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Investigating the Role of Honeybee (*Apismellifera* L.) Pollination on Seed Yield of Soybean (*Glycine max L. Merril*)

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Abstract: Insect pollinators are essential in increasing seed set of many flower and fruit crops. This experiment was carried out to evaluate the effect of the honey bee pollination in seed yield and oil contents of *Glycine max* L. The plots were grouped into three treatment groups with three replications. The results of the study showed that the yield obtained from plots pollinated by honeybee was superior with the mean yield of 3945kg/hectare followed by plots left open under natural conditions with the mean yield of 3754kg/hectare. The lowest mean yield of 2483kg/hectare was recorded for the plots excluded any pollinator. The results also revealed that honey bee pollination increases *Glycine max* L fruit yield by 38.9% over natural pollination. The pod number in covered treatment with honey bees was 93.75 higher than in the covered treatment without honey bees. The average weight of 100 seed was larger in the area covered with honey bees and without honeybee. The average oil content was 19.21%, 19.39% and 18.47 in uncovered area, covered with honey bees and caged without any pollinator respectively. The germination test show differences ($P<0.05$) among the seeds in different treatments. It was concluded that the honeybee pollination in the soybean increased the seeds production. Therefore, moving honeybee colonies to *Glycine max* farm during the flowering period is one of the most essential inputs to maximize *Glycine max* seed production.

Keywords: *Apis Mellifera*, *Glycine max*, Honeybees, Pollination, Seed Yield

1. Introduction

Flowering plants and honey bees have a special relationship in which both are benefited from each other; where honeybees get nectar (as feed) and in turn facilitate the pollination process [8, 9]. The world's diet of fats and oils comes from oil seeds: coconuts, cotton, oil palm, olives, peanuts, rape, soybeans and sunflower. These plants are dependent upon or benefited by insects pollination. Of these insects, honey bees are responsible for more than 80% of the total pollination of major crops and contribute a lot for the increased production [10, 15]. In adequate pollination can result in reduced, delayed yield and a high percentage of inferior fruit/seed yields with ample pollination, the plant can set fruit early and the grower may harvest quality and good crop a head of inclement. Soybean (*Glycine max*) is one of the most valuable crops in the world, due to its multiple uses as a source of livestock and aqua culture feed, human food, industrial purposes and more recently, as a source of bio-energy [13, 14].

Soybean production in Ethiopia was 38,166.04ha from which 81241.833 tons produced with productivity of 2.129tons ha^{-1} and in Oromia region 14,626.78 ha was cultivated with production of 31,832.611 tons and a productivity of 2.176tha^{-1} in 2015/2016 cropping season (CSA. 2016). The current five-year plan, GTPII, has given due consideration for soybean production as industrial crop and its production is expected to increase from 0.72 million quintals in 2015 to 1.2 million quintals by the year 2020 to meet the demand of the market by creating a linkage with the industry and export market [6]. Since it is well adapted to lowland to mid altitude agro-ecologies of the country. The entire low to mid altitude maize belt are as of the country are also suitable for soybean production. The realization of all these potentials and targets are expanding soybean production vertically and horizontally without adequate understanding of its production economics. The efficiency pollination of honey bees is due to their great numbers, their physique and their

behavior of foraging on only one plant species at one time. Usually a honeybee can visit between 50-1000 flowers in one trip, which takes between 30minutes to four hours. Honey bee is also believed to play a significant role in the economy of Ethiopia through pollination services. The previous studies on the value of honey bees as pollinators in Ethiopia indicated that honeybees can increase the yield of Niger (*Guizotia abyssinica*) by 43% [7] and *Allium cepa* by 84%.

Honeybee is an efficient pollinator for soybean varieties which result an increase in the seed production by 95% [3]. But no study has been conducted to see the effect of honeybees 'pollination on soybean yield under Ethiopian condition. This study was conducted with the following objectives; to investigate the effects of honey bee pollination on soybean seed yield and oil contents; to identify potential pollinators other than honeybees.

2. Materials and Methods

2.1. Study Site

The experiment was conducted at Adami Tulu Agricultural Research Center (ATARC) under rain-fed condition. Adami Tulu Agricultural Research Center (ATARC) is located in the mid Rift Valley of Ethiopia 167km South of Addis Ababa on the road. It lies altitude of 7°9'N and longitude of 38°7'E. It has an altitude of 1650m.a.s.l, and It receives a bimodal un evenly distributed average annual rain fall of 760.9mm per annum (ATARC, 1998). Rain fall extends from February to September with a dry period in May to June, which separates the preceding "short" rains from the following "long" rains. The long-term mean minimum and maximum temperatures are 12.6°C and 27°C, respectively. The soil is fines and loam in texture with the soil type of sand, clay and silt in proportion of 34%, 48% and 18% respectively and a pH of 7.88 [1].

