



Comparison of the pollination effectiveness between different honeybee nuclei and bumble bees on summer squash in plastic house

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ABSTRACT

*Experiment was carried out in (Amman-Jordan) during 2018/2019 growing season, to compare pollination efficiency between the different Honeybee nuclei's of (*Apis mellifera* L) and imported Bumblebee (*Bombus terrestris* L.) in pollinating summer squash (*Cucurbita pepo* L.) grown in plastic house within the evaluation of summer squash potential for the pollen and nectar production with some idea about plant parameter to achieve the best pollination integral management, The effectiveness of honey bees and bumble bees on summer squash pollination was compared under field condition, but not studied in plastic house cultivation in Jordan, were the major problems for fruits production is insufficient pollination due to confined condition and limited wind resulting in low production; large sticky pollen grain has unusual properties such as the absence of dehydration and rapid loss of viability. Therefore *C. pepo* requires pollination agents to transfer pollen in plastic house. Both types of flower in plastic house are open from early morning until mid-day. Male flowers open and close half an hour earlier than female flowers, male flowers produce more nectar and visited more often by the bees than female flowers, to determine the foraging activity of bees visiting its flowers, the time and type of provision obtained. Comparison was made among treatments of summer squash flowers pollination by using a number of measures including single bee visits to marked flowers and flowers parameter. Demarcated 10 male and 10 female plants in each section (5 male and 5 female in each row) labeled plant; the highest fruits weight was obtained from three honeybee nuclei's, whereas lowest weight in bumble bees, Moreover fruits number showed significant differences between the 3 treatments. Marketable Squash fruits (more than 12 cm. length) were harvested each two days a week during ripening period. In conclusion, treatment of plastic house summer squash with three honeybee's nuclei increases fruit weight, size and production of sufficient pollen and nectar sugar concentration for their pollinator's agent.*

Keywords: - *Cucurbita pepo*, pollination, honey bee, Bumble bees, pollen, nectar.

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INTRODUCTION

To meet the increasing population demands for vegetables fruits plastic house cultivation is spreading rapidly among farmers in Jordan. Plastic house vegetable cultivation is a production system providing a high income per unite area due to various benefits, such as year round production, improvements in crop quality and increase in yield. Plastic houses allow a more efficient use of water, fertilizer, pesticides and labor [23], for all these reasons protected culture environments have been increasing during the last few decades, It is necessary to get maximum yield in agriculture by using available pollination in order to get maximum profit from per unit area because existing agricultural land and pollination are rapidly diminishing due to the rapid industrialization and urban development. Therefore; we need to know the right pollination needed for the plants, and insect relationship. Furthermore, it is essential to develop the most suitable pollination schedule to get maximum plant yield for different ecological regions as plant pollination depends mostly on plant flowers. Pollination plays an important role in flowering plant reproduction and fruits set for wild plant community [5]. Estimates showed that up to 90 % of all flowering plant species rely on pollination by insect such as bees [5]. So the animal pollinator's contribute approximately between 15%-30% of global food production [25]. In Jordan there are few studies on the pollination of plastic house crops of major commercial importance like broad bean [1], [20], eggplant, sweet and chill pepper [2], and the pollination efficiency on field-growing tomato [3], while squash is one of the main ones without bees and other insect visitors didn't produce any fruits [9], the unusual properties such as the absence of dehydration and rapid loss of viability [18] especially large sticky pollen

grain which is not easily blown by wind [19,11] is the main constraint of production. This paper investigates more directly comparison of different honeybee nuclei's and bumble bee pollination visit to squash flowers particular attention given to pollination regiments of flowers. Bees are the principle means by which pollen is transferred from the male to the female flower, this paper observed that honey bees worked on the flowers from 5.00 am to mid-day around 11.00 am. In plastic house with maximum activity from 7 to 9 am. [21]. According to Jordan [10], Summer squash consider the most among vegetable crops based on cultivated area, with approximately 2757 hectares for commercial production which rank fourth crops of the total vegetable area in the country, produced about 72 thousand tons of squash in 2017. The crop is grown throughout the country, where irrigated water and arable land are available, and it is mainly grown by small holders, who employ relatively poor crop management practices. To maximize fruit and pod set in different vegetable crops. Honeybees [17] and bumble bees [14]; are frequently used. The production of fruits and seeds of many crops is increased when bees visit their flowers pollinating them, it is necessary to evaluate the need of controlled pollination of crops dependent on pollinating agents [12]. The objective of this study To evaluate pollination in summer squash to determine the density and diversity of pollinators foraging behavior on flowers and their resultant effects in terms of fruits production, and to evaluate the potential of *C. pepo* for anther pollen amount and nectar sugar concentration, that could help maintain colonies placed in the plastic house, and bee's pollination activity at different times of the day, in relation to temperature, and to appraise knowledge of farmers about bee pollinators, their natural history and crop pollination, and develop protocols to be used by them for integrating pollinators into farming systems.

