**Journal of Plant Protection and Pathology**

**Journal homepage & Available online at: www.jppp.journals.ekb.eg**

# Effect of Pollination by Honey Bees on Seed Yield and Its Attributes in Egyptian Clover (*Trifolium aalexandrinum* L.)

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## ABSTRACT

The effect of honey bees as a pollinator on quality and quantity of Egyptian clover (*Trifolium alexandrinum* L.) seeds was studied to get acquainted of the colony numbers (No. of frames) on seed production during 2019/2020 and 2020/2021 winter seasons. The honey bees, *Apis mellifera*, had significant effect in all seed treatments and four frames gave the highest seed setting. Over the two seasons, the maximum seed setting was recorded with four honey bee frames (81.6%) followed by three frames (75.5%), open pollination gave 72.1%, two frames gave 64.7%, one frame gave 59.6%, and without honey bee pollination in greenhouse gave 11.5%. Maximum seed yield was obtained by treatment of four frames (1.03kg/plot) followed by thee frames (0.944 kg/plot), two frames (0.822 kg/plot) and one frame (0.7 kg/plot). While the open pollination gave 0.89 kg/plot and without honey bee pollination gave 0.155 kg/plot. Maximum 1000-seed weight was increased by number of honey bee frames since the weight by four frames was 3.23 g/plot, by three frames was 3.07g/plot, by two frames was 2.77g/plot, and by one frame was 2.68 g/plot. The 1000-seed in open pollination treatment weighted 2.95 g/plot and 2.60 g/plot in free-honey bee pollination. Maximum seed germination was recorded in four frames of honey bee (94.5%), followed by three frames (93.5%), open pollination (92.8%), two frames (92.2%), one frame (89%), whereas the minimum seed germination was recorded in treatments of free-honey bee pollination (83.5%).

***Keywords:*** *Apis mellifera*, *Trifolium alexandrinum*, Honey bee-frames, Seed setting, Seed weight and Seed germination,

## INTRODUCTION

Egyptian clover or berseem (*Trifolium alexandrinum* L.) is a main annual winter leguminace fodder in Egypt. Berseem is popular due to its high yield of green fodder, its multicut about 4-7 cuts (40 ton/fed. Bondok *et. al.* 2016) and better quality of fodder (20% crude protein), high digestability (up to 65%) and high palatability (Singh *et al.* 2019) and (FAO, 2011). Berseem is characterize by succulent, high palatability, green manures, soil fertility (Graves *et al.* 1996, EL Nahrawy, 2005, Bakheit, 2013 and Kumar, *et al.* 2021), and nitrogen- fixing (Stewart *et al.* 2008 and Singh *et al.* 2019). Egyptian clover has been established as one of the best winter fodder crop in Egypt, it is the king fodder in Egypt (Badawy *et al.* 2018). Berseem is high in protein and mineral content (Laghari, *et al.* 2000).

Egyptian clover is grown for forage and seed yields, its also the essential honey producing crop (Bakheit 1989). Synergistically, honey bees are necessary for berseem clover pollination and to increase seed yield (Roy *et al.*, 2005; Goodwein *et al.*, 2011; Abrol, 2012, Jat *et al.*2014 and Kumar, *et al.* 2021 ). About 75% of the world's flowering plants are need to insects for pollination (Bezabih and Gebretsadikan, 2014).

Seed production of berseem requires cross pollination by insect pollinators because of self-incompatibility in Egyptian clover (Free, 1993). Further, Zeidan *et al.* (2012) revealed that honey bees play an important role in floret pollination, which leads to increase seed production.

