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BEE HONEY AND MEDICINAL PLANTS IMPACT IN LEARNING AND MEMORY OF HONEY BEE WORKER *APIS MELLIFERA* L.

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ABSTRACT

This study has been conducted in the Faculty of Agriculture - University of Kufa. The main objective of the current study is to see the effect of some natural products (plant extracts and honey) which are commonly used in the folk medicine in Iraq for their impact on the memory. We tested Arabic gum, rosemary, coriander and different types of honey on the learning and memory of honey bees. The results of the current study showed that the natural products that have been used had an impact on the learning and memory of workers honey bees compared to 1M sucrose as a control. The highest rate of learning was from multiflower honey followed by the coriander extract, then Eucalyptus honey, where they reached 58.8, 57.6 and 56 % respectively, while it reached 51.2, 48.4, 46.8% for the Arabic gum, desert honey and the aqueous extract of rosemary leaves respectively compared to 30% in control. The results also showed that the natural products had an impact on the nervous system of honey bee workers by strengthening their memory. The results gave better responsiveness 12 hours post treatment compared with their response after 24 hours. These products had an impact on the memory of honey bee workers, where it was found that the treatment consisted of Eucalyptus honey gave the highest percentage of memory with 68 % compared with the 18% for the control. The rest of the treatments which are composed of multiflower honey, coriander plant extract, desert honey, Arabic gum and rosemary, were as follows: 65,61,58,55 and 53% respectively.
Keywords: Honeybee, plant extracts, learning and memory, honey.

Introduction

Honey bees and other pollinators are responsible for pollinating about 35% of global agricultural land, including 87 of the leading food crops worldwide (FAO., 2018). The total economic value of the 46 insect-pollinated direct crops (mostly are stimulant crops, nuts, fruits, edible oil crops, and vegetables) was €625 billion in 2005, that is 39% of the world production value (Gallai *et al.*, 2009). In addition to their widespread use as pollinators, honey bees have long held the interest of researchers for their social behavior and society complexity (Toth and Robinson, 2007). Honey bees also act as a model for exploring genetics as their genome shows greater similarities to vertebrate genomes than other well-known insects such as *Drosophila* and *Anopheles* (Weinstock *et al.*, 2006). Also, researchers use honey bees as a model for social evolution, and memory and learning development in invertebrates (Fahrbach and Robinson, 1995).

The division of labor and changing tasks from work inside the cell and shifting to work outside is affected by internal factors such as lack of food stored, as well as the small number of house bees(Huang and Robinson, 1996). This shifting might be related to the shift changes of mRNA amount in the brain, where the gene expression increases in the brain of foraging bees compared to nurse bees (Whitfield *et al.*, 2003). Also, some researchers noticed that the foraging behavior has related with increasing in the levels of certain gene called Amfor (Heylen *et al.*, 2008), JH hormone (Huang *et al.*, 1994), and Octopamine concentration (Barron *et al.*, 2002). In addition, Kather *et al.* (2011) found that the foragers had a high level of n-alkane compared to house bees.

Learning is defined as the acquisition of information and skills, while subsequent retention of that information is called memory. Learning flower cues and flower location in fields is the most important factor in foraging and honeybees acquire skills for effective foraging by two means: exploratory experience from foraging and the information resulted from the dance performance of recruiting bees (Von Frisch, 1967; Menzel and Müller, 1996). Honey bees are considered as active insects, as they rely on the scent of flowers in determining of their food sources in the first trip, then they distinguish the smell of pollen and nectar in order to be able to return to the food sources. This information is kept in the olfactory memories for several days (Beekman, 2005). Long-term memory in bees can last for several months, as in the winter bees or about 2 weeks as in summer bees (Menzel and Müller, 1996).

