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THE ROLE OF BROWN MUSTARD *BRASSICA JUNCEA L.* CZERN AND BORAGE *BORAGO OFFICINALIS* L. IN SUPPORTING HONEY BEES *APIS MELLIFERA* L. WITH POLLEN GRAINS AND NECTAR THRONGH THE WINTER CRITICAL PERIOD WITHIN BASRAH ENVIRONMENTAL CONDITIONS

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Abstract

This study was conducted in a private farm 20 km NE of Basrah on the Shatt al-Arab river during winter 2018-2019 where two annual herbaceous plants, brown mustard *Brassica juncea* (L.) Czern (Brassicaceae) and Borage *Borago officinalis* L. (Boraginaceae) were grown to indicate their role in supporting the foraging honey bees with pollen and nectar during the period where sources of these supplements are scarce. It was clear that there were no significant differences in the foraging rates or number of visits to flowers of both plants by honey bees. The environmental conditions had a considerable role in this respect, that correlations showed that foraging rate of bees was affected by temperature and relative humidity. The results also showed that foraging speed of honey bees on flowers of both species was nearly equal. Mean number of pollen grains / anther (1412500) and average pollen load of one bee (541500) of borage had significant difference with that of brown mustard (671100 grain / anther and 152820 grains) respectively. There is also a considerable variation in the pollen morphology of the two species including shape, size and sculpture. The current study recommended cultivation of the two plants for nectar and pollen supply.

Key words: Brown Mustard, *Brassica juncea* L., Borage, *Borago officinalis* L., Honey Bees.

Introduction

Man attention to honey bees is ancient, where he take care of bees mainly for its honey and wax. Recently this attention developed to include other tools like pollen, royal jelly, bee poison and propolis etc. Bees role in pollinating agricultural crops may equal to the effect of fertilizers or supplementary irrigation (Haddad *et al*., 2010). As living beings bees need carbohydrates, proteins, lipids, minerals and water for growth and reproduction which are collected as water, nectar and pollen. Nectar is collected from floral nectaries which is rich with carbohydrates providing bees with energy. Pollen is the source of protein, lipids, vitamins and minerals. Colony consumes some of the collected nectar and pollen to maintain its different necessities and store excess in the hexagonal eyes to be used when needed (Herbert, 1993). Honey bees have an important role in pollination that is essential to increase fruits and seeds production in fruiting trees, field crops, aromatic herbs as well as wild plants. The distinctive behavior of honey bees in searching for and foraging on opening flowers of plants increasing production to 37% or sometimes 80-100% (Clarence *et al*., 2004; Glaiim, 2007). Cultivation of plants rich with nectar and pollen such as borage and brown mustard helps honey bees to continue their activity. Borage (*Borago officinalis* L.) of the family Boraginaceae is an annaul herbaceous and hairy plant, height 70-100 cm. Stem branched. Leaves simple, alternate. Flowers are often blue or violet, pentamerous, hypogynous. Plant can grow well in wet soil with good drainage and pH of 4.5-8.2 and can be cultivated in early spring, autumn and late winter. Borage may originated from West Meditterranean and North Africa and cultivated in wide range area of Asia inclnding Arab countries. Plant is highly intra-plant pollinated (geitonogamy) and cultivated for different purposes, for its seed oil, medicinal uses or foraging honey

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bees, to support colonies especially that it flowers in the critical winter period where pollen and nectar sources are nearly lacking. Brown mustard *Brassica juncea* (L.) Czern (Brassicaceae) is an annual herb with alternate leaves. lnflorescence racemose, flowers hermaphordite (bisexual), actinomorphic, hypogynous. Sepals 4, petats 4, free, cruciform. Stamens 6, tatradynamous. Four nectaries located around the base of filaments. Ovary of 2 united carples, bilocular. Style solitary, stigma capitates to bilobed. Fruit siliqua (Townsend and Guest, 1980). Brown mustard is self-compatable or self incompatable crop in various regions and countries, depending on environmental conditions (Stewart 2000; Devi *et al*., 2007). It is a good source of pollen and nectar and attractive to pollinating insects as well as it is drouglt resistant. Many species in the family Brassicaceae are among the important sources of pollen and nectar such as rape (*B.napus*), black mustard (*B.nigra*), white mustard (*Sinapis alba*) and rocket *Eruca sativa*. Due to the poverty in sources of honey bees nutrition represented by nectarine and pollen rich plants during the criticle periods of winter in Basrah, two crops brown mustard and borage were grown which importance was determind by their success to tolerate local environmental conditions in the previous season, as well as the rarity in publications about honey bee breading and sustenance which have great importance to increase bees consistency, this research is suggested.