As number of honey bee workers increased, the seed set in *Trifolium alexandrinum* increased as well. Beri *et al.* (1985a) found higher seed setting under open pollination (53%) than that under free honey pollinators (26.3%). Further, Roy *et al.* (2005) reported a 12.3- 99.2% reduction in seed setting under conditions of non-insect pollinators compared to open pollination conditions. Bakheit (1989) indicated that seed setting percent was higher under uncaged condition (51.9%) followed by hand pollination (17.1%) and lower in caged conditions (0.96%). Similarly, Singh *et al.* (2012) revealed the highest seed setting was under open pollination condition (62.46%) and and the lowest without insect pollination (40.33%). Jat *et al.* (2014) found the seed setting percentage was the lowest under no insect pollinators (34.06%), but it increased under bee pollination (81.4%). Also, Bondok *et al.* (2016) reported that the seed setting percent was minimum in treatments under no insect pollinators (25.7%) and maximum in treatments with bee pollination (80.5%). The input of bee pollination at low intensity of bee pollination (BP) with two frames, medium intensity of BP with four frames led to increase seed setting by 277 and 288% over those with no insect pollinators. Moreover, BP with six frames led to an increase in seed setting by 318 % over those with no insect pollinators. The same trend, many workers have studied modes of pollination (Hassanien ,1953, Narayanan *et al.*1961, Chowdhury *et al.* 1966 ; Malavia *et al.*1999). Similarly, Kumar *et al.* 2021 found the minimum seed set was recorded in the without

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**DOI: 10.21608/jppp.2023.180766.1121**

insect pollination (6.45%), while hand pollination was found over without insect pollination (8.65%). The plots for open pollination showed 74.86 percent of seed setting.

Seed setting reduced by self-pollination. The average of seed set under open pollination 73.95%, reducing by 56.25%, while under selfing without tripping seed setting was 6.60% greater reduction by (67.35%) EL-Shahawy and Gheit (2001). Also, the same results were reported by Bakheit (1989). Berseem dependence on insect pollination for seed production (Klein *et al.* 2007 and Zidan *et al.* 2019).

The objective of this study therefore to examine effect of frame numbers of honeybee hives on seed production of berseem and to determine the appropriate number of beehives that could be used per feddan to increase production of seeds. The quality and quantity parameters of the resulting seeds are evaluated under bee pollination and no bee pollination conditions.

## MATERIALS AND METHODS

The current studies were undertaken during the two successive winter seasons of 2019/2020 and 2020/2021 in the Experimental Farm at Sakha Agricultural Research Station, Kafr EL-Sheikh, Governorate, Egypt.

The Egyptian clover cultivar Helaly (*Trifolium alexandrinum* L.) was used and treated by the recommended cultivation practices. The field experiments were designed with six treatments in a randomized complete block design with three replicates, the plot size was (10 m2). The honey bees *Apis mellifera* was evaluated as pollinators. Seed setting, seed yield and their components were investigated in six treatments as follows:

1- One-frame (1F/plot) 130 bee workers equal 1 colony/feddan (54000 bee workers) or one beehive.
2- Two-frames (2F/plot) 260 bee workers equal 2 colony/feddan.
3- Three-frames (3F/plot) 390 bee workers equal 3 colony/feddan.
4- Four-frames (4F/plot) 520 bee workers equal 4 colony/feddan.
5- Open-pollination (OP).
6- Without honey bee pollinator (WHP) under caged conditions.

The bee pollinated plots were caged in nylon net. The nets were got stitched using nylon cloth of 16-mesh size sufficient to exclude the entry of insects in the cage but did not hinder other factors. Cage height was 2.5 m to impede any movement of honey bees inside the nets. Cages were erected with the help of silver poles. The net touching the ground was sufficiency pressed under the soil to prevent entry of any insects.

Care was taken to immediately zip-close the cage after entry/exist. After taken the 4th cutting every season (25/4/2020 and 20/4/2021). The colony was regularly provided with water and sugar solution weekly. After the bloom was maturity completely as change the color.

**The fifth treatment open pollination (OP):** these plots were not caged and remained exposed to open access for receptive pollination by all agencies at all times.

The sixth treatment without honey bee pollinator (WHP), plants in the plots was caged without honey bees in nylon-net. The plants were sprayed with 20 EC to kill all insects. When the flower reached the brown color in the beginning of June, nutrition was stopped and the bee frames and cages were removed.

