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Effect of Some Substances as Therapy Treatments On The Hygienic Behavior of Honey Bee Worker, *Apis mellifera* L.

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Abstract

Hygienic behavior of honeybee workers involves inspection, uncapping and removal of diseased and dead brood from the colony cells. The present study was conducted to clarify the effect of three compounds as therapy treatments against certain disease and pests on honey bee workers hygienic behavior as percentage in the presence of pin-killed brood. Colonies were more efficient in the removal activity of dead brood, 24 hours after application. The removal activity of dead brood was significantly higher after treatment as compared with that recoded before treatment, being 5.00, 3.67 and 4.33 % after using marjoram oil, oxalic acid and Oxytetracycline (Terramycin), respectively. The corresponding percentages before treatment were 17.33, 14.00, and 13.67 % of brood, respectively.

Keywords: Hygienic behavior, honey bee workers, dead brood, antibiotics.

Introduction

Hygienic behavior is one of several known mechanisms of resistance against *V. destructor* (Peng *et al.*, 1987; c.f. Boecking and Spivak, 1999). Hygienic honey bee workers are able to detect, uncap the wax covering over the brood cells and remove diseased larvae and pupae out of their cells. The bees uncap and remove the majority of mite infested cells 4–7 days after the cell is capped (Spivak 1996, Thakur *et al.* 1997), when off spring of the invading foundress mite are developing on the capped pupa. The removal of infested pupae, thus, limits the number of offspring of the mites by interrupting their reproductive cycle (Fries *et al.* 1994). Hygienic behavior is predominantly performed by the middle-aged worker bees (5-20 day-old workers) that have not yet begun foraging and about 18% of the bees in the colony are actually involved in the task at any given time (Arathi *et al.*, 2000). The expression of honey bee hygienic behavior was measured using a modification of the pin-killed brood (PKB) assay, described by Keffus *et al.* (1996).Hygienic behavior is an economically important form of social immunity in honey bee colony (Wilson-Rich *et al.*, 2009). Hygienic behavior is a genetic trait, thought to be controlled by two to seven loci (Rothenbuhler, 1964; Milne, 1985a, b; Moritz, 1988; Kefuss *et al.*, 1996; Thakur *et al.*, 1997; Gramacho, 1999; Lapidge *et al.*, 2002). Individual adult bees that express the hygienic trait uncap and

remove diseased brood from combs before the pathogen is transmissible, thus reducing the spread of infection in the colony (Rothenbuhler, 1964a, b). At the colony level, hygienic behavior is a mechanism of reissuance to two brood diseases, American foul-brood and chalk brood (Park *et al.*, 1937; Woodrow and Host, 1942; Rothenbuhler, 1964a, b; Gilliam *et al*., 1983; Milne, 1983; Taber, 1986; Spivak, 1996; Palacio *et al*., 2000; Spivak and Reuter, 2001). Hygienic behavior also has been studied as one colony-level mechanism of resistance to the parasitic mite, *V.destructor*, through the bees' removal of infested pupae (Boecking and Drescher, 1992,; Moretto, 1993; Spivak, 1996; Reuter and spivak, 1998). Hygienic bees respond to odour cues that stimulate them to detect, uncap and remove diseased, parasitized and dead brood (Rosenkranz *et al*., 1993; Palacio *et al*., 1996; Masterman *et al*., 1998; Gramacho, 1999). In lab studies, Masterman *et al*. (2000) demonstrated that bees bred for hygienic behavior were able to discriminate better between odours of healthy and diseased brood compared to bees not bred for hygienic behavior (non-hygienic bees) and concluded that the two lines of bees displayed deferential olfactory sensitivity. Gramacho and Spivak (2003) reported that honey bees collected during uncapping had greater olfactory sensitivity than honey bees collected during brood removal, which could explain task partitioning of uncapping and removing brood among bees within a hygienic colony (Arathi and Spivak, 2001). Colonies that removed more than 95% PKB within 24 hrs were considered super-hygienic, those that removed 90-95% PKB in the same interval were considered hygienic, whilst non-hygienic were colonies that removed less than 90% PKB (Stanimirovic et al. 2002). Only those super-hygienic colonies that had also good reproductive and productive features were considered breeder colonies and used. Heritability of hygienic behavior in grey honey bees (*Apis mellifera carnica*) for rearing queens during the experiment.