Materials and Methods

Study site

This study is conducted in a private farm on Shatt al-Arab River, 20 km NE of Basrah where an ideal apiary was constructed in area of 12×12 m and a height of 3 m. Three sides were externally surrounded with reed shoots acting as a heat insulator while internally lined with jute fabric (gunny). The ground was leveled where 30 homogenized bee hives in terms of strength were located.

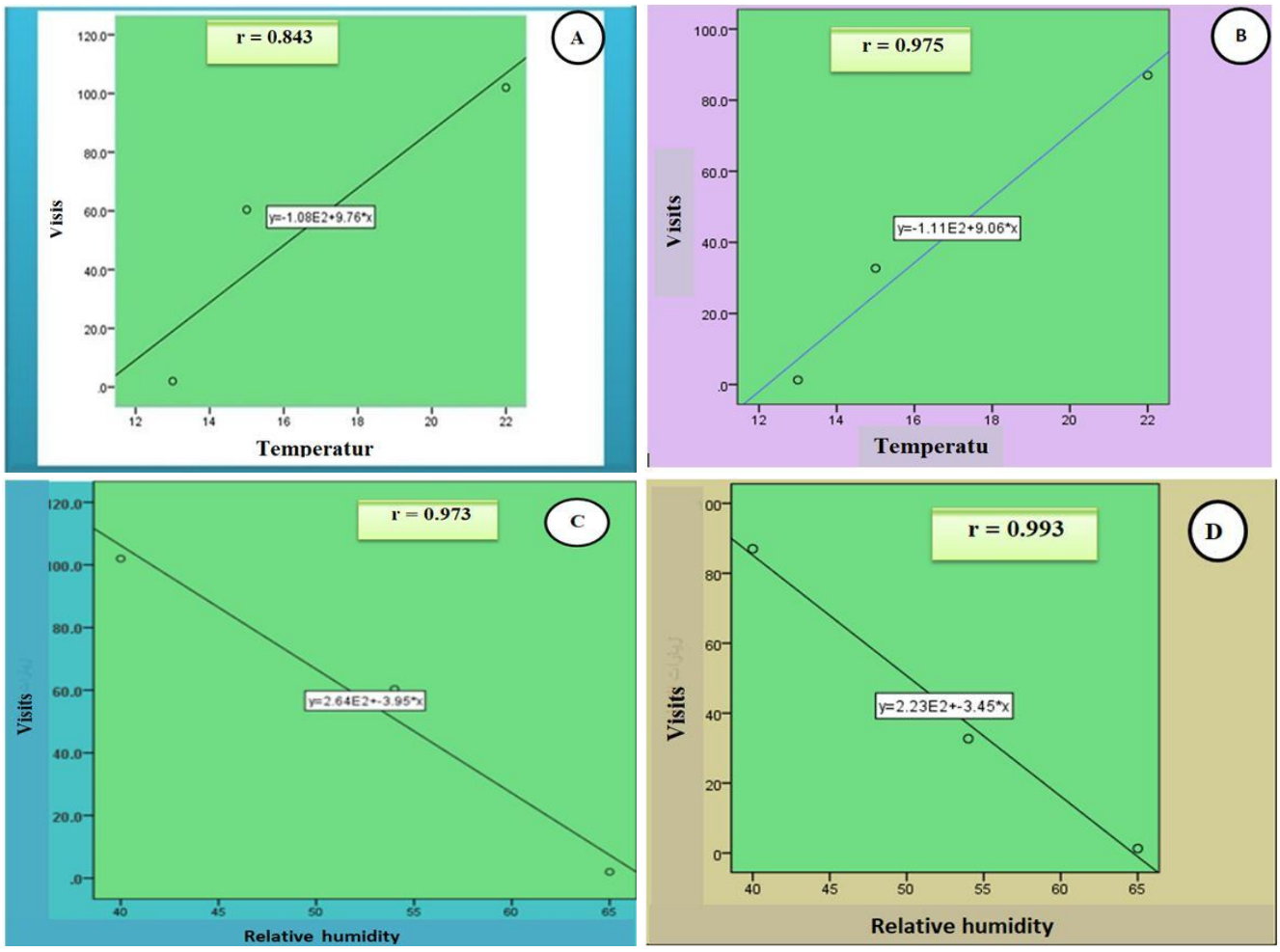

Fig. 1: Correlations between honey bee visitings to borage, brown mustard flowers and environmental conditions.
A. Correlations of temperature and honey bee visitings to borage flowers.
B. Correlations of temperature and honey bee visitings to brown mustard flowers.
C. Correlations of humidity and honey bee visitings to borage flowers.
D. Correlations of humidity and honey bee visitings to brown mustard flowers.

Borage (*Borago officinalis*) and brown mustard (*Brassica juncea*) plants were cultivated 100 m apart from apiary. Land was ploughed, smoothed and incorporated with farm yard manure then 30 rows of 15m long were prepared for sowing borage and brown mustard seeds. Row-row spacing was 30 cm while plant plant spacing was 30 cm for brown mustard and 50 cm for borage. Dropping irrigation was applied.

Bee foraging on plant flowers

In order to recognize bees virility their visits number was recoded three times aday, 8-10 am, 12-2 pm and 4-6 pm, on certain flowers through the whole flowering period (about 45 days), with 3 days intervals between records.

Foraging speed (Sec.)

Speed of foraging was visually observed starting from 9:00 am to 12:00 at noon with naked eye and stop-watch. Foraging speed in seconds was recorded. Speed was recorded when bee become steady on the flower with 10 replicate in locations with high blooming (Kearns and Inouye, 1993).

Foraging rate (Min.)