**Data recording**

Thirty flower heads were randomly selected in each replicate of different treatments and tagged. The flower heads were manually harvested and the following quantitative and qualitative parameters were recorded.

**Number of florets per head:**

Florets per head of each treatment from each replicate were counted and their mean was calculated.

**Number of seeds per head:**

The number of seeds of the above selected heads was counted and weight and the mean was determined.

**Seed settings (%):**

Seed setting percentage was counted by:

**Seed setting (%) = No. of seeds per flowers /No. of florets per flowers * 100**

**Fertility index:**

Fertility index, expressed in present (FI %) was calculated according to Beri *et al.* (1985b) as follows:

**Fertility index = No. of seed setting under cage condition (6) / No. of seed setting under condition (1→5) x100**

**Seed yield:**

Total of threshed seeds obtained for each treatment then seeds cleaned, weighted per kg/ plot and the mean of replicates was determined.

Thousand seeds from each replication of individual treatments were weighed in grams.

**Seed germination (%):**

Fifty seeds of berseem (Helaly c.v) for each treatment in three replication were taken. These seeds were put into wet cotton in a petri dish.

Petri dish was kept in an incubator 23ºC for one week daily to determine the germination percentage of the tested seeds according to I STA (1993).

**Seedling length (cm):**

Ten normal seedlings were randomly selected from seed germination test and length between tips of the primary shoot to tip of primary root was measured.

**Vigor index:**

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

The vigor index was calculated by using

**Vigor index = Seed germination (%) × Total seedling length (cm).**

**Statistical Analysis:**

Analysis of variance (ANOVA) for different qualitative and quantitative parameters were statistically analyzed by using MSTAT-C program V.4 (1986), combined analysis over years was done according to the method of Snedecor and Cochran (1989). Combined analysis over years was performed, when the assumption of homogeneity of error was non- significant as suggested by Winer (1971). The differences between the means performed using L.S.D′s test at 0.05 and 0.01 probability levels. Further, simple correlation was determined on mean treatments and seasons as described by Steel *et al.* (1997).

## RESULTS AND DISCUSSION

**1- Seed setting and fertility index:**

Means of flowers/head, seeds/flower and seed setting percentage affected by honey bee pollinators in 2019/2020

and 2020/2021 winter season and their combined as well as relative to open pollination and without honey bee pollinator presented in Table (1).

The obtained data revealed significant differences among treatments for seeds/flower and seed setting percentage and not significant differences among treatments for florets/ flower. Where it recorded a range of 68.3 to 70.3 and from 66.7 to 70.7 in the two seasons, respectively.

**Table 1. Means of fertility index and seed setting under different honey bee intensity of the two season and their combined**

| No. of Frames | Mean Fertility index | | | Mean Seed Setting | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2019/2020 | 2020/2021 | Combined | 2019/2020 | | | 2020/2021 | | | Combined | | |
| | Fertility Index % | Fertility Index % | Fertility index % | Florets/ Flower (No.) | Seeds/ Flower (No.) | Seed setting ( % ) | Florets/ Flower (No.) | Seeds/ Flower (No.) | Seed setting ( % ) | Seed setting ( % ) | over open pollination ( %) | Over WHP (%) |
| 1 frame | 19.42 | 18.75 | 19.11 | 69.0 | 41.2 | 59.7 | 67.3 | 40.0 | 59.5 | 59.6 | 82.7 | 518.3 |
| 2 frames | 17.70 | 17.20 | 17.45 | 69.3 | 45.2 | 65.2 | 68.0 | 43.6 | 64.1 | 64.7 | 89.7 | 562.6 |
| 3 frames | 15.36 | 14.42 | 14.89 | 68.3 | 52.1 | 76.3 | 69.7 | 52 | 74.6 | 75.5 | 104.7 | 656.5 |
| 4 frames | 14.03 | 12.91 | 13.47 | 70.3 | 57.0 | 81.0 | 70.7 | 58.1 | 82.2 | 81.6 | 113.2 | 709.6 |
| Open-pollination | 15.72 | 15.59 | 15.66 | 69.7 | 50.9 | 73.0 | 67.7 | 48.1 | 71.2 | 72.1 | 100 | 627 |
| (WHP) | - | - | - | 68.3 | 8 | 11.7 | 66.7 | 7.5 | 11.2 | 11.5 | 15.9 | 100 |
| Mean | 16.45 | 15.77 | 16.12 | 69.2 | 42.4 | 61.1 | 68.4 | 41.6 | 60.5 | 64.8 | - | - |
| F test | - | - | - | N.S | ** | ** | N.S | ** | ** | ** | - | - |
| L.S.D 0.05 | - | - | - | - | 4.37 | 2.05 | - | 4.19 | 1.92 | 1.15 | - | - |

