All the collected parameters were subjected to ANOVA using the GLM procedure of Statistical Analytical System computer software (SAS).

RESULTS

Self-sterility was found to be higher in the treatment excluded from insect pollination of lucerne implying that cross-pollination is necessary if payable seed yields are to be produced. Cross-pollination may be achieved through the action of insects, primarily honeybees, foraging on lucerne flowers for nectar and pollen collection. Honeybee pollination was identified increasing the percentage of flowers forming seed pods, the number of seeds per pod and the size of seeds.

Flower heads per plant: The average numbers of flower heads recorded from plots prior to application of the treatments were 11.33, 9.47 and 12.20 for caged honeybee, cage without honeybee and open pollination treatments, respectively. In terms of numbers of flower heads, there were no significant differences found between different plots prior to the application of treatments.

Pods per plant: Significantly ($p<0.05$) the highest seed pods per plant (10) was found in plots received caged honeybee treatment followed by open pollination (6.3) treatment. Whereas significantly ($p<0.05$) the least seed pod (4.2) was recorded from plots received cage without honeybee treatment. However, there was no significant difference between open pollination and cage without honeybee pollination. The honeybee pollination had increased the pod formation with the rate of 58% over the cage without honeybee treatment and 37% in open pollination. The pod number difference between the treatments indicated that the crop requires insect pollination particularly honeybee and other insect pollinators for pod development which could subsequently result in high seed yield.

Seeds per pod: Significantly ($p<0.05$), the highest seeds per pod (3.92) were recorded in caged honeybee treatment. The number of seeds per pod in caged honeybee treatment was observed with 37.8 and 31.4% increase over caged without honeybee and open pollination treatments, respectively. The least seed per pod was recorded in caged plot without honeybee. The presence of insect pollinators significantly increased seed setting in the plant. This by default implied that honeybee pollination increased significantly the percentage of seed setting and number of filled seeds in the pod.

Pollination Efficiency (PE): Responses of pollination efficiency were different across the experimental treatments. Pollination in cages with honeybees showed significantly ($p<0.05$) higher pollination efficiency (61%) compared with pollination in cages without honeybees (34%) and pollination in natural condition (46.67%). Pollinations in cages with honeybees were more efficient as compared with pollination in cages without honeybees and open pollination. As a result, honeybees increased the pollination efficiency by 44 and 23% over cages without honeybees and open pollination, respectively (Table 1).

Seed yield: Seed yield of alfalfa was found to be significantly different between the treatments. The yield obtained from the plots pollinated by honeybee colonies was statistically significant with highest mean seed yield

Table 1: Analysis of variance for effect of pollination on yield components of alfalfa (Mean±SD)

Treatments	Total flowers	Total pods	Proportion of flowers producing pod	PE (%)
Cage with honeybee	11.48±0.70^{a}	10.0±1.0^{a}	87.42±11.11^{a}	61.00±6.00^{a}
Cage without honeybee	9.47±3.00^{a}	4.2±0.7^{b}	47.30±14.88^{b}	34.00±1.00^{c}
Open	12.20±2.46^{a}	6.3±1.4^{b}	52.88±15.05^{b}	46.67±3.79^{b}
LSD (0.05)	4.68	2.23	27.57	8.56
CV	20.45	15.82	22.07	8.76

Averages in the same column followed by the same letter are not statistically different, p>0.05; Duncan's multiple range test. PE: Pollination Efficiency; CV: Coefficient of Variation

Table 2: Analysis of variance for effect of pollination on yields and yield components of alfalfa (Mean±SD)

Treatments	Seed/pod	Seed/plant	Seed yield (kg ha^{-1})
Cage with honeybee	3.92±0.88^{a}	130.8±28.60^{a}	118.8±5.0^{a}
Cage without honeybee	2.44±0.23^{b}	39.66±18.66^{b}	43.2±6.0^{c}
Open	2.69±0.81^{ab}	51.89±41.75^{b}	98.5±7.5^{b}
LSD (0.05)	1.40	62.21	19.41
CV	15.67	42.02	18.91

Means with the same letter are not statistically different at p>0.05; LSD: Least Significance Difference; CV: Coefficient of Variation

Table 3: Mean thousand seed weight (g) and germination (%) of alfalfa as affected by honeybee pollination

Treatments	TSW	Germination
Cage with honeybee	2.69±0.14^{a}	24.00±1.00^{a}
Cage without honeybee	1.89±0.19^{c}	10.50±0.95^{c}
Open	2.41±0.24^{b}	19.83±0.63^{b}
LSD (0.05)	0.41	3.82
CV	8.48	5.56

Averages in the same column followed by the same letter are not statistically different, p>0.05; Duncan's multiple range test; TSW: Thousand Seed Weight

of 118.8±5.0 kg per hectare followed by the plot left open to be pollinated by all visiting insects produced the yield of 98.5±7.5 kg per hectare. The lowest yield (43.2+6.0 kg) per hectare was obtained from treatment of which all pollinators excluded (Table 2). On the basis of the results obtained in the caged plot without honeybee and in the cage with honeybee, the seed yield of alfalfa was 2.75 times (or 63.6%) better in the presence of honeybees than in the absence of any pollinator whereas it was better than open pollination by 1.2 times more yield (or 17%).