This experiment was carried out in the field between 9:00 am and 12:00 at noon by naked eye and using manual counter and stop watch to calculate number of flowers visited by bee workers in the main field in each minute with 10 replictes per bee taking in consideration selecting the period of full blooming. Observations were recorded in a half circle of 3m diameter oppositing sun rays taking calm position to avoid any movement stimulating bee workers. lf bees disappear before the end of minute or depart from the front half circle, counting is neglected (Kearns and Inouye, 1993).

Quantity of pollen held by honey bees

Amount of pollen grains held by a single bee was counted according to Fhaid, (1988) and Kraidy, (2016) with some modifications where 5 bees were randomly collected after 5 days of blooming, its load of polled basket was discharged with small needles on a glass slide. 30 ml glass vials were filled each with 10 ml of distilled water. Each bee was located inside a vial and shaken well to extract pollen suspension. The bee was caught with tweezers and washed with 5 ml distilled water. 0.1 ml of the 15 ml of pollen suspension collected was laid on a glass slide, the slide was then placed on a hot plate to 40°C till dryness. A drop of fuchin glycerin gel was mounted on a cover slide cross shaped and set down gradually on the slide. The slides were examined under the high power of light microscope. Number of pollen counted was multiplied by 150 to get the real number of grains in 15 ml of pollen suspension.

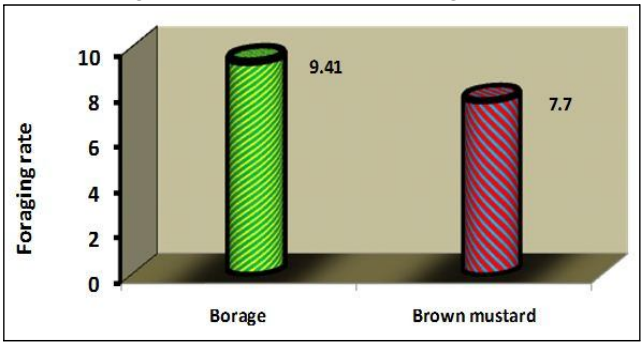

Fig. 2: Foraging rate of honey bee on flowers of borage and brown mustard/ second.

Quantity of pollen / anther

Number of anthers were set down in 2 ml centrifugal tubes, dried in an oven at 25°C for 24 hours. Tubes were vibrated for craking the anthers to set pollen free from the pollen sac. 1 ml of distilled water was added to the tube and shaken well for 5 minutes to prepare water pollen suspension. One drop of the pollen suspension was placed on haemocytometer. Number of pollen per anther was evaluated according to the equation cited in Gupta *et al*., (2017).

$$\text{The quantity of pollen / one anther} = (2 \times \frac{n}{10^{-4}}) \times a$$

Where 2 = 2 ml of pollen suspension

n = average number of pollen in the large square

10^{-4} = volume of the large square

a = number of anthers.