In most studies concerning hygienic behavior, the bees were not presented with diseased brood instead, hygienic behavior was experimentally elicited either by piercing healthy pupae through the cell capping with a pin to kill it (Cosenza and Silva, 1972; Newton *et al*., 1975; Gilliam *et al*., 1978; Message, 1979; Milne, 1982; Taber, 1982; Spivak and Gilliam, 1991) or by freezing a section of wax-capped pupae (Goncalves and Kerr, 1970; Reuter and Spivak, 1998). The hygienic behavior of honeybees, *A. mellifera*. involves the workers' recognition and removal of diseased or parasitized brood (Arathi and Spivak 2000). It is well known that hygienic behavior confers resistance to honeybees against several brood diseases (Rothenbuhler 1964a, 1964b; Spivak and Gilliam 1993) as well as against the parasitic mite, *V. destructor* (Spivak 1996; Boecking and Spivak 1999; Arechavaleta-Velasco and Guzma´n-Novoa 2001; Spivak and Reuter 2001; ibrahim *et al*. 2007).oxalic acid is used as a compound in eliminating *v.acobsoni* which it external parasitic that attacks the honey bees *Apis cerana* and *A. mellifera* and the disease caused by the mites is called varroatosis. such ectoparasite can reproduce in honey bee colony and attaches to the bee body causing weakness the bee by sucking the body haemolymph. Marjoram oil used as a natural substance in eliminating american foulbrood symptoms as honey bee disease and *v. Jacobsoni* as ectoparasite.Oxytetracycline hydrochloride is the active ingredient of Teramycin. Tetracyclines are antibiotics used for treatment of bacterial brood diseases such as american foulbrood and european foulbrood. The efficacy of this antibiotic, for control of EFB has been widely demonstrated by Katz nelson *et al*. (1952), Lehnert and Shimanuki (1980) and Hornitzky *et al*. (1988).

In the present study, sub lethal effects of the treatment with Marjoram oil, oxalic acid and Terramycin (antibiotic) on behavior of *A. mellifera* were investigated to find out their effect on the in-hive behavior of worker bees.

Materials and Methods

Experiments were carried out at the department of Apiculture, plant protection Research Institute, Agriculture Research Center, Dokki, and Giza, Egypt on the first hybrid carniolan bee colonies during summer 2013, season. To assess hygienic behavior, three honeybee colonies were used for each tested compound. An area (2.5 × 2.5 cm) of centered sealed worker brood (100 cell/one comb/colony) was bordered and killed by piercing a fine wooden pin into each cell and then the comb was returned to its hive (Sammataro, 1996). The percentages of brood removal in each colony were recorded after 24 hours. The following treatments were used.

1- Marjoram oil (Origanum Majorana)

Marjoram oil used as a natural substance in eliminating american foulbrood symptoms as honey bee disease and *V. Jacobsoni* as ectoparasite .was used as suspension by mixing 3ml of crude oil dissolved with 3ml of tween 80 (emulsifier**)** added to

500 ml of the sugar solution (1:1) It was used in honey bee colonies as a feeding process and repeated every four days, for four times.

2- Oxalic acid (dicarboxilic acid) was used at the concentration of 2% by adding 2.0g of oxalic acid to 100 ml of water and sprayed in honey bee colonies between bee combs to obligate diseased honey bees; the treatment was done every 4 days and repeated four times (16 Days). Oxalic acid spray treatments were carried out during the brood less period between the beginning of November and the end of December 2012.

3-Terramycin (antibiotic): The active ingredient of this compound is Oxytetracycline hydrochloride. Tetracyclines are antibiotics with four hydrocarbon rings derivation defined as" a subclass of polyketides having an octahydrotetracene-2-carboxamide skeleton" and used for treatment of bacterial brood diseases american and european foulbrood). Terramycin used as suspension by mixing 2ml of antibiotic (as 2% concentration) and added to 100 ml of the sugar solution (1:1) which is used in honey bee colonies as a feeding process and repeated one time every seven days for three weeks.

The number of dead brood cells per 100 randomized 100 were recoded in treated and untreated (control) honey bee colonies and the reduction percentages in dead brood cells for each treated colony were obtained according to the formulation given by Henderson and Tilton (1955) as follows:

$$\text{Reduction \%} = \left[1 - \frac{\text{No. in control before application}}{\text{No. in control after application}} \times \frac{\text{No. in treat.after application}}{\text{No.intreat. before application}} \right]$$

The obtained results were analyzed using ANOVA Program, with probability $p<0.05$ as critical value for all tests. Least significant differences (L.S.D.) test was used for separation of statistically significant means according to Duncan's Multiple Range Test for comparing means in analysis of variance.