****: Significant at 1% level probability; N.S: Not significant; WHP: Without honey pollination**

Concerning seeds/ flower character, the results revealed that, there were significant differences among treatments in both seasons. The average in the first seasons ranged from 8 to 57 and 7.5 to 58.1 in the second season.

Regarding the percentage of seed setting, the obtained data expressed significant differences between WHP and OP treatments, where seed setting under OP recorded an average of 72.1% significantly higher than that of WHP which recorded an average of 11.5% in agreement with Beri *et al* (1985) and Roy *et al* (2005).

High seed setting (%) was recorded under the treatment of 4 frames (81.0, 82.2 and 81.6%) significantly higher than that of other treatments at the first and second seasons and their combined, respectively.

It is obvious that increase over open pollination (OP) for seed setting with mean 13.2%. While, increase over without honey pollinator (WHP) recorded with mean 609.6 percent over two seasons.

In addition, seed setting increased by 418.3, 462.6 and 556.5% more than without honey pollinator for 1, 2 and 3 frames were less than open pollination, but had increased at 3 frames by 4.7%, it indicated that the three frames were the first of effectiveness than open pollination.

The obtained data are in agreement with what reported by Jat *et al* (2014) and Bondok *et al.* (2016) whom mentioned that, high seed setting may be attributed to the role of honeybees density per unit area as an agent of pollination where honey bees are the major factor causing seed setting in the Egyptian clover.

**2- Seed yield:**

The relationship between honey bee as a pollinator and Egyptian clover as a source of nectar and pollen grains is a symbiotic relationship reflects on seed quantitavely production , so that high yield of seed would be obtained in the presence of honey bee as a suitable pollinator.

Concerning seed yield, data in Table (2), indicate significant differences among all treatments in the two seasons and their combined. Open pollination treatment ranked the third in seed yield (0.89 kg/plot) lower than the treatment of 4 frames which recorded (1.03 kg/plot) with 15.7% increasing. The lowest yield obtained from without honey bee pollinator less than what obtained from open pollination treatment with 82.58%. The treatment of 4 frames produced the highest significant yield (1.03 kg/plot) where 3 frames treatment ranked the second (0.944 kg/plot) significantly higher than that of 2 frames treatment (0.822 kg/plot) and 1frame treatment (0.7 kg/plot), over two seasons.

Increased with honey bee intensity may be attributed to increase seed yields by 351.6 to 509 percent over without honey bee pollinator lacking but increase of 564.5 percent was recorded when high intensity of honey bee pollination in 4 frames.

It gave 6.1 percent higher yields were medium level 3 frames and 15.7 percent over open pollination. In addition, open pollination was higher seed yield than one and two frames by 27.1 and 8.3% over the two seasons, respectively.

Honey bees pollinator increased seed yield of Egyptian clover by 351.6 to 564.5% more than without honey bee pollinator as one to four frames, respectively, it is due to effect of honey bees intensity on seed setting and mass seed of Egyptian clover.

