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USE OF HONEYBEE PRODUCTS FOR MANAGING ROOT-KNOT NEMATODE IN TOMATO CROP AND THEIR EFFECT ON SOIL HEALTH AND GROWTH PLANT PARAMETERS

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ABSTRACT

This study was conducted to assess the effects of different honeybee products including propolis, bee venom, and drone milk extract against *Meloidogyne javanica*, on tomato plants. Results showed that all tested products significantly reduced nematode parameters when compared with infected plants with nematode alone. Applying drone milk extract at the rate of 0.5 ppm was highly effective in-vivo and in-vitro studies followed by propolis in reducing nematode parameters. The highest percentages reduction in gall counts, egg masses, females/root, numbers of juveniles / 250 g soil, the final population of nematode (PF) as well as the reproductive factor (RF) were demonstrated by 89.52; 80.68; 89.16; 91.24; 94.18 and 94.2%, respectively with drone milk. The treatment of propolis was the second most effective one with no significant differences between them. The lowest reduction was obtained with the bee venom at the same concentration. The results also showed that the tested honeybee products at this concentration significantly improved plant growth parameters of plant height; root length; the number of leaflets; fresh shoots, and root weight. We quantified soil fauna in multiple taxonomic groups to determine how species abundance, richness, diversity, consistency, and community composition of species were affected by these simulated products. The fauna was minimally affected by the treatments with the three products. However, in the drone milk treatment, the richness and diversity increased, consequently, the plant parameters were improved. Finally, natural honeybee products may be employed as nematode management alternatives to control nematode infection in tomato fields.

Key words: Tomato; Root-knot nematode; soil microorganism diversity.

INTRODUCTION

No doubt, the utilization of pesticides has problems for the environment and human toxicity. **(EL Roby et al., 2015; Abd-Elgawad, 2008; EL Roby and Darwish 2018**). Honeybee products are an effective and successful tool for the improvement of an effective, more eco-friendly, and less hazardous use in the pest control strategy **(Mahdy and Abdel-Aal, 2014).** Honeybee products are promising materials in controlling root-knot nematode, Meloidogyne javanica. **(Abdel-Aal, and Galal 2013**). They have different biological effects such as antibacterial **(Menezes et al., 1997**); antifungal (**Cafarchia et al., 1999; Millert Clerc et al., 1987**); antiviral (**Amoros et al., 1992**) nematicides (**Mahdy and Abdel-Aal, 2014; Abdel-Aal, and Galal 2013**). **Noweer and Dawood (2009**) showed that some honeybee product extracts increased the protein content of faba bean plants and reduced the juvenile-Meloidogyne sp. population density in soil and the number of root galls on roots. (**Taha and Ibrahim, 2020, Abdel-Aal and Galal, 2013**). They noticed that honeybee products recorded a highly significant increase in proline concentration which may be increased tomato resistance to nematode infection. **Noweer and Dawood, (2009**) found that the qualitative of some honeybee product extracts contain sterols, flavonoids, and phenolic compounds as well as a few numbers of phenolic acids i.e., coumaric, ferulic, salicylic, and benzoic acid (**Freires et al., 2016**). **Taha and Ibrahim, (2020)** noticed that honeybee products induced plant resistance to nematode infection. **Noweer and Dawood, 2009; Freires et al., 2016 and Ghanem, 2011)** indicated that propolis, bee venom, and royal jelly are promising materials that have antagonistic and medicinal properties against pathogens. The main objective of this study is to assess the effectiveness of honeybee products i.e., drone milk, bee venom, and propolis, as agents to develop efficient, more eco-friendly, and less hazardous products that can be used in minimizing Root-knot nematodes infections or used as a good element in tomato pest control management and conserve the health of the soil.

MATERIALS AND METHODS

Three different honeybee products i.e., drone milk, bee venom, and propolis were applied as foliar sprays with a concentration of 0.5 PPM and 0.05 mg / 100 cc incorporating with the top 10 cm layer at the transplanting date/ pots. The honeybee products were obtained by collecting from honeybee hives in the apiary of the Faculty of Agriculture, Minia University. Minia, Egypt. The bee venom and drone milk concentrations were prepared by mixing the bee venom ampoule contents with tap water to prepare the concentrations of 0. 5 ppm. The experiment was carried out under greenhouse conditions at the Experimental farm of Fac. of Agric., Minia Univ., Minia, Egypt, in pots (20 cm in diam.) filled with sandy-clay soil (1:1, v/v). All treatments were applied at the same time as three-week-old tomato transplants *(Lycopersicon esculentum* Mill cv. GS) transplanting into pots (3 plants /pot).