Results and Discussion

The artificially killed brood from wax cells by adult workers in the experimental colonies and removed them outside the hives is considered as an indicator of hygienic behaviour. The data given in Table (1) clearly show that the mean number of dead brood in 100 wax cells before treatments ranged between 13.67 and 17.33 individuals .These number highly significantly decreased after marjoram , oxalic acid and terramycin treatments (F.value = 17.98) , being 5.00 , 3.67 and 4.33 dead brood / 100 brood cells , respectively.

To evaluate the efficiency of the tested compounds, Henderson and Tilton formulation was Applied as reduction in the number of dead brood .as shown in Table 1 , the most efficient Compound was oxalic acid which reduced the dead brood by 84.85% , followed by marjoram oil (83.32 %) and Terramycine (81.68 %) . The difference between these percentages Proved to be statistically insignificant, where F.value= 1.01, p= 0.42 and L.S.D. =5.46. On the other hand significant differences were found between the numbers of dead brood in 100 wax cells before and after treatment with each of the single tested compound (Table 1). this means that hygienic behavior is the first defense mechanism in honeybee against at least two diseases; American foulbrood and chalk brood. Hygienic behavior of workers differentiate and removes the infected brood before sporulation of the pathogen.

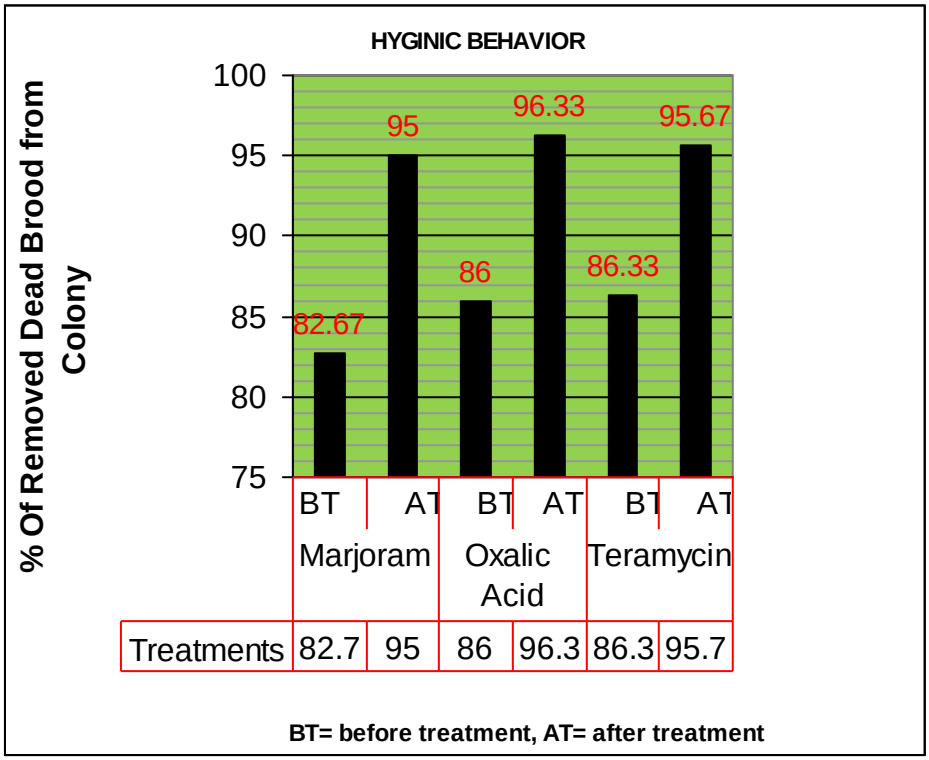
According to Rothenbuhler (1964a,b) two variable genes were suggested to be responsible for this behavior; one gene for uncapping cells and the other one for removing diseased brood . Mortiz (1988) reported that there are many genetic factors responsible for this complicated behavior which is expressed in a phenotype. on the other hand, Lapidge *et al*. (2002) proposed that there are seven genetic loci controlling hygienic behavior, which is very obvious in A. cerana. These bees remove Varroa mites of infected brood and stop reproductive cycle as well as killing immature stages (Fries et *al*., 1994). Zakaria and Allam (2007) found that the formic acid and black cumin oil treatments increased the biological activities of treated bee colonies (Number of covered combs with bees & sealed worker brood area). (Gappriela chioveanu**,** 2010*)* found that *Apis mellifera* carpatica colonies remove worker brood infested with Varroa destructor mites from the nest (hygienic behavior), and groom the mites off them- selves and from other adult bees (grooming behavior) after using oxalic acid. Kamel *et al.* (2003) mentioned that A.mellifera.

