Effect of Honey Bees Pollination on Egyptian Clover Seed Yield

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ABSTRACT

Pollination behaviour and extent of self-incompatibility were studied in Egyptian clover, an important fodder legume cultivated in Egypt. The effect of pollination mode on quantitative and qualitative parameters of Egyptian clover seed yield has been studied at Gemmiza Research Station during 2012-2013 and 2013-2014 winter seasons. Maximum seed setting percentage (80.5%) was recorded with first hybrid Carniolan bees pollination and 6 frame colony (BP – six f) followed by frame colony (BP- four F) with seed setting percentage (73.1 %) and two frame colony (BP-two F) with seed setting percentage (71.6%).

Maximum seed yield (2346.6 seeds) was observed with treatment BP- 6 F followed by BP- 4 F (2250.0 seeds) and BP – 2 F(1826.6 seeds). Still the lowest seed yield was found with the without insect pollination (WIP) treatment. Also, minimum 1000 – seed weight with WIP was observed (2.56g). The 1000 seed weight of BP – four F, six F and BP-two F were 3.56, 3.30, 3.80g, respectively.

The highest seed germination percentage (95.7%) was recorded with BP– six F and the lowest germination percentage (38.5 %) was found with WIP.

Keywords: Bee pollination, Egyptian clover, self – incompatibility, Seed Viability, Seed weight.

INTRODUCTION

Trifolium alexandrinum L., commonly called Egyptian clover or berseem is very important winter forage crop in Egypt, owing to higher quantitative yield parameters viz, green fodder yield (40 T/ fed) and multicut nature (4-7 cuts) along with qualitative parameters namely, succulency, high palatability, nutritve value (20% crude protein), digestibility up to (65%) and continuous supply of seven months (Oct to May) .

Egyptian clover is one of the most entomophilic crop requiring insects, especially bees for cross – pollination. Singh *et al.* (2012) also reported higher seed yield per plot (18 m^2) in plots caged with *Apis mellifera* colony (2-frame), (4-frame) and (6- frame) as compared to plots caged without insect pollination (WIP).

Honey bees are reported to be the primary and absolutely essential pollinators of Egyptian clover, visiting it avidly for nectar and pollen and significantly increasing seed yield according to Sharma and Singh, (2003) *A. dorsata* (6.5 bees) was the most abundant floral visitor followed by *A. mellifera* (4.4 bees). With immense scope to increase production of biomass and seed of Egyptian clover, a variety of pollinators has been used by the man for guided pollination that included *A.mellifera* and *A. cerana*, (Pinzauti and Martiniello, 2003).

The aim of the present study was to determine the effect of the honey bees pollination on quantitative and qualitative yield parameters of Egyptian clover seed yield and to find out colony strength (frames/colony), of *A. mellifera* required for enhancing different parameters of Egyptian clover seed yield .

MATERIALS AND METHODS

Plant materials:

One cultivar of Egyptian clover, (Gemmiza1) was used for the present study.

Bee pollination (BP): The effect of Honey Bees pollination on quantitative and qualitative parameters of Egyptian clover was investigated using the following four treatments :

T1 – Caged without insect pollination (WIP):

In (WIP) or caged without bees (CWB) treatment the plants in the plot were caged in a nylonnet (50m^2) of 16mm mesh size as explained below in bee pollination. However, in WIP, no honey bee colony was placed inside. The net prevented pollination by any insect or other pollinators.

Bee pollination (BP):

The bees 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 physical factors. Cage height was 2.5m sufficient to allow unhindered movement of honey bees inside. These cages were erected with the help of bamboo poles and tightened with the help of cords. The net clothing touching the ground was sufficiently pressed under the soil so as to prevent entry of any insects. A zipped entry was kept at one corner of the net to allow manual entry for data recording and other operations. Care was taken to immediately zip-close the cage after entry/ exit. The crop was caged immetediately before the beginning of flowering time. (10/5/2013), (13/5/2014). After that, a colony of *A. mellifera* in standard deep LT wooden hive was kept

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Received August15, 2016, Accepted September 21, 2016

inside the net just at the start of flowering (10/5/2013) or not later than 10 percent flowering. The colony strength depended upon the treatments given below. The colony was regularly provided with water. The bee colony was removed when all the tertiary bloom was complete. Based on the colony strength (frames / colony), the following four treatments were taken:

T1 : Without insect pollination (WIP).