**Table 2. Means of seed yield under different honey bee intensity of Egyptian clover Helaly in the two seasons, their combined and relative to open pollination and without honey pollination.**

| No. of frames | Seed yield kg/plot | | | | |
|---|---|---|---|---|---|
| | 2019 /2020 | 2020 /2021 | Over two years | over O.P % | Over WHP% |
| 1 frame | 0.750 | 0.650 | o.7 | 78.7 | 451.6 |
| 2 frames | 0.840 | 0.803 | 0.822 | 92.4 | 530.3 |
| 3 frames | 0.957 | 0.930 | 0.944 | 106.1 | 609.0 |
| 4 frames | 1.060 | 1.000 | 1.03 | 115.7 | 664.5 |
| O.P | 0.900 | 0.880 | 0.89 | 100 | 574.2 |
| WHP | 0.16 | 0.15 | 0.155 | 17.4 | 100 |
| Mean | 0.788 | 0.736 | 0.762 | - | - |
| F test | ** | ** | ** | - | - |
| L.S.D 0.05 | 0.003 | 0.058 | 0.05 | - | - |

****: Significant at 1% level probability; WHP: Without Honey pollination; O.P: Open pollination**

It is obvious that honey bee pollination increased yield in berseem. The same trend was reported by Narayanan et al. (1961) and Bakheit (1989) indicated high seed number per 100 flower heads by 1958 and 2671 seeds with open pollination treatment in two plots compared to 27 and 28

seeds per 100 flowers resulted from self-pollinated treatment in cages.

While, in another season he reported 4360 and 7054 seeds/ 100 flowers under open pollination treatment compared to 64 and 43 seeds/ 100 flowers resulted from without honey bees treatment.

The same trend was obtained by Jat et al. 2014 as he mentioned that low intensity of honey bees (BP-2F) recorded 2235.5 seeds/50 flower heads lower than obtained seeds from open pollination treatment (2316.3 seeds and BP-4 F treatment which yielded 2373.8 seeds/ 50 flower heads. The higher yields in present study might be due to the role of honey bees/unit area intensity 4-frames A. mellifera colony.

On the other hand recorded higher seed yields in open pollination (Singh et al. 2012) at 136g/18 m2 plot followed by plots with 4-frames A. mellifera colony (130.67 g) and least (60.50 g) in plots caged without insect pollination.

Mallingar and Prasifka (2017) found that sunflower seed mass was 26% higher on open flowers than on bagged ones. Furthermore, seed mass on open flowers increased significantly with pollinator visitation rates. Mallinger, et al. (2019) illustrate that the benefits of insect pollination to crop yields, depend on genetic and environmental factors including plant self-fertility, pollinator visitation rates, and pollinator efficacy.

**3- 1000-seed weight:**

Seed weight is an indicator of seed health and vigor which determine quantitative (seed yield) and qualitative parameters of obtained yield of such crop.

Data present in Table (3), show the effect of using honey bee as pollinators in different densities on 1000-seed weight of Egyptian clover.

It is obvious that the 1000-seed weight is an index of basic seed characteristics.

The treatment of BP- 4-frames offered the maximum density of bee pollinators for the Egyptian clover which resulted significant heavier 1000-seed weight (3.23 g) than other treatments. In addition, Bp-1f and Bp-2f recorded significant reduction compared to open pollinated treatment but in WHP without honey bee pollination, the previous ratio treatment were higher than that obtained in the absence of honey bee pollinators (WHP) with 3.08 and 6.54% in Table (3) at the first and second seasons, respectively.