Many studies during the past years have focused on the therapeutic effects of herbs on the learning and memory (Myhrer, 2003). We have used in our experiments some natural products, including Arabic gum, rosemary, coriander, as well as some types of honey (Eucalyptus, multiflower and desert). Arabic gum is used to increase the ability to focus and improve the memory (Mahboubi *et al.*, 2016). The improvement in memory when using rosemary may be due to an inhibition of the Acetylcholinesterase in the brain. In addition, rosemary has an antioxidant property that may also be responsible for affecting cognitive function and anxiety (Miraj, 2016). some studies showed that the coriander has negatively affected on the memory and learning of mice after 1 hour, but it positively affected after 24 hours and 7 days (Zargar-Nattaj *et al.*, 2011). In this regards, some studies have proven the positive effect of honey on memory and learning (Othman *et al.*, 2015).

The aim of this study is to determine the biological effect of some medicinal herbs and honey on the honeybee learning and memory.

Materials and Methods

1. Preparation of aqueous extracts of plants

The plant extracts was prepared by taking 10 gm plant in a volumetric flask of 250 ml and 100 ml of distilled water was added to it and then placed in a water bath at a temperature of 50 ^{o}C for rosemary and coriander, or 100 ^{o}C for Arabic gum for 30 minutes. Then the mixture was filtered through a piece of gauze, the filter was taken, and it was centrifuged in a cooled centrifuge (HERMLE, Labortechnik GmbH) at 3000 rpm for 15 minutes. After which the filtrate was taken (Amin and Hamza, 2005; Asad and Alhomoud, 2016).

2. Proboscis Extension Reflex (PER)

In this experiment, we used foraging bees by collecting them from the hive entry (Figur.1). We had seven treatments: control 1M sucrose, Arabic gum aqueous extract, rosemary leaf aqueous extract, coriander seed aqueous extract, Eucalyptus honey, multiflower honey, and desert honey. All applications were used by dissolving 1 ml of treatment in 25 ml of 1M sucrose. Individuals from all treatment groups were tested at the same time.

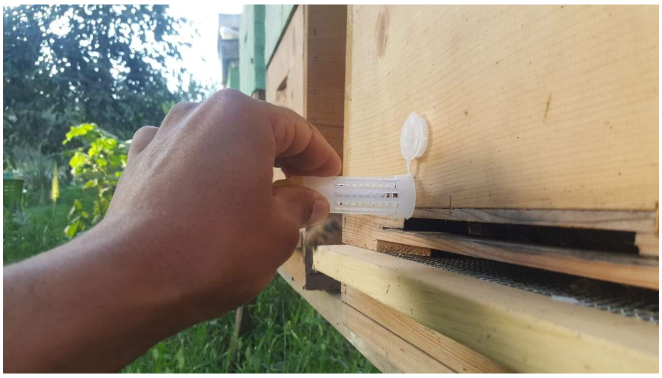

Fig. 1 : Collecting foragers when they return to the hive

A 10 bees were used for each treatment and they were placed under freezing until immobile (Mustard *et al.*, 2012). Once immobile, they then safely restrained in plastic insulin syringe (U-100 syringe, with the orange cap, copyright device, figure 2), leaving only their heads free. Conditioning and extinction experiments described below occurred between 1 and 10 April, 2020. Treatment delivery occurred to immobilized, restrained honey bees in the morning; bees that did not feed were not retained for experiments. After 2 h starvation, honey bees were fed 1M sucrose solution using a wooden toothpick and left for ~15m before PER trials (Guo *et al.*, 2017). 10 ml syringe was used with a piece of filter paper was placed in it, which was saturated with one of the treatments. PER started when we offered the treatments odors (olfactory stimulation) as a conditioned stimulus CS for 6 seconds on the bee antennae and when responding was occurred, we had given a drop of sugar solution as a reward or an unconditioned stimulus US. This process was repeated 5 times in a row to confirm the learning experiment. After that, bees were kept at room temperature waiting the memory experiment. The memory experiment was done by recording the proboscis extension responses to the treatment odors (CS) only without reward (US), this has been done after 12 and 24 hours (Hammer and Menzel, 1995) (Figure 2).