T2 : *A. mellifera* colony of two-frame strength.

T3 : *A. mellifera* colony of four – frame strength.

T4 : *A. mellifera* colony of six-frame strength.

All these treatments were replicated three times in a plot area of $50m^2$.

Recording of observations:

Forty flower heads were randomly selected and tagged in each replication of different treatments. At maturity they were manually harvested – the following quantitative and qualitative yield parameters were recorded on these heads :

Number of florets per head:

After harvesting twenty flower heads were randomly selected from each replication of the treatment. Number of florets per head were counted and their mean was calculated.

Number of seeds per head:

After counting the number of florets from above selected heads, number of seeds per head was also counted and mean seed number was determined.

Seed setting (%):

From randomly selected and marked 20 heads, number of florets per head was counted. At harvesting number of seeds per head was counted. Seed setting percent was calculated as follows:

Seed setting (%)= No. of seeds per head/No. of florets per head × 100.

Seed Yield:

Total number of seeds obtained from the threashing of 40 heads was counted with the help of seed counter.

1000 – seed weight:

Thousand seeds from each replication of individual treatments thus counted were weighed with the help of electronic weighting machine (fcosct) to get 1000 – seed weight in grams.

Seed Viability:

To determine berseem seed viability, a hundred berseem seeds of variety, was put into a petri dish on a small piece of wetted cotton and each treatments replicated and put into an incubator 25 °C and treatments were examined daily to determine the percentage of germinated seed as a viability percent.

The experimental design was a randomized complete block design three replications, the treatment means were compared using the least significant differences (L.S.D).

Honey Bees Materials:

The present study was conducted in Gemmiza Agricultural Research Station, Gharbia governorate, during the two winter seasons of 2012 and 2014, Six honey bee colonies in the same strengthes combs covered with bees hived, having mated queens of the same age were selected. These colonies were divided into two groups (each contains 3 colonies) randomly selected for each of control and caged group.

Berseem clover flowering hive yield of honey and pollen 2012/2013

	Cage I		Cage II		Control	
Reading	**Honey in inch.**	**Pollen**	**Honey in area**	**Pollen**	**Honey in inch.**	**Pollen**
	40,46,52	11/10/9 gm	-	-	49,51,53	10/8/6
	75,70,65	13/15/17 gm	-	-	75,76.5,78	15/13/11
	100,105,110	10/12/14 gm	-	-	120,123,126	15/17.5/20
	143,146.5,150	9/10/11 gm	-	-	149,151,153	10/10.5/11
Total	1057.5	-	-	-	12045	147
Mean	88.12	-	-	-	100.37	12.25

Berseem clover flowering hive yield of honey and pollen 2013/2014

	Cage I		Cage II		Control	
Reading	**Honey**	**Pollen (gm)**	**Honey**	**Pollen**	**Honey**	**Pollen (gm)**
5/2014	37, 35, 33	11/10/9	-	-	45,49, 53	7/6/5
5/2014	49,50,51	12/13/14	-	-	70,71.5,73	9/13/17
5/2014	98,99,100	9/10/11	-	-	110,112, 114	10/11.5/13
5/2014	130,132,134	-	-	-	130,135,140	3/3.5/4
Total	316	33	-	-	3675	102
Mean	79				91.87	8.5

Honey area was measured at seven days intervals using wire frame divided into square inches (Moellar 1967). Pollen was weighted every seven days. Statistical analysis of the obtained data was carried out adopting the analysis of variance procedure and the means were compared by L.S.D test(Fisher,1944).