MATERIAL AND METHODS

The selected plastic house dimensions were 60m length, 10m width and 3m in height and the area of the plastic house was divided in to three sections, the area of each section was 200 m² equipped with closing apparatus by using fine mesh. Summer squash after germination the young seedling were transferred to plastic house section nominated for pollination, each section planted with grand total of 100 in two rows (50 seedlings for each) at apart of 40 cm. space between the plants. The plastic house was drip irrigated with needed water. The mineral nutrient solution containing all the essential elements was provided. For periodic observations in each section 20 plants demarcated with iron pole, ten in each line five male and five female flowers were labeled, the following parameters were studied in experiment.

Plant material

In order to manage summer squash production effectively, it is necessary to know how many bee visits are required for adequate fruit set and fruit size, summer squash male and female flowers occurring individually in the axils of the leaves in the same plant, female flowers are borne on very short stems and easily distinguished due to its large ovary at the base of the flower, with a thick style and three stigmatic lobes. The male flowers are borne on long stems, has five stamens with united filaments and anthers [11]. Generally the cucurbits are considered to be naturally cross-pollinated. Pistillate flowers occur after staminate ones and remain receptive more than 12 hours, large sticky pollen grain [28]. Fertilization occurs in the early morning according to environmental factors. In each section randomly selected and demarcated twenty male and female flowers diameter, female flower ovary and petal length, anther and pollen weight amount, male and female flower sex ratio, , fruit weight and number, nectar sugar concentration,

Bumble and honey bees management

Bumble bees (*Bombus terrestris* L.) colony housed in a box containing 50-60 worker bees with a queen (mainly imported from Europe) with a small opening which allows the bees to enter and exit, were placed in section nominated for bumble bees pollination. The bumble bees are regarded generally as the most important group of insect pollinators [4], the nest provided with a sugar-water solution, a substitute to the nectar. Honeybee's nuclei containing from 1500 to 2000 bees ideal for pollination in enclosures. [13], bees moved after sunset to avoid losing foraging bees and during flower blooming to provide pollen and nectar, sugar solution and fresh pollen were provided to prevent insufficient requirements in small plot size, in most circumstances honeybees' nuclei containing 3-4 combs of brood, queen and bees. Nuclei's were managed [7]. To ensure that the maximum number of bees visit the crops to be pollinated. Water for bees in each section introduced in stock tank with wooden floats on which they can land, it's important for brood rearing and later for cooling the nuclei. The value of the honeybees as a pollinator is far greater than it is value as a honey producer, for that reason three kinds of methods for pollination were selected. The first plastic house section provided with one honeybees nuclei, the second provided with three honeybees nuclei and the third section provided with bumble bees nest, all of them placed 1 meter above the ground at equidistantly, in a place that is opposite to the sun rise to lead early foraging

activities and to encourage the bees to work over the whole area. The flight holes were open after closing plastic house sections with a fine mesh to prevent honeybees from going outside the plastic house.

Bumble and honey bees monitoring activity

Honey and bumble bee's activity parameters such as squash line foraging, male female visitation rate, pollen loaded and un-loaded bees trip number, monitoring of the two flowers carried out by labeling visited flowers and demarcated plant keeping determined each week during growth period was made through visual observation by counting every 50 min from the start of visitation, for 10 min each hours /section., at the experiment of the three treatment sections during different periods of growing season and flowering period. Data were collected between 5.00 am to 11.00 am. because the observation showed that after 11.00 o'clock fewer bees visited the flowers and each bees spent less time at each flowers.