**Table 3. Means of 1000-seed weight under different honey bee intensity of Egyptian clover Helaly in the two seasons, their combined and relative to open pollination and without honey pollination.**

| No. of frames | 1000-Seed weight (g) | | | | |
|---|---|---|---|---|---|
| | 2019 /2020 | 2020 /2021 | Over two years | Over O.P % | Over WHP% |
| 1 frame | 2.75 | 2.61 | 2.68 | 90.8 | 103.08 |
| 2 frames | 2.82 | 2.71 | 2.77 | 93.9 | 106.54 |
| 3 frames | 3.08 | 3.06 | 3.07 | 104.1 | 118.08 |
| 4 frames | 3.37 | 3.15 | 3.23 | 109.5 | 124.23 |
| O.P | 2. 97 | 2.92 | 2.95 | 100 | 113.46 |
| WHP | 2.67 | 2.52 | 2.60 | 88.1 | 100 |
| Mean | 2.94 | 2.83 | 2.88 | - | - |
| F test | ** | ** | ** | - | - |
| L.S.D 0.05 | 0.13 | 0.15 | 0.09 | - | - |

****: Significant at 1% level probability; WHP: Without honey pollination; O.P: Open pollination**

Also, the treatment of Bp-4f indicates significant higher 1000-seed weight (3.23g) than that obtained from open pollination (2.95g) and WHP treatments (2.60 g) as an increased with 9.5% and 24.23%.

Our results here were harmony with these gained by Free, 1993 who revealed that higher 1000-seed weight heads in bee pollination plots by 13.8 g compared to 0.2 g in without insect pollination but was significantly lower in op 6.1 g. Also, Jat *et al.* (2014) involved the treatment BP-4F, BP-2F and op significantly heavier than without insect pollination (3.30, 3.17 and 3.03 g, respectively) in seed weight.

Similarly, Bondok *et al.* (2016) showed the BP-4 F and BP-2 F treatments were significantly heavier than without insect pollination 3.56 and 3.30 g, respectively. The same trend Kumar *et al.* (2021) found OP recorded higher 3.01g/ 1000 seed weight, while without insect pollination lighter 2.57 g. Also, Singh *et al.* (2012) indicated lower 1000-seed weight (2.47 g) in plots caged with *A. mellifera* colony compared to open pollinated plot (2.51 g) and was the lowest under without insect pollination 2.22 g).

**4- Seed germination:**

The seed germination is an indicator viability of seed. It reflects in seed quality.

Data obtained in Table (4), recorded seeds with minimum germination 83.5% in WHP treatment, while the maximum germination in combined data was decided in other treatments 1-f (89%), 2-f (92.8%), 3 f (93.5%) and 4 f (94.5%).

These results showed increase over op with seed germination, while the lowest increase over op (90%), but all other treatments 1 f (95.9%), 2 f (99.4%), 3 f 100.8% and 4 f (101.8%). Also, these results involved the lower treatment 1 f (106.6%) over WHP. The other treatments were 2 f (110.4%), o.p (111.1%), 3 f (112%) and the higher 4 f (113.2%). These results agree with Singh *et al.* 2012 revealed that increased seed germination by 7.1- 11.8 per cent. Also, Jat *et al.* (2014) recorded the highest germination from O.P plots (90.67%), caged with 4-frame *A. mellifera* colony (88.67%) and the lowest in without insect pollination plots (80%). Similarly, Bondok *et al.* 2016 found the lowest seed germination (38.5%) in WHP. The treatment Bp-4F was recorded (95.1%) and BP-2 F (94.5%). The same trend, Kumar *et al.* (2021) recorded the minimum seed germination in without insect pollination (79.33%) but (91.17%) in open pollination.

**5- Seedling length and vigor index:**

Data in Table (4), cleared that highly significantly and no variation could be estimated for seedling length and vigor index of seeds harvested from plots, whereas, the maximum seedling length was recorded for treatments 1f (4.89 cm), 2 f (4.93cm), op (5.34cm), 3 f (5.45cm), and 4 f (5.50cm), respectively. These results were increased over pollination with seedling length by (2.06%) and (3.00%) for 3 f and 4 f, respectively.