RESULTS AND DISCUSSIONS

Effect of pollination on seed setting:

Berseem flower head on an average contained 61.9 - 67.9 florets (Table 1). The treatment where crop was caged to avoid any contact with floral visitors (WIP) resulted in minimum seed of 15.9 seeds/head while seed setting in BP-2f, BP-4f and BP–6P treatments were 44.5, 46.8 and 54.7 seeds/ floret, respectively.

Seed setting:

Percentage seed setting as minimum in WIP (25.7) and significantly higher in BP-2f (70.4), BP-4F (72.7) and BP-6f (80.5) treatments. The input of bee pollination through managed at low intensity (BP-2f), medium intensity bee pollination (BP-4F) led to increasing seed setting by 277.0 and 288 percent over WIP Moreover (BP-6F) resulted in an increase of 318 percent over WIP. The present study thus clearly indicated that, employing honey bee as an agent of pollination, led to significantly increasing seed set.

These findings are amply supported by the work of Singh *et al.,* (2012) who reported higher seed setting under open pollination condition (62.46%) and bee pollination with 4-frame, *A.mellifera* colony (61.01%) and the lowest (40.33%) in without insect pollination. Similarly, many studies reported higher seed set in bee pollination than open pollination. Hassanein (1953) recorded highest seed setting (38.9%) in BP followed by Op (23-5%) and minimum in WIP (1.9 seeds/head). Similarly, Bakheit (1989) found highest seed setting in BP with *A. mellifera* (51.9%) followed by hand pollination (17.1%) and least in caged conditions (0.96%).

Like in the present study, many researchers have tried various modes of pollination (Chowdhury *et al.,* 1966; Narayanan *et al.*, 1961; Singh *et al.,* 2012) and accepted the need of honey bees to increase seed yield (McGregor, 1976; Free, 1960). But there is a clear lack of efforts on utilizing various densities of bee populations and that may be the reason of lower seed set recorded by these workers. However, these studies clearly indicate the highest benefits of saturated pollination where maximum (80.5%) seed setting was recorded.

Effect of the Honey Bees pollination on number / 40 heads:

The quantitative seed yield produced by Egyptian clover is the ultimate reflection of the symbiotic relationship it had with various floral visitors resulting from the process of pollination and fertilization.

The lowest yield (Table 2) was obviously recorded in plots without insect pollination as only 906.6 seeds/40 heads heads were obtained compared to all other treatments of pollination component involved. Relatively gradual higher seed yields were obtained in bee pollination (BP-2f) (1786.6 seeds), bee pollination (BP-4f) (2245.0 seeds) and bee pollination (BP-6f) which gave the maximum seeds (2353.3).

The study thus, clearly indicated the immense value of bee pollination in achieving higher seed yield in Egyptian clover. The previous studies reported higher seed yields in bee pollination (BP-4f) (130.67g) and least (60.50g) in plots caged without insect pollination (Singh et al., 2012). Similarly, Narayanan et al., (1961) reported higher (1958 and 2671 seeds/100 flower heads) yield in two plots compared to only 27 to 28 seeds with automatic self pollination in cages during 1957 and also during 1960 at 4360 and 64 to 43 with WIP.

Table 1. Effect of bees pollination on seed setting of Egyptian clover

Levels of pollination	Mean seed setting parameters					
	2012/2013			2013/2014		
	Florets / head	Seeds / head	% Seed setting	Florets / head	Seeds / head	% Seed setting
Cage without insect pollination (WIP)	61.9	16.1	26.1	62.00	15.7	25.3
Bee pollination. (BP) two frams	62.9	43.5	69.2	63.5	45.5	71.6
Bee-pollination – $BP_{-4}F$	63.8	46.1	72.3	65.0	47.5	73.1
Bee pollination- $BP_{-6}F$	67.9	54.7	80.5	68.1	54.8	80.5
L.S.D 5%	3.42	5.42	6.21	5.28	6.28	8.26