Anther pollen amount

40 flower anthers in each section used for this evaluation, Male flowers were randomly selected and bagged before anthesis to prevent visits by bees. After anthesis the flowers were unbagged, the anthers of each flower were removed, placed in vials with 70% ethanol was washed with it until all pollen grains were removed [30], and all pollen grains were weighted in milligrams. The total weight of pollen grains in the flowers samples was calculated by estimating the weight of pollen grain per anther and multiplying by the average number of anthers. Also this procedure was based on [29] with some modification; this provided an estimate of total pollen output per flower (using Fisher made in Japan device range 0-32 μ g) also the anther weighted and the average anther weight calculated.

Nectar sugar concentration

Nectar sugar concentration measured in bagged and un-bagged 10 female and 10 male flowers for each section every two hour at 5, 7, 9, 11 am. Twice during the growth period, ten replicates (flowers) were evaluated, five male and five female in each treatment. [31]. The experiments Randomized Complete Block Design (RCBD) with split plot arrangements were used and analysis according to SAS/STAT user's guide [26]. Comparison between means was made using Least Significant Differences (LSD) test at 5% probability level.

RESULTS AND DISCUSSION

Temperature

Temperatures maintained in the various growth stages of the crops, the temperature integrals were recorded weekly within a half hour integral daily, during the growing season **Table (I)**. Honeybee's different nuclei and Bumble bees' maximum flowers visitation increased between 7.00-9.00 am. At morning (Cady 1993) and (Nicodemo *et al.* 2009) were the plastic house temperature range between 16 to 25 C° before the temperature fluctuations increase up to 36 C° whereas foraging activities still limited toward mid-day so all the investigation recorded before this time.

Table1: Plastic house average weekly and daily mean temperature within half hour integrals observed during flowers blooming and bee visitation.

Week Time	1 C°	2 C°	3 C°	4 C°	5 C°	6 C°	7 C°	8 C°	9 C°	Aveg. C°
5 am.	11	10	11	14	8	8	8	9	9	9.8
5.30 am.	13	12	14	16	10	8.5	9	11	10	11.5
6 am.	15	12	16	17	12	9	10	12.5	11	12.7
6.30 am.	16	13	16	18	13.5	10	11	14	12	13.7
7 am.	19	15	17	18.5	14	11.5	11.5	15	16	15.2
7.30 am	22	16	18	18.5	15.5	13.5	15	16	18	17
8 am.	22	18	20	21	19	18	18	18	20	19.3
8.30 am.	24	20	23	23	21	21	19	20.5	22	21.5
9 am.	26	26	25	26	25	24	23.5	23	25	24.8
9.30 am.	28	27	26	26.5	26	29	25.5	24	26	26.4
10 am.	30	27.5	28	27	28	31	26.5	26	29	28.1
10.30 am.	31	28.5	29	28.5	31	33.5	28	28	31	29.8
Average c°	21.4	18.7	20.2	21.2	18.5	18	17	18	19	19.1

Plant parameter

Flowers dimension (Characteristic)

There was a difference among flowers diameter and petal length between the male and female flowers calculated over 30 samples (5 male and 5 female flowers in each section). Male had the widest flower diameter is 13.2 cm; and the longest petal 9.7 cm; whereas the female flowers diameter and petal length

is 12, 8 cm; respectively Table (2). Therefore the male flowers large size lead to the containment of high sugared concentration in nectar Table (3), and the large petals 8 -9.7 cm long with campaniform, provide suitable landing platforms for the bees. The slightly scented, bright yellow flowers seem to be more attractive for bee visitation. Also there was statistical difference among ovary length in opened flowers which range from 5 to 9 cm. recorded from the labeled female flowers which distinguished by the presence of an ovary at the base Table (2).

Table (2). Male and female flowers average dimension recorded over 10 samples from each section

Male and female flowers dimension	Male	Female
Flowers diameter cm.	13.2	12.00
Petal length cm.	9.7	8.0
Flower sex ratio	27.27	16.06
Ovary length range cm.	5-9 cm.	

Yield data

Cucurbit crops are dependent on insect pollinators for fruit set. The absence of these vectors would result in more than 95% fruit production loss in these crops (Klein *et al.*, 2007). For the demarcated plant Fruits were removed when they reached commercial size (12 cm in length) were harvested by hand each 2–3 days, the demarcated plant crops weighted. The number of fruit was also counted. The fruit weight was 68480 g. 42371 g. and 40140 g. The number of fruits was 423, 274, and 282 for the three honeybee's nuclei, one honeybee's nuclei and bumble bees treatment respectively. Table (3), three honeybee's nuclei pollinated fruits obtain the highest fruits weight result analysis indicated that a good correlation existed between the weight and the number of fruits for each treatment, the approximate percentage indicates that the individual fruits are superior, good firmness and quality, presence of pollinators, to maximize fruit size (Walters and Taylor 2006) but the lowest weight obtained from the treatment with bumble bees.