Pollen morphology

Pollen morphology of the two cultivated plants was evaluated, where slides for pollen were prepared and examined under high power of Zeiss Primostar Microscope with graded eye piece. Pollen morphology included shape, size, number, design of apertures and sculpture. Pollen were described according to Erdtman, (1952) and Hesse *et al*., (2009). Data are summarized in table 2 and plate 2.

Statistical analysis

Data were analysed by Statistical Analysis Programm SPSS, Version 23 following complete randomized design. Correlations were analysed by the same programm.

Results and Discussion

Vitality of workers foraging on plant flowers:

Statistical analysis in table 1A and 1B illustrates significant differences in visits number of bees during the study period where March had the highest mean number (94.83) through 5 minutes, while January had the lowest mean number (1.66). The same table shows

Table 1A: Visiting honey bee to plants flowers in five minutes.

Month	Time / Plant	8 - 10	12 -2	4 - 6	Average Month and plants	Average time and plants	Average Month
January	Borage	0	3.6	2.4	2	55.33	1.66
	Brown mustard	0	2	2	1.33	72	
February	Borage	64	59.8	57.6	60.46	60.27	46.6
	Brown mustard	36.6	37.2	24.4	32.73	33.53	
March	Borage	102	119.2	86.8	102.66	48.93	94.83
	Brown mustard	179.4	61.4	20.2	87	15.53	
Average Time		63.6	47.2	32.23	47.7		
Average Plants			Borage = 55.04			Brown mustard = 40.35	
L.S.D to plants = 19.4 L.S.D to month and plants = 3.3 L.S.D time and plants = 33.62 L.S.D to all factors = 5.77							

significant differences in the number of visits along day times were early morning visits were 63.6, decreased at noon to 47.2 and 32.23 at afternoon. Borago and brown mustard shows no significant variation in bee visits, but the variation was in relation to a period of time and plant species where it was 102.66 and 87 respectively January recorded the least number of bee visits to both plants arrived at 2 and 1.33 respectively. Bee vigor depends on several factors, bee itself, field and environmental factors that are bees intensity, health of the hive if it is infected with pests or not, bee if it is old or young, household or foraging. Field if it is rich in nectar and pollen resources as well as environmental factors, including temperature and relative humidity. Bees vigor stops near 8°C while there is appreciable activity at 8-16°C, while optimum is between 16-32°C (Kanr and Kumar, 2013). In Basrah city the mean temperature of December and January is about 12.5°C, but it is less than 8°C at early morning most days of these months (Al-Mayah *et al*., 2016). Due to the decrease of temperature in winter, especially in the early morning, foraging is nearly missing. The maximum vigor of bees is in March where the temperature is nearly optimum (Fig. 1). Number of visits is positively correlated with temperature where R of Borago is 0.843 and 0.975 respectively (Fig. 1, A,B). Relative humidity has a highly negative effect on bee visits to both plants where R is - 993 and -973 respectively, although there is no direct effect of this factor on honey be action, including searching for food (Joshi and Joshi, 2010). The integration between temperature and relative humidity is important for anther dehiscence and availability of pollen for visiting bees (Abou shaara, 2012). The optimum conditions for pollen release is 20°C or more and 70% relative humidity or less. Low temperature and high relative humidity have multiple effect on decreasing bee vitality, anthers dehiscence and release of pollen. In general the two plant species were attractive to honey bees with nonsignificant difference between them except distinct periods of the day especially in early morning hours (Fhaid, 1988). Both species provide bees with pollen and nectar. Prefering any plant by bees depends on the quantity of pollen and nectar. Borage is rich with these nutrients, that its flowers are star-shaped clustered in a blue or violet inflorescence. Flower diameter is 2.2 cm, dangle to permit bees hanging down with the flower to suck honey and collect pollen easily. So bees spend more time in foraging to collect the maximum food as possible. Borage is composed of many inflorescences. Sometimes reaching about 250, each of nearly 20 flower or about 5000 flower/plant (Kadhim, 1986). Brown mustard flowers are smaller, cruciform, aggregated in a bright yellow racemose inflorescence. Diameter of the flower is about 1.3 cm.