Thousand seed weight and seed germination: Regarding the 1000 seed weight, treatment one (caged with honeybees) was significantly different (p<0.05) from caged treatment without honeybees and open treatment (Table 3). The highest seed weight was observed in the treatment received caged honeybee (2.69±0.14 g) followed by open treatment (2.41±0.24 g) while the least was recorded for plots received the caged without honeybees. The low seed weight in caged without bees might be contributed to the smaller seeds in self-pollinated flowers. From this investigation honeybee, pollination has significant impact on quality of alfalfa seeds.

There were statistically different (p<0.05) germination percentages among treatments. Statistically, the highest germination percentage was recorded in caged treatment with honeybee (24.00±1.00) followed by open treatment (19.83±0.63) with the least germination percentage in caged treatment without honeybee (10.50±0.95) (Table 3). The caged treatment with honeybee showed 17.4 and 56.25% increase over caged without honey and open pollination treatments, respectively.

DISCUSSION

Impact of pollination on seed yield: Pollination was identified as a major limiting factor to higher, more reliable seed yields and improved seed quality. Self-sterility was found to be higher in the treatment excluded from insect pollination of lucerne implying that cross-pollination is necessary if payable seed yields are to be produced. Cross-pollination was identified increasing the percentage of flowers forming seed pods, the number of seeds per pod and the size of seeds.

Seed yield of alfalfa was found to be significantly different between the treatments (P<0.05). The yield obtained from the plots pollinated by honeybee colonies were superior with highest mean seed yields of followed by the plot left open to be pollinated by all visiting insects. The lowest seed yield per hectare was obtained from treatment of which all pollinators were excluded. This result is in agreement with results by Cecen *et al.* (2008) who identified that honeybee pollination of lucerne in the west Mediterranean region of Turkey had significantly increased seed production when compared to plants from which pollinators were excluded. Similarly, Free (1970) and Leaderhouse *et al.* (1968) showed that pollinating insects particularly honeybees have a great contribution for improving the seed yield of the *A. cepa* crop. McGregor and Pesson and Louveaux have also investigated that honeybees are used to pollinate the *Allium cepa* in large cages for hybrid seed production. Experiments conducted in Australia by Mothorpe and Jones also found that physically excluding honeybees from lucerne crops resulted in significantly reduced seed yield compared to open or free access plots of lucerne. In

agreement with this study, using cages with insulation, Vansell and Todd (1946) showed that the honeybee had an essential role in the pollination of alfalfa where flowers on plants that were placed in cages without bees had not even opened nor produced seed. In contrast, in the plants in cages with bees and in the open field there was an abundance of seed.

Pods per plant and seeds per pod: Significantly ($p<0.05$) the highest seed pods per plant were found in plots received cage honeybee treatment followed by open pollination treatment. Whereas, the least seed pod was recorded from plots received cage without honeybee treatment. Honeybee increased the pod formation with the rate of 58% over the treatment without honeybee. Significantly ($p<0.05$), the highest seeds per pod were also recorded in caged honeybee treatment. Honeybee treatment also increased the number of seeds per pod with 37.8 and 31.4% over caged with no honeybee and open plots, respectively while the least seed per pod was recorded in caged plot without honeybee. This result is in agreement with the results obtained by Cecen *et al.* (2008) who reported that pollination increases the percentage of flowers forming seed pods, the number of seeds per pod and the size of seeds.

Pollination Efficiency (PE): Pollination efficiencies were statistically ($p<0.05$) different across the experimental treatments where pollination in cages with honeybees showed significantly higher pollination efficiency compared with pollination in cages without honeybees and pollination in natural condition (open pollination). Pollinations in cages with honeybees were more efficient as compared with pollination in cages without honeybees and open pollination. As a result, honeybees increased the pollination efficiency by 44% (1.8 times) and 23% (1.33 times) over cages without honeybees and open pollination, respectively. Similar finding was reported by Bosnjak and Stjepanovic that the tripping of flowers and pollination of alfalfa was 3-6 times better in the presence of honeybees than in their absence. Schmidt, however, suggests that the honeybees are good pollinators only if there is no other nectar flora except alfalfa.

Thousand seed weight and germination percentage: The highest seed weight was observed in treatment that received caged honey bee followed by open treatment while the least was recorded for plots received the caged without honeybees. This is in line with the finding by Sajjad *et al.* (2009) who reported similar trend in *Sesbania sesban* pollination by honeybee. This may be due to smaller seeds in self-pollinated flowers that might have led to gain low seed weight which in turn, produced less vigorous and less viable seedlings. The same researcher also stated that cross-pollination of lucerne is vital to ensure the maximum quantity and quality of seeds.

CONCLUSION

Honeybee pollination played significant roles in reproductive success of alfalfa by increasing seed yield, seed weight and germination as compared to other two treatments of self (only cage) and open pollinations. Hence, honeybee pollination was identified as a major limiting factor to higher, more reliable seed yields and improved seed quality. This calls for cross-pollination if payable seed yields are to be produced. The current study found that physically excluding honeybees from lucerne crops resulted in significantly reduced seed yield compared to free access plots of lucerne whereas the physically enclosing honeybees in a lucerne crop had almost doubled the seed yield compared to the self-pollinated treatment.

From the current study, it is noted that interdependency exists between lucerne production and honeybee pollination which implied the need of integrating both sectors to increase values of both industries in the future for scaling up the seed production at farmer level or on the large commercial basis. The relatively low seed yield recorded in the present study in general was attributed to excessive soil moisture during the experimental period that induced too much vegetative growth as the expense of seed production.

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