Table 2. Effect of bees pollination on the number of 40 heads of berseem clover

Bees of pollination	Mean number of seeds / 40 heads	
	2012 – 2013	2013 - 2014
	Mean	Mean
Without insect pollination (WIP)	866.66	946.66
Bee pollination. $BP_{-2}F$	1746.66	1826.66
Bee-pollination – $BP_{-4}F$	2240	2250.00
Bee polliuation- $BP_{-6}F$	2360	2346.66
L.S.D 5%	22.94	24.63

Table 3. Effect of bees pollination on 1000 – seed weight of Egyptian clover

Bees of pollination	1000 – seed weight / in different seasons (g)	
	2012 – 2013	2013 - 2014
	Mean	Mean
Cage without insect pollination (WIP)	2.63	2.49
Bee pollination. $BP_{-2}F$	3.32	3.29
Bee-pollination – $BP_{-4}F$	3.59	3.54
Bee pollination- $BP_{-6}F$	3.83	3.77
L.S.D 5%	1.94	1.29

The higher yields in the present study could be explained by the employment of the concept of "saturated pollination" where higher intensity of honey bees/unit area was used by keeping a six-frame. *A. mellifera* colony as the previous workers only went up to four-frame colony.

Effect of the honey bees pollination on seed viability

The seed viability is the most important index of the seed germination (Table 4). It is a solid indicator of the history of processes of pollination fertilization and subsequent seed development. The success or failure (complete or partially) will be ultimately reflected in seed quality and govern the furtherance of the germination (Chaudhary, 1998).

Table 4. Comparative effect of bees pollination methods on clover seed viability during 2012-2013 and 2013-2014 seasons

Treatments	% Viability	
	2012-2013	2013-2014
Plant caged with honey bees	96.0	95.5
Plant caged without honey bees	36.6	40.4
Open field	87.4	89.5
L.S.D 5%	5.41	22.41

In plots, devoid of any insect pollination activity (WIP) seed with minimum viability (38.5%) were recorded. This level was under the minimum germination required for seed standards (80.0%). The maximum viability however, was recorded in all bee pollination levels i-e; BP-6f (96.0%), BP-4f (95.1) and BP-26 (94.5).

Employing these pollination inputs led to increasing seed viability by 7.1-11.8 per cent (Sigh *et al.,* 2012) while acknowledging the role of different levels of pollination reported contradictor findings of highest seed viability from bees pollination plots caged with a 4-frame *A. mellifera* colony (95.1%) and the lowest in without insect pollination plots (38.5%).

Effect of the honey Bees pollination on 1ooo - seed weight of Egyptian clover:

Seed weight is a direct index of the seed health and vigour that eventually determines quantitative (yield) and qualitative parameters (Table 3). A bolder and heavier seed is the result of proper post pollination and fertilization development processes and is supposed to be better than normal or shrivelled seeds. Especially in a crop like Egyptian clover where seed has no other purpose like food or feed, a bolder seed is an indicator of better germination by providing more food reserve to the germination seedlings. The 1000 seed weight thus is an index of basic seed characteristics

The saturated bee pollination treatment with 6 – frame first hybrid carniolan bees *A.mellifera* ensured maximum pollination of available Egyptian clover florets , thus resulting not only in higher number but also heavier seeds with maximum1000 – seed weight of 3.8g and in absence of any pollination input (WIP). Lightest seed weight with 2.56g/ 1000 seeds was recorded. The seed weight of Bp-4f and BP -2f levels though significantly heavier than WIP; 3.56 and 3.30g, respectively. The results while signifying the need of insect visitors also show a moderate increase in seed weight by employing different levels of pollination as compared to WIP. These results are considered full

support from Free (1993) who also reported higher seed weight /1000 heads in bee pollinated plots (13.8g) compared to only 0.2g in without insect pollination (WIP).