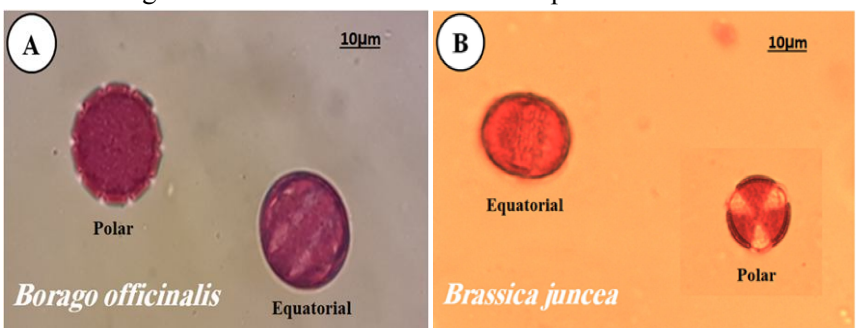

Plate 1: Shape and dimentions of pollen grains in the studied plants.

Table 1B: Visiting honey bee to plants flowers in five minutes.

Time \ Month	January	February	March	Average Time
8 - 10	0	50.3	140.7	63.6
12 - 2	2.8	48.5	90.3	47.2
4 - 6	2.2	41	53.5	32.23
Average month	1.66	46.6	94.83	
L.S.D. Time = 11.68, L.S.D of months = 2.32, L.S.D. of Time and months = 20.2				

Flowers are vertical with peduncle and have landing platform to bees and other pollinating insects to facilitate honey and pollen collection. These small flower cause less foraging speed and more foraging rate due to the relative shortage in pollen per flower, which was 671100 pollen / anther in comparison with 1412500 pollen / anther in borage (Fig. 4), or due to competition between honey bees and other pollinating insects forcing bees to visit flowers within short time (Al-Amery, 2009).

Table 1B, demonstrates effect of study period on visits number through day time where signifcant difference was recorded. The highest mean was during early hours of March days reaching 140.7 visits, while no visits were recorded in the early hours of January days, because of the high decrease in temperature which leads to stop bees vigour and foraging. Younis, (2009) stated that vitality set back at 8°C or less and 38°C or more.

Foraging rate (flowers / second)

Fig. 2, shows that there is slight and non-significant difference in foraging rate on both plants. Differenes may be related to amount and concentration of nectar. Mitchell and Waser, (1992) emphasized that flowers rich in nectar are visited successively by bees. Whenever sugar concentration is high in nectar, foraging will be high, when concentration is low flowers become unattractive to the extent that bees stop visits.

Foraging speed

Fig. 3, shows that there is no significant differences in the foraging speed of bees on the two plant species, but there is a simple difference in attraction factors of

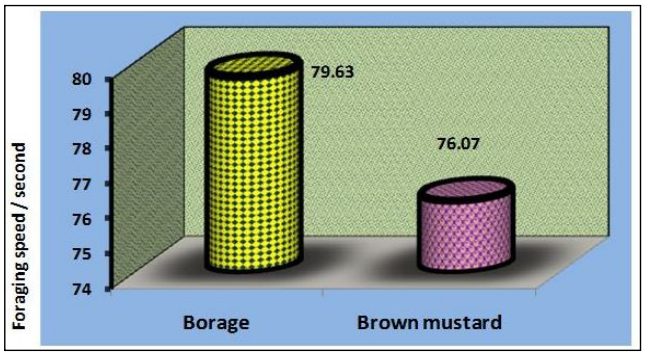

Fig. 3: Foraging speed of honey bees on flowers /second.

Table 2: Pollen morphology of borage and brown mustard.

Parameter	Borage	Brown mustard
Size	Medium	Medium
Dimensions µm	21.9 (26.**50**)36	26(33.46) 52
Shape	Spheroidal	Oblate spheroidal
Aperture	10 – Colporate	Tricolpate
Ornamentation	Microgemmate	Microreticulate
Exine thickness µm	7.36	4.12

the flowers including pollen and nectar Stout, (1994) and Suzuki, (2000) confirm that size, structure of flower, size, morphological structure of the insect including length of proboscis and head breadth of the insect have an important role in the foraging speed, that large insects are incapable to enter inside the flower as in borage, so foraging rate is low. This forced the insect to visit more flowers.