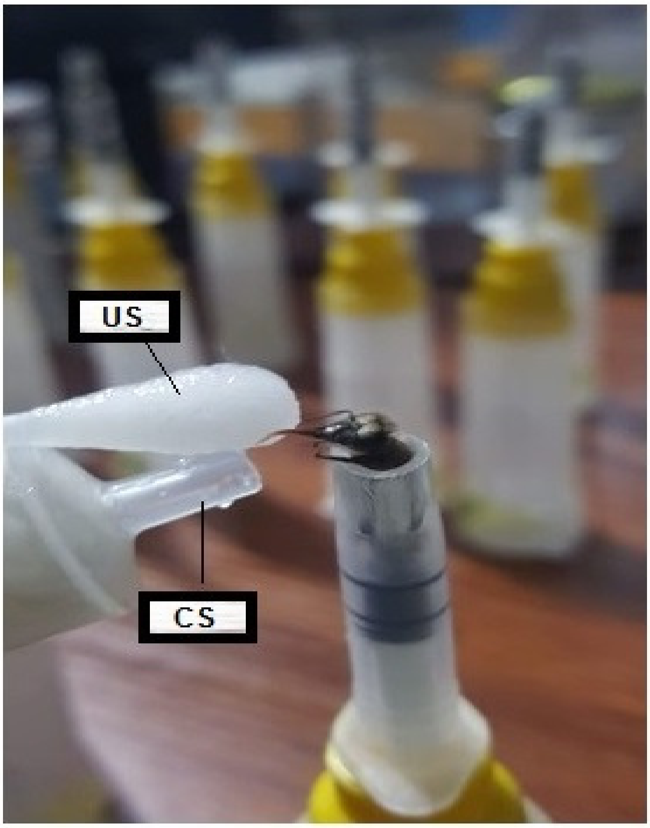

Fig. 1 : Overall view of the setup used for olfactory conditioning. A honeybee was placed in a holder. Olfactory stimulation provided by odors (CS) was applied to the antennae, whereas the proboscis was rewarded with 1M sucrose solution (US). Thus, the bee was trained to associate the CS with the US.

Statistical analysis

Repeated-measurement analysis of variance ANOVA was used among groups as well as within-group comparisons. LSD with post hoc comparisons was used. The α-level was set to 0.05 (two-tailed) for all analyses. For all the experiments, the responses of subjects or PER were scored as binary variables. JMP Pro 14 software was used for data analysis.

Results and Discussion

1. The effect of plant extracts and honey types on the honey bee learning.

This experiment was conducted in the laboratory to test the effect of some natural products for their effect on learning behavior. The results of the current study (Figure 3) showed that there is a significant effect between the treatments used in this experiment to measure the level and degree of learning in honey bee foragers compared to control (p = 0.0 076, $F_{6,349}$ = 2.9737). As shown in the Figure (3), bees in the multi-flower honey had the highest rate of learning (58.8%), followed by the aqueous extract of coriander seeds then the Eucalyptus honey (57.6 and 56%) respectively. While, the following treatments came after, Arabic gum, desert honey, and rosemary extract (51.2 and 48.4 and 46.8% respectively) compared to the control treatment represented by 1M sucrose which gave the lowest learning rate (30%).