Also, increase over without honey pollination with seedling length was 1 f (7%), 2 f (7.88%), op (16.84%), 3 f (19.25%) and 4 f (20.35%), respectively. Also, the data showed that the maximum vigor index was recorded in treatments 1 f by (434, 79), 2 f (453.85), op (495.09), 3 f (509.62) and 4 f (519.3), respectively. These results increase over open pollination with vigor index were (2.93%) and (4.89%) for 3 f and 4 f. while, increase over without honey

pollination with vigor index was 1 f (13.89%), 2 f (18.89%), op (29.69%), 3 f (33.5%) and 4 f (36.03%), respectively.

However, lower seedling length (4.57cm) and vigor index (381.75) was recorded in the plot with without honey pollination. This result was an agreement with Kumar *et al.* (2021), who found that lower seedling length (4.58cm) and vigor index (383.58) was mentioned in the plot with without honey pollination.

**Table 4. Means of seed germination, seedling length and vigor index under different honey bee intensity of Egyptian clover Helaly in the two seasons, their combined and relative to open pollination and without honey pollination.**

| Treatment | Seed germination | | | | | Seedling length | | | | | Vigor index | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2019/ 2020 | 2020/ 2021 | Mean | Over (O.P) % | Over WHP (%) | 2019/ 2020 | 2020/ 2021 | Mean | Over (O.P) % | Over WHP (%) | 2019/ 2020 | 2020/ 2021 | Mean | Over (O.P) % | Over WHP (%) |
| 1 frame | 90 | 88 | 89 | 95.9 | 106.6 | 4.91 | 4.86 | 4.89 | 91.57 | 7.00 | 441.9 | 427.68 | 434.79 | 87.82 | 13.89 |
| 2 frames | 92.3 | 92 | 92.2 | 99.4 | 110.4 | 4.96 | 4.89 | 4.93 | 92.32 | 7.88 | 457.81 | 449.88 | 453.85 | 91.67 | 18.89 |
| 3 frames | 94 | 93 | 93.5 | 100.8 | 112 | 5.54 | 5.36 | 5.45 | 2.06 | 19.25 | 520.76 | 498.48 | 509.62 | 2.93 | 33.5 |
| 4 frames | 94.7 | 94.3 | 94.5 | 101.8 | 113.2 | 5.57 | 5.42 | 5.50 | 3.00 | 20.35 | 527.48 | 511.11 | 519.3 | 4.89 | 36.03 |
| O. P. | 93.3 | 92.3 | 92.8 | 100 | 111.1 | 5.53 | 5.34 | 5.34 | 100 | 16.84 | 497.29 | 492.88 | 495.09 | 100 | 29.69 |
| WHP | 85 | 82 | 83.5 | 90 | 100 | 4.67 | 4.47 | 4.57 | 85.58 | 100 | 396.95 | 366.54 | 381.75 | 77.12 | 100 |
| Mean | 91.6 | 90.3 | 90.9 | - | - | 5.20 | 5.06 | 5.11 | - | - | 476.81 | 457.76 | 467.29 | - | - |
| F test | ** | ** | ** | - | - | ** | ** | ** | - | - | ** | ** | ** | - | - |
| L.S.D 0.05 | 1.81 | 2.39 | 1.4 | - | - | 0.06 | 0.04 | 0.35 | - | - | 8.43 | 10.93 | 4.01 | - | - |

****: Significant at 1% level probability; N.S: Not significant; WHP: Without honey pollination; O.P: Open pollination**

**Correlation matrix:**

Data in Table (5) indicated that correlation was highly significantly among study characteristics seed setting, seed yield, 1000-seed weight and germination percentage. Seed setting was positively correlation with seed yield (0.748**), 1000- seed weight (0.840**) and seed germination (0.941**). This result agreement with Kumar *et al.* (2021) who reported the bee visit was positively correlation with seed setting (0.176), seed yield (0.685*), 1000 seed weight (0.671*) and germination percentage (0.437). Long and Morandin (2011) revealed that a positive correlation between honey bee activity and onion seed set, as well as between number of bee visits and seed yield.