Table (3). Summer squash different demarcated plant parameter in different treatment section

Plant different parameter	Plant parameter type	One honeybees nuclei	Three honeybees nuclei	Bumble bees nest
Marketable fruits weight and number	Number	274	423	282
	Weight (kg.)	42.371	68.480	40.140
Male and female flowers ratio	Male flowers	24.10	30.58	34.10
	Female flowers	11.50	20.08	18.24
Anther and pollen weight	Anther weigh g.	1.648125	1.817075	1.933975
	Pollen weight g.	0.06645	0.0176	0.193225
Un-bagged flowers sugar concentration	Male flowers µg.	40.965	38.412	37.837
	Female flowers µg.	26.547	21.962	29.837
Bagged flowers sugar concentration	Male flowers µg.	35.106	31.188	34.856
	Female flowers µg.	31.612	30.837	27.221

Male female flowers ratio

During the main growing season there were differences among number of female and male flowers per plant which recorded each hour each five day, **Table (3)** the ratio of male to female flowers is usually occupy approximately 2:1 or higher calculated at 21C⁰ average temperature in plastic house (K. Abu-Hammour and D. Wittmann 2010).

Anther pollen amount

Three treatment Pollen average production's data showed in **Table (3)**. The amount of pollen produced did not vary significantly between the three treatments, with a mean production per anther of 0.06645, 0.0176, and 0.193225, for the one honeybees, three honeybees and bumble bee's treatment respectively. Average amount of pollen grain with and without anther found in flowers recorded twice during squash growth period from 40 sample for each section (Fisher made in Japan 0-32), pollen production are important for the honey-producing potential of *C. pepo* by bees, and further investigation will lead to an increased understanding of bee-flower relationship in squash, the amount of pollen grains weight did not differ in the three treatments [30].

Bagged and un-bagged flowers sugar concentration

If we know the quantity and quality sugars percent of the nectar, we can determine the relative attractiveness of the crop to bees [8, 24]. The amount and composition of floral nectar can vary from hour to hour and from day; this parameter was evaluated in the same flowers used for nectar production Table

(4). Nectar secretion is important for attracting bees to determine the effectiveness of visits by three treatments at different hours, 5 male and 5 female flowers for each line in each section were protected before anthesis, and same numbers left Un-protected and the nectar sugar concentration recorded at four different period at 5.00, 7.00, 9.00 and 11.00 am (as sucrose equivalent) each treatment included 20 flowers (replicates) totaling 60 flowers for three treatments, twice per season. Sugar concentration measurement recorded by using a pocket refractometer range concentration 0-50°, this parameter was evaluated the same flowers used for nectar by capillary and a drop of 5 µL was placed in the refractometer. The reading was rapidly performed to avoid alterations due to evaporation, there was a highly significant positive correlation between temperature and nectar sugar content, sugar total daily nectar production was not influenced by removing nectar several times per day, indicating that nectar secretion is not stimulated or inhibited by frequent removal [30], most bee-pollinated flowers, producing 22-40 mg sugar per flower in 6 H. female flowers produced significantly more nectar sugar than did males [18] for the bagged flowers whereas the bees decrease the un-bagged flower nectar concentration

Bees Parameters

Pollination agent honeybees consider the main summer squash pollinator, in this study the bees visitation to summer squash male and female flowers counted regularly and number of bees enter the hives even pollen loaded or un-loaded and the foraging activities around section lines were calculated periodically according the below parameters.

Visitation Rate

Demarcated squash male and female flowers mean visit rate weekly activity in ten minutes each daily hour during growth period [31]. *C. pepo* male and female flowers visitation by different bee's treatment showed significant differences between the three treatment and between the male and female flowers (Table 4) which indicate honeybees visit female flowers more than male flowers whereas the bumblebees has the similar visit for male and male flowers, and the highest flowers visitation %50 represented by the three honeybees nuclei and %38, %12 for the one honeybees and bumble bees respectively. The daily time recorded highest activity start early morning between 7.00 Am.-9.00 am [6] and [22]. The number of visit for each flower was different between daily hours and between male and female flowers, for the harvest of nectar in female and male flowers, so it is necessary to know the required number of visit for adequate fruit set and size, as well as conditions that limit fruit set besides the lack of pollen.