Quantity of pollen / anther

Fig. 4, shows that the average number of pollen in borage is 1412500 while brown mustard had 671100 grain / anther. This quantity is genetically constant in all species. The size and shape of pollen grains attribute number of pollen in the anther. When grains are large and irrigular, anther can carry only small quantity. The size of 26-50 µm and subspheroidal shape of grain of both borage and brown mustard let the anther to carry large number of pollen. The quantity is not equal in the two species. This may partly owing to anther size in borage which contain pollen more than two times greater than brown mustard. Both species are highly cross pollinated depending on the quantity of pollen for successful pollination and production of seeds (Table 2 and Plate 2).

Honey bees load of pollen

Fig. 5, explains the quantity of pollen of borage and brown mustard held by honey bee. There is a significant difference in this quantity that the load of borage is 541500 pollen grains in comparison with brown mustard (152850 pollen grain). This may depened on flower size in borage which is larger (2.2 cm) than brown mustard (1.3 cm) (Plate 1, A,B), in addition to shape and size of anthers. Data shows that the anther shape of borage is triangular of 1 cm long while anther of brown mustard is spherical

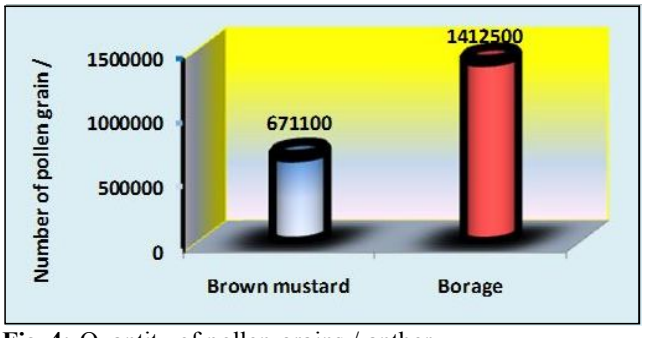

Fig. 4: Quantity of pollen grains / anther.

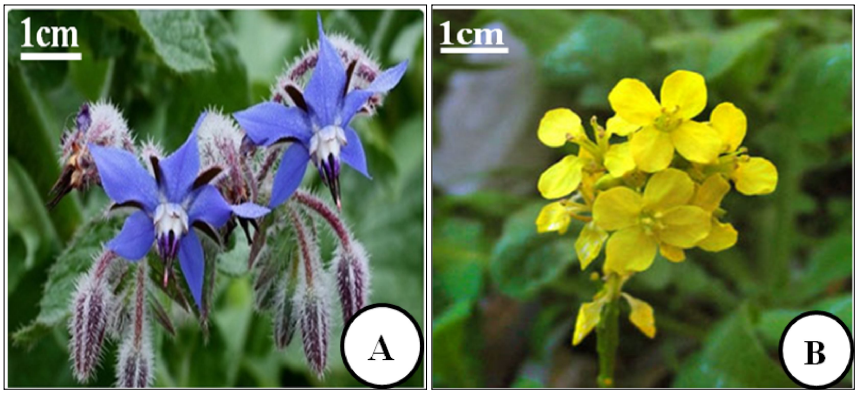

Plate 2: Flowers of borage (A) and brown mustard (B).

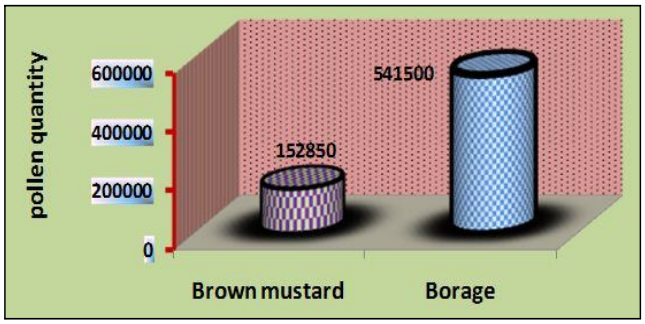

Fig. 5: Quantity of pollen grains / bee.

with diameter less than 0.3 cm. So content of borage anthers of pollen is much higher than of brown mustard. Ultimately the two plant species are recommended to be cultivated for their richness in pollen and nectar, their attractive properties to honey bees, their highly cross-pollination and blooming within the winter critical period especially that flowering is the most sensitive phase in plant for temperature stress and climate change.

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