However, the finding of Singh *et .al.* (2012) do not support present study who reported lower 1000 - seed weight (2.47g) in plots caged with *A.mellifera* colony compared to without insect pollination (2.22g).

CONCLUSION

It is clear that the seed setting (%), seed yield, 1000 seed weight and percent viability of Egyptian clover significantly increased under bee pollination levels in comparison with no bees pollination.

Saturated bee pollination by employing an 6-frame of *A. mellifera* colony resulted in significantly higher seed count over lower intensity bee pollination (4 and 2 frame colonies). The utility of Egyptian clover crop as highly valuable nectar and pollen sources was confirmed for *A. mellifera* colony development.

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الملخص العربي

تأثير التلقيح بنحل العسل على محصول بذور البرسيم المصري

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يتميز البرسيم المصري بوجود ظاهرة عدم التوافق الذاتي Self –Incompatibility بدرجات متفاوتة في جميع أصنافه لذلك فمن الضروري دراسة سلوك التلقيح في البرسيم المصري بالحشرات الملقحة "نحل العسل".

لذلك أقيمت تجربة حقلية في محطة البحوث الزراعية بالجميزة خلال موسمي٢٠١٢-٢٠١٣، ٢٠١٣-٢٠١٤ حيث كان الهدف من البحث دراسة محصول بذور البرسيم المصري ومكوناته، وكانت أهم النتائج المتحصل عليها هو كالآتي:

نسب العقد:

- الحد الأقصى للنسبة المئوية للعقد(٨٠,٥%) مع العاملةSix Frame في الموسمين ٢٠١٣-٢٠١٤.
- النسبة المئوية للعقد (٧٣,١%) مع المعاملة Four Frame في موسم ٢٠١٤ (٧٢,٣%) في موسم ٢٠١٣.
- النسبة المئوية للعقد (٧١,٦%) مع المعاملة Two Frame في موسم ٢٠١٤ و(٦٩,٢%) في موسم ٢٠١٣.

إنتاجية البذور:

- الحد الأقصى لإنتاجية البذور كانت (٢٣٤٦,٦) بذرة مع المعاملة Six Frame في موسم ٢٠١٤ و(٢٣٦٠) بذرة في موسم ٢٠١٣.
- إنتاجية البذور كانت (٢٢٥٠,٠) بذرة مع المعاملة Four Frame في موسم ٢٠١٤ و(٢٢٤٠) بذرة في موسم ٢٠١٣.
- إنتاجية البذور كانت (١٨٢٦,٦) بذرة مع المعاملة Two Frame في موسم ٢٠١٤ و(١٧٤٦,٦) بذرة في موسم ٢٠١٣.
- أقل إنتاجية بذور كانت مع المعاملة(WIP) بدون حشرات ملقحة.
- أيضاً وزن(١٠٠٠) بذرة كانت مع المعاملة(WIP) أقل وزن وهو(٢,٥ جم) في موسم ٢٠١٤ و(٢,٦ جم) في موسم ٢٠١٣ بينما مع Four Frame كانت(٣,٥٦ جم) في موسم ٢٠١٤ و(٣,٥٩ جم) في موسم ٢٠١٣ وكذلك مع المعاملة Two Frame كانت(٣,٣٠ جم) في موسم ٢٠١٤ و(٣,٣٢ جم) في موسم ٢٠١٣ على التوالي وكانت مع المعاملة Six Frame (٣,٨٠ جم) في موسم ٢٠١٤ و(٣,٨٣ جم) في موسم ٢٠١٣.

نسب الإنبات:

- أعلى نسبة إنبات كانت(٩٦%) مع المعاملة Six Frame في موسم ٢٠١٣ و(٩٥,٥%) في موسم ٢٠١٤.
- أقل نسبة إنبات كانت(٣٦,٦%) مع المعاملة(WIP) بدون حشرات ملقحة في موسم ٢٠١٣ و(٤٠,٤%) ٢٠١٤.