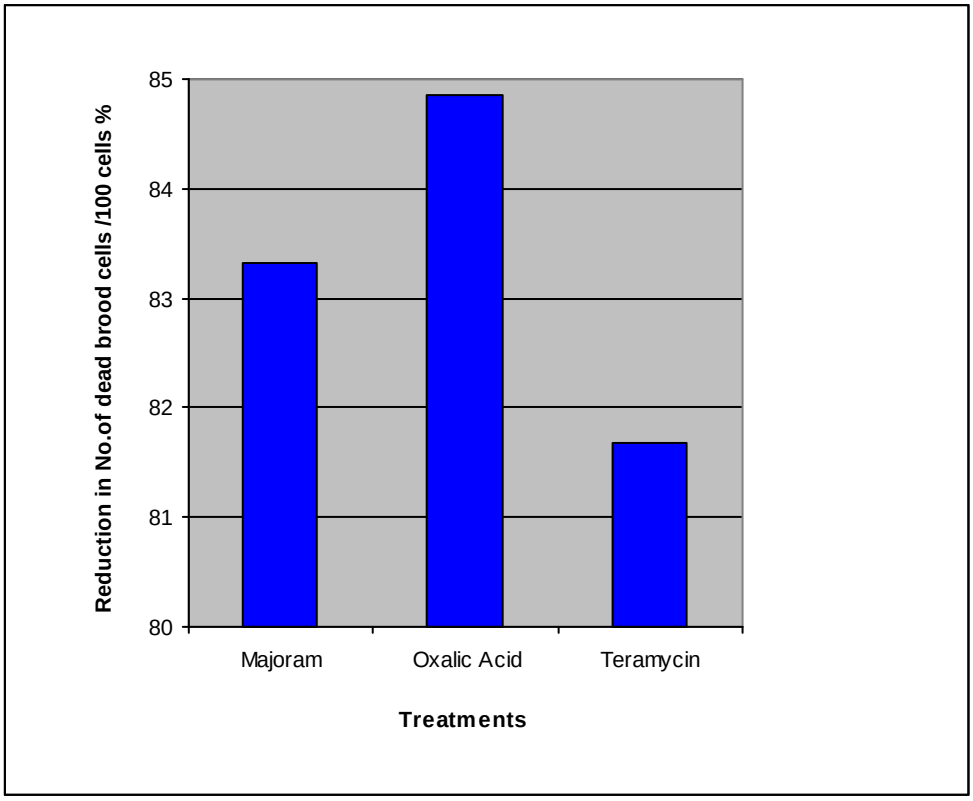
Lamarckian removed 72.5% of dead brood, while it was 35.6% for A.m. carnica, but after 24 h they were 90.5 and 59.4% for the two races, respectively. Al-Medani (2004) reported that *A.mellifera jemenitica* removed 85.5% of dead brood during 48 h. Sensitivity to odors of diseased brood is increased in bees' exhibit hygienic behavior. This mechanism is enhanced by the modulatory effect of octopamine, a noradrenaline-like neuromodulator (Goode et al., 2006). Palacio *et al.*, 1996; Spivak and Downey, 1998, reported that honeybees removed pierced (pin-killed) brood faster than frozen brood.

Table (1): Mean number of dead brood cells in 100 randomized brood cells found in the experimental honey bee colonies (Means ±S.E.)

Treatments	No. of dead brood in wax cells/ 100 cells		F.value	L.S.D and P value	Reduction in No. of dead brood in Cells /100 brood cells (%)*
	Before treatment	After treatment			
Marjoram	17.33±2.12	5.00 ± 0.19	22.5 * *	7.103 0.0090	83.32
Oxalic acid	14.00 ± 2.37	3.67 ± 0.14	15.62**	7.9812 0.0168	84.85
Teramycin	13.67 ± 1.98	4.33± 0.17	19.04**	6.245 0.0120	81.68
Control (untreated check)	16.00 ± 1.87	27.67± 2.94	--------		----------
F.between treatments	———	———	1.01(Insig)		

***** Reduction (%) was calculated according to the formulation given by Henderson and Tilton (1955).





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