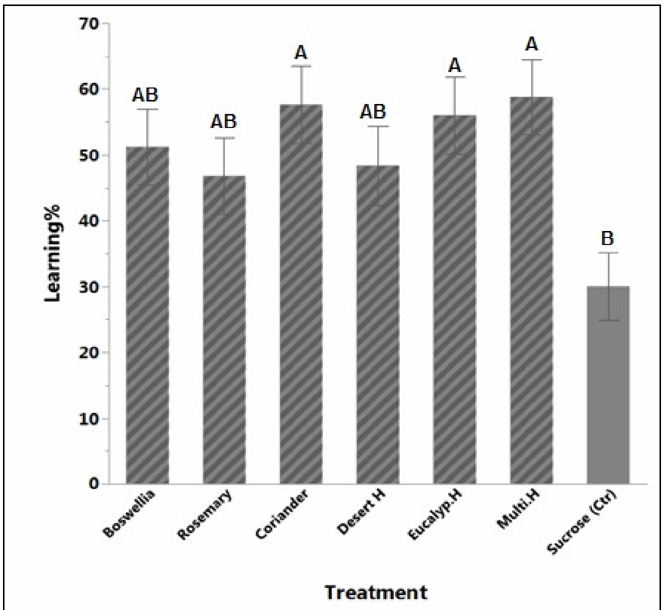

Fig. 2 : Learning behaviour of foraging honey bees *A. mellifera* Fed different plant extracts and honey compared to control (1M sucrose. columns represent average values. Columns containing the same letters do not differ significantly with each other by probability p = 0.05, N=60.

The results shown in figure (3) shows that the learning behavior was on the top when admitting multi flower honey, Eucalyptus honey and coriander extracts compared to 1M sucrose. The reason for these responses may be attributed to the occurrence of behavioral changes associated with the olfactory perception and memory in the brain (Fernandez *et al.*, 2009; Locatelli *et al.*, 2013). It is worth noting that some previous studies confirmed that Arabic gum had an effect on the learning and memory of animals, where *Boswellia serrata* gum resin increased the spatial learning and the dendritic tree of dentate gyrus granule cells in aged rats (Hosseini-Sharifabad *et al.*, 2016). Other studies have shown that rosemary *R. officinalis* improved memory by inhibiting the action of acetylcholinesterase in the brain. In addition, rosemary may act as an antioxidant, which may be responsible for affecting cognitive function (Miraj, 2016) .Some studies have also shown that exposure to coriander volatile oil had a positive effects on spatial memory formation of rats (Cioanca *et al.*, 2013).

2. The effect of time on honey bee memory

The results of the current study (figure 4) showed that the time had a significant effect on the memory of honey bee workers ($p < 0.001$, $F_{2,419}$ = 10.0734), where the memory was better after12 h compared to 24 h, where its average was 70 and 50 % respectively. The results confirmed that the effect of the natural products under study was higher in the short-term memory, and the more time, the lower their response to the smell of the natural substance. Some studies found that a single learning trial resulted in time-dependent processes, leading to high memory just after the trial, low memory within 2-4 min later, very low memory within the next 10-15 min, and vanishing memory retention over several days (Menzel, 1968; Erber, 1975b; 1975a). The performance of honey bee workers may decrease after long periods of exposure to stimuli due to the deterioration of memory and learning which makes the results at a lower rate (Smith and Burden, 2014). Another study has confirmed that the memory of honey bees has decreased within the time when they directly exposure to gas diesel exhaust (Reitmayer *et al.*, 2019).