Table (4). Honey bees different activities on *Cucurbita pepo* inside treatment section in plastic house

Bees different parameter	Bees parameter type	One honeybees nuclei	Three honeybees nuclei	Bumble bees nest
Mean visit rate each 10 min. /H. weekly	Male flowers	1.2	2.3	0.5
	Female flowers	1.7	1.7	0.5
Pollen loaded and un- loaded bees hive entrance 10 min. / H weekly	Loaded bees	1.7	2.5	0.2
	Un- loaded bees	1.3	2.9	0.3
Foraging activities along squash line 10 min. /H. weekly	Line 1	2.6	3.7	1
	Line 2	2	3.3	1.1

Number of pollen loaded and un-loaded bees enter hive

Cucurbit flowers during growth season are attractive to pollinators as sources of either pollen or nectar, both male and female flowers produce nectar, which provide carbohydrate to pollinators [9]. Also, offer pollen to bees. Pollen loaded and un-loaded bees entered the hive counted each ten minutes each hour daily hour from 5.00 until 11.00 H. weekly during squash growth period; significant differences were occurs in the treatments and among the pollen loaded and un-loaded bees Table (4). Pollen loaded and un-loaded bees average entrance to hives was 1.7, 2.5 and 0.2 for one honeybees nuclei, three honeybees nuclei and bumblebees respectively, whereas the most loaded bees occur in three treatment and the lowest load occurs in bumble bees treatment, the un-loaded bees enter the hives was 1.3, 2.9 and 0.3 for the one honeybees nuclei, three honeybees nuclei and bumble bees respectively whereas is the unloaded pollen Bumble bees indicate the lowest entrance.

Foraging activities along squash lines

Bees foraging activity along squash section lines were recorded ten minutes each hour at 5.00, 6.00, 7.00, 8.00, 9.00, 10.00, 11, weekly along the squash growth period .Bees foraging activates along section lines in each treatment show no deference, but show high differences between section treatment, The total

number of bees per two section lines was 4.6, 6.9 and 2.1 for one honeybees nuclei, three honeybees nuclei and bumble bees respectively. Table (4) which indicate that the honeybees visit section lines flowers in three honeybees treatment higher than the bees in one honeybees treatment whereas the lowest visit recorded in bumble bees nest, the bees spent more time collecting nectar in the male flowers which produce more nectar than the female flowers [22].

CONCLUSION

This Study lay out an overview of cucurbit pollination. It describes the importance of bees in cucurbits plant and provides aspects of cucurbit floral biology and pollination requirements intends to give cucurbit growers, beekeepers, and scientists the best recommendations on how to choose pollinators and how to apply them to optimize cucurbit production in plastic house. Moreover, it is necessary to understand cucurbit plant floral biology as well as the pollinator's biology and behaviors to develop suitable management strategies in order to maximize fruit set. The results of this work demonstrated that summer squash flowers produce abundant nectar and pollen, which seem to be available in quantities sufficient to sustain pollinating colonies in plastic house production. Activity of bees visit was at peak in between morning (7.00 until 9. 00 am), while the lowest activities were recorded at mid-day when the plastic house temperature increased towards 12.00 o'clock. During this time of the experiment it was showed the importance of using honeybees as a pollinator in summer squash. The results indicated that three bee nuclei could be used successfully in plastic house for summer squash pollination. The three bee nuclei treatment gave highest yield, higher number of fruits than other treatments. *Cucurbita pepo* has sufficient pollen and nectar in their attractive yellow flowers enhance to managed pollination program with low cost. However, honey bee use is usually far more economical since *Apis mellifera* nuclei contain several thousand more foragers than bumble bee's species. Intensive honeybee's pollination is essential in modern agricultural setting for high yields of fruits, nuts, and seed for the most economically crop plant. It can be recommended that the use of three honey bees containing 1000 to 2000 bees as a pollinator to increase yield and improve quality of summer squash inside the plastic house. Pollination research can highlight the need for pollination management of certain crops with unsuitable yield, and can help growers to select appropriate pollinating species and management options and to utilize their resources effectively.

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