2.2. Experimental Management

The experimental land was prepared according to required standard. The plots were leveled manually and the sowing was done in June under rain fed conditions. The seeds will be planted by hand at a specified spacing by placing two seeds per hill and thinning will be done to one plant at each specific intra row spacing ten days after seedling emergence to achieve the desired plant density in each row. Recommended spacing was used with plot size of 4m×4m ($16m^2$) with seeding rate of 134kg/ha. All the other agronomic practices were followed as per the recommendation for the crop.

2.3. Treatments and Experimental Design

The experiment was laid out in RCBD with three replication per treatment. At 25% of the soybean blooming, bee colonies were transferred to the cage. Supplementary feed were given for bee colonies. After flowers shed, taking out of the bee colonies from the cages. Every pollinator recording was done between 06:00 and 18:00 hours at every hour interval. The experiment was conducted with the following treatments.

1. T1: Plot of soybean was caged with mesh wire inclusive of hived bee colony.
2. T2: Plot of soybean was caged to exclude any pollinators.
3. T3: Plot of soybean was left open for natural pollination.

Different data were collected from each treatment:-Seed yield, pod average weight, Weight of 100 seeds in (g), seed size, number of pods per plant, number of seeds per pod, germination capacity of seeds from different treatments, number of seed set, percentage of matured and aborted grains per different treatments, number of pollinators other than honey bees and cash obtained from the sale of improved seeds of soybean will be collected.

The bee activity were observed on the plot left open for any pollinator for five consecutive days to assess which and how many insect species were visiting the soybean plants. soybean plants that have approximately above 40 flowers were selected and number of honey bees visiting at these flowers was recorded for 15 minutes at 1h interval from 6.00 to 18.00h (7–8h, 9–10h, 11–12h, 13–14h, 15–16h, 17-18h). As low walk a long all labeled flowers of treatment, the identity of all insects that visited soybean flowers was recorded. All insects encountered on flowers were recorded and the cumulated results expressed in number of visits to determine the relative frequency of honeybee.

Observations were also taken on germination percentage of the seeds of all three treatments. For germination potential test 100 seeds of soybean were sprinkled on a 10cm diameter Petri dish plate which was covered with moist filter paper. Moisture was maintained by spraying water. The filter paper was removed after the germination was over and the number of seeds germinated out of hundred was counted. The experiment was replicated three times. A germination success study was conducted by considering the principle of maximum percentage germination, following the necessary steps used by the International Rules for Seed Testing. Finally germination percentage of seeds was determined by using the following formula:

$$\text{Germination percentage} = \frac{n}{N} \times 100\%$$

Where: n=Total number of germinated seeds

N=Total number of seeds in the sample

Mass of 100 seeds and total fruit yield (g) per $24m^2$

Yield increment (%) =yield from honey bee pollinated–yield from insect pollinated (100)

Yield from open pollinated

All collected data were subjected to Analysis of Variance (ANOVA) using SAS software (The SAS system for windows 9.2) appropriate to the design of the experiment. Mean separation was done using LSD test at $p<0.05$.

3. Results and Discussions

In this study the effect of honey bee pollination on seed yield and various yield attributing parameters of *Glycine max* were studied as compared to open pollination and without any pollinator.

3.1. Total Fruit Yield (Kg/Ha)

The findings demonstrated that the yield obtained from plots pollinated by honey bee was superior with the mean yield of 3945kg/hectare followed by plots left open under a natural condition with the mean yield of 3754kg/hectare. The lowest mean yield of 2483kg/hectare was recorded for the plots excluded insect and honey bee pollinator (Table 1). Honeybee pollination increases the yield of *Glycine max* by 38.9% over the treatment without any pollinator. Honey bee can increase *Glycine* seed production from 9-81% [3]. These results suggested that the pollination made by insects, in particular *A. Mellifera* was responsible for the increase in the productivity of *Glycine max*. The seed yield difference between the treatments indicated that the crop requires insect pollination particularly honeybees for seed production. The *A. Mellifera* honey bees were efficient to accomplish the cross-pollination works in the soybean flower and their uses in the agriculture bring considerable gain.

3.2. Average of Seed Weight and Pod Diameter

Seeds from honey bee pollinated of *Glycine max* were weighed more compared to those from control *Glycine max*. The average weight of *Glycine max* in honey bee pollinated plots was 13.75gm as compared to open pollinated plots 12.25gm, revealing 38.2% increase in fruit weight as a result of honeybee pollination while the least 10.0gm was recorded for plot received the caged without any pollinator (Table 1). *Glycine max* weights increased when honey bee colonies were included [5]. Pod diameter Maximum pod diameter 6cm was recorded in honey bee pollinated plots followed by open pollination 5cm and caged without honey 3.8cm (Table 1). Statistical analysis of data shows significant differences between honeybee's pollinated and open pollinated treatments but no statistical significance difference between pollination caged with bees and open [2, 12]. That lower pod diameter was found in pollination caged without honeybee.