**Table 5. Simple correlation between honey bee pollination quality and quantity seeds in 2019/2020 and 2020/2021 seasons.**

| | Seed setting% | Seed yield/g | 1000 seed weight/g | Seed Germination% |
|---|---|---|---|---|
| Seed setting % | - | 0.798** | 0.840** | 0.941** |
| Seed yield/g | - | - | 0.673** | 0.812** |
| 1000-seed weight/g | - | - | - | 0.816** |
| Seed germination% | - | - | - | - |

**** Correlation is significant at the 0.01 level probabilities.**

## CONCLUSION

It could be concluded that use of honey bee pollinators in Egyptian clover have a positive effect on seed setting, seed yield, 1000- seed weight and seed germination. The different application of honey bee pollinators improved the quantitative and qualitative parameters of berseem seeds. Employing BP of four frames of *A. mellifera* hives led to significant increase in clover yield compared with other treatments of low intensity of honey bee pollinator (3 frames, 2 frames and 1 frame) and open pollination. The treatment without honey bee pollinators recorded the lowest values all parameters estimated. In addition, it can be recommended with using three beehives per feddan in the open field.

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# تاثيرتلقيح نحل العسل على محصول البذرة وصفاتها فى البرسيم المصرى

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## الملخص

تمت دراسة تأثير تلقيح نحل العسل على جودة وكمية إنتاج بذور البرسيم المصري *Trifolium alexandrinum* L شملت تأثير أعداد الطوائف (عدد البراويز) على إنتاج البذور خلال مواسم الشتاء 2020/2019 و 2021/2020 . وقد أثبتت النتائج أن لنحل العسل *Apis mellifera* تأثير معنوي في جميع معاملات البذور حيث أثبتت النتائج أن أربعة براويز أعطت اعلى نسبة عقد على مدار الموسمين ، وقد تم تسجيل أعلى نسبة عقد للبذور بأربعة براويز نحل عسل (81.6٪) تليها ثلاثة براويز (75.5٪) ، والتلقيح فى الحقل المفتوح سجل نسبة عقد 72.1٪ ، وسجل بروازين 64.7٪ ، وبرواز واحد سجل 59.6٪ ، وبدون تلقيح نحل العسل فى الصوبة سجلت نسبة العقد 11.5٪. وقد تم الحصول على اعلى محصول للبذرة بمعاملة أربعة براويز (1.03 كجم/ المعاملة) ، تليها ثلاث براويز حيث سجلت (0.944 كجم/المعاملة) ، وبروازين (0.822 كجم/المعاملة) ، وبرواز واحد سجل (0.7 كجم/المعاملة) ، بينما أعطى التلقيح المفتوح 0.89 كجم/المعاملة ، وبدون تلقيح نحل العسل أعطى 0.155 كجم/المعاملة. وقد تم الحصول على أعلى وزن لـ 1000 بذرة بزيادة عدد براويز نحل العسل ، حيث سجل أعلى وزن باستخدام أربعة براويز 3.23 جم/المعاملة ، وثلاثة براويز 3.07 جم/المعاملة ، وسجل بروازين 2.77 جم/المعاملة ، وبرواز واحد سجل وزن 2.68 جم/المعاملة. وقد سجلت نتائج الوزن لـ 1000 بذرة في التلقيح المفتوح 2.95 جم/المعاملة ، وفي تلقيح نحل العسل الحر أعطت 2.60 جم/المعاملة. وقد تم تسجيل أعلى نسبة إنبات للبذور باستخدام أربعة براويز من نحل العسل (94.5٪) ، تليها ثلاثة براويز (93.5٪) ، والتلقيح المفتوح سجل (92.8٪) ، وبروازين (92.2٪) ، وبرواز واحد (89٪) ، بينما كان الحد الأدنى لنسبة إنبات البذور في معاملات تلقيح نحل العسل الحر (83.5٪).من خلال النتائج يمكن التوصية باستخدام عدد ثلاثة خلايا نحل للفدان بالاضافه الى الحشرات الزائرة تحت ظروف الحقل المفتوح.