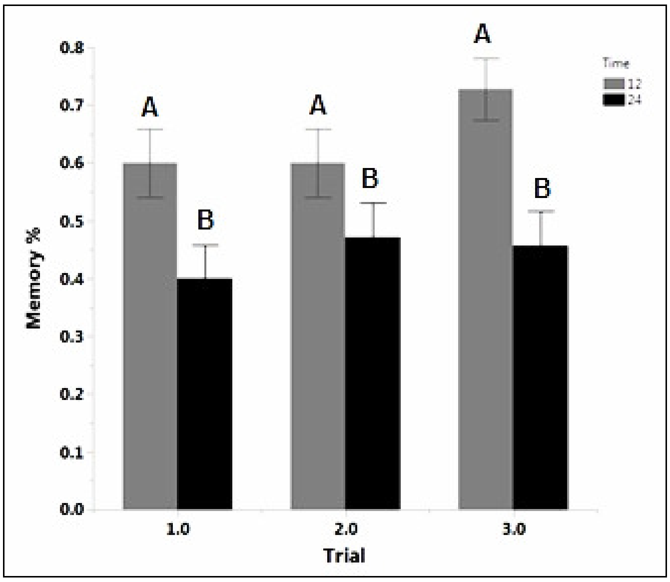

Fig. 3 : The memory of the honey bee *A. mellifera* fed on different plant products and different honeys after 12 and 24 hours. Columns represent average (N= 60). Columns containing the same letters do not differ significantly with each other by probability (p = 0.05).

3.The effect of natural products on the honey bee memory

Figure (5) shows that the natural products used in this experiment had a significant effect on the memory of the honey bee workers compared to control (p = 0.003, $F_{6,209}$ = 3.38). From the figure (5) we can see that the Eucalyptus honey gave the highest percentage of memory (68%) compared to 18% in the control (1M sucrose). While it reached 65, 61, 58, 55, 53% in multiflower honey, coriander extract, desert honey, Arabic gum extract and rosemary extract respectively. We can conclude that honey in general has had a greater effect on the bee memory and this agree with the study of Chepulis *et al.* (2009) who found that honey can improve memory loss and cognitive decline associated with aging in rats. They attributed the reason of the memory improvement to the sugar content of honey, so they used sucrose as a control. In another study by Akanmu *et al.* (2011) they concluded that honey improved spatial working memory and possesses anxiolytic, antinociceptive, anticonvulsant, and antidepressant effects in mice. Also, honey might improve the special memory area in the brains of bees, or the harmful oxidative stress to the brain has been reduced by breaking down acetylcholinesterase (Othman *et al.*, 2015) . Regarding the plant extracts effect on the memory, some studies have found that coriander leaves extract at 5, 10 and 15% w/w of diet enhanced the memory of young and aged mice (Mani *et al.*, 2011). In another study, the mice treated with Arabic gum *B. papyrifera* at 50, 100 and 150 mg/kg showed a significant decrease (P< 0.01) in time taken to find food by the learned mice in radial arm maze (Farshchi *et al.*, 2010). Finally, a study has found that rosemary extract (40% carnosic acid) may improve the memory score and oxidative stress activity in rats at a concentration of 100mg/kg (Rasoolijazi *et al.*, 2015).

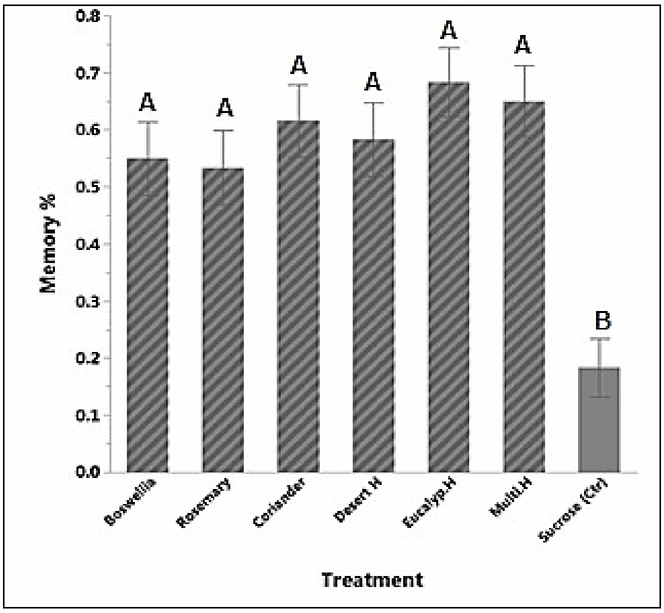

Fig. 4 : Memory of honey bee *A. mellifera* fed different plant extracts and honey compared to sucrose as a control. Columns represent average (N = 60). Columns containing the same letters do not differ significantly with each other by probability (p = 0.05).

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