3.3. Number of Seeds, Pods and Oil Content

The pods and seeds number in uncovered area, covered with honey bees and covered without honeybees, presented differences between themselves. The average numbers of pods produced per treatments were 84, 93.75 and 68 open pollination, plots caged with honey bee and caged without any pollinator respectively. The average numbers of seeds produced pretreatments were 51, 63 and 45 open pollination, plots caged with honeybee and caged without any pollinator respectively (Table 2). Seeds produce per *Glycine max* was found to be significantly different between the treatments.

Analysis of seeds sampled in three treatments shows that There was no difference (P>0.05) between the treatments. Crude oil extract were in the normal rate to the soybean grain. The average content of crude oil extract in the seeds were 19.21±0.112%, 19.39±0.052 and 18.47±0.192 plots open for any pollinator, caged with honey bees and caged without any pollinator respectively (Table 2). There was no statistically significant difference between open, caged with honeybee and caged without any pollinator [4, 11]. Crude protein content and ether extract were in the normal rate to the soybean grain.

Table 1. *Effect of honeybee pollination on Glycine max seed yields, weights of 100 seeds in (g) and pod diameter.*

Treatments	Seed yield kg/ha	Weight of 100 seeds (mg)	Pod diameter (cm)
Uncovered area	3754±13.74^{a}	12.25±1.109^{b}	5±0.354^{b}
Covered area with honeybees	3945±20.42^{b}	13.75±0.854^{b}	6±0.408^{b}
Covered area without honeybees	2483±38.39^{c}	10±0.408^{a}	3.875±0.239^{a}
CV (%)	1.62	12.8	13.36

Averages followed by different small letters, in the same column, are different by Turkey's test (P<0.05)

Table 2. *Average number of pods, oil content and seeds counted from samples of plants of soybean Glycine max.*

Treatments	Number of pods	No. of seeds	Oil content
Uncovered area	84±1.080^{b}	51±6.721^{b}	19.21±0.112^{a}
Covered area with honeybees	93.75±1.315^{a}	63±9.1^{a}	19.39±0.052^{a}
Covered area without honeybees	68±1.080^{c}	45±3.559^{c}	18.47±0.192^{a}
CV (%)	2.95	22.24	1.26

Averages followed by different small letters, in the same column, are different by Turkey's test (P<0.05)

3.4. Germination Potential

There were statistically different (p<0.05) germination percentage among three treatments. Statistically, the highest germination percentage 27.25% was recorded in caged with honey bee treatment followed by open left treatment 21.7% while the least 16.5% germination was in caged treatment without any pollinator (Table 3). The increase in germination rate in the open plots is a result of a superior pollinating efficiency of honey bees. The germination rate was greater on average by 12% in onion with honey bee activity [5].

Mean while, the normal plant number was superior (P<0.05) treatments covered with honeybees, in relation to the un covered area and covered without honeybees.

Table 3. *The percentage of Glycine max seeds germination, normal plants and abnormal plants.*

Treatments	Germination (%)	Normal (%)	Abnormal (%)
Un covered area	21.25±1.315[b]	40.25±1.702[b]	9±0.707[b]
Covered area with honeybees	27.7±0.854[a]	45.5±3.32[a]	6.75±0.629[a]
Covered area without honeybees	16.5±1.37[c]	36.75±1.93[c]	10.5±0.654[b]
CV (%)	11.8	10.62	14.75

Averages followed by different small letters, in the same column, are different by Turkey's test (P<0.05)

3.5. Insect Visitors on Glycine Max Flowers

Various insects visited *Glycine max* flowers during its flowering time. These include honeybee, butter flies, flies, Bumble bee and moths. Among the insect visitors recorded on *Glycine max* flowers during the study period, honeybee is the major pollinator of *Glycine max* with 60.9 visits in the open pollination on the field, while moth was the least abundant visitors in the open pollination (figure 1).

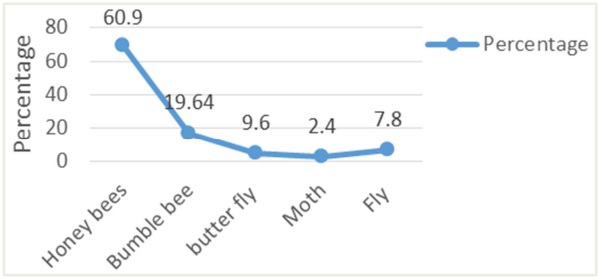

Figure 1. *Percentage of various pollinators collected from soybean during the flowering period.*

4. Conclusions and Recommendation

The study revealed that soybean (*Glycine max* L.) largely dependent on intensive honey bee pollination. The results of the study also revealed that honey bee pollination increases *Glycine max* seed yield by 38.9% over natural pollination. It was concluded that the honey bee pollination in the soybean increased the seeds production. Therefore, moving honeybee colonies to *Glycine max* farm during the flowering period is one of the most essential inputs to maximize *Glycine max* seed production. It is important also to create awareness on the value of crop pollination in boosting crop yield and to solve the challenges they are facing.

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