2.1. Propolis collection

The Propolis samples used in bioassays and greenhouse tests were prepared as propolis solution, (0. 5 PPM) according to **(Strehl et al., 1994**) propolis collected from hives placed in the experimental apiary of the University of Minia, using fine nylon mesh placed above the combs, in May 2021 (i.e., two months before the beginning of the experiments). Then, Propolis samples were separated from the net, and cleaned, by removing visible impurities. The harvested propolis was first heated in a saucepan filled with water to remove the debris (wax, pollen, etc.) stuck to it. The mixture of propolis and water was filtered. The propolis was stored in the freezer at -4°C for about two days and then crunched into a fine powder. and stored in a freezer at −18°C. frozen samples were homogenized using a coffee mill, ground propolis was weighted with an analytical balance and extracted three consecutive times with methanol/water solution (80/20, v/v) and centrifuged for 12 min at 4000 r.p.m. and 10C°. The resulting solution was evaporated under vacuum at room temperature, to obtain a paste, and stored at −18C° until use. (**Ghanem, 2011; Cornara et al. 2017 and Abdel-Aal, and Galal 2013**) and used to prepare concentrations

2.2.Collection of Drone milk (DM).

Drone milk was prepared during blooming and harvested honey by separation from the drone larvae and pupae in the late spring (first half of May and 2nd week of June). The raw liquid material was divided into plastic tubes and was stored at −20°C until the beginning of the investigation.

2.3. Extraction of juveniles

The second-stage juveniles were extracted from roots using the combination of Baermann funnels with elutriation and sieving technique A modified method from the method of **Thorne 1961**.

2.4. Effect of different products on mortality of *M. javanica*

To study the effect of the products on the mortality of juveniles (J2), a 6 mL of concentration was poured into a sterilized Petri dish (6 cm diameter), and 50 ± 4 juveniles were added and replicated three times. Then, they were incubated at 26 ± 2. Distilled water was used as a control. The mortality of juveniles was assessed after 72 h. The juvenile was dead when did not move on probing with a fine needle. Treatments were each replicated three times and the percentage of death per each treatment was calculated according to the following formula:

Juveniles' mortality = (dead Juveniles /total no. Juveniles) X100. Also, the percentage of mortality in comparison with control (corrected mortality) was determined by using the Abbott formula, (Abbot 1925).

Corrected Mortality = ((MT _ MC))/((100 –Mc)) *100

MC: The percentage of mortality in control; MT: The percentage of mortality in the treatment

2.5. Greenhouse treatments

Pure culture from root-knot nematode *Meloidogyne javanica* was reared on *Solanum lycopersicon*) cv. Super train B plants grown under greenhouse conditions at 25±2C° when plants were heavily infected (6-7 weeks after infestation) nematode eggs were extracted from galled roots using 0.5%

sodium hypochlorite solution (NaOCl) (**Hussey and Barker,1973**) and used in infection of new tomato plants in the experiment., at the same time of transplanting five hundreds of nematode larvae JV2 were inoculated by pipetting into three holes made around the tomato root zone. Each treatment was replicated three times and the non-treated plants served as a control treatment. Plants were arranged in a completely randomized block design at approximately 25±2C°. Plants were watered daily and fertilized weekly with 5 ml of 2 g/l N:P: K (20:20:20), obtained from the International Egypt Company for Agricultural and Industrial Developing. Eight weeks after nematode inoculation, the number of galls, egg masses, females and developmental stages/root system, number of eggs/egg mass, number of juveniles (J2)/250g soil, nematode final population (Pf), and reproduction factor (Rf) (**Goodey, 1957**) were calculated according to the equation:

PF = ((No.of egg masses*NO.of the egg for each egg masses) +No. of females per root + No. of juveniles in soil250g soil /pot) -

The reproduction factor (Rf) was calculated according to the equation: RF=PF/PI (Norton, 1978) (Pi = initial population).

Egg masses were stained before counting by dipping the infected roots in phloxine-B solution (0.15 g/l tap water) for 20 minutes as described by **Daykin and Hussey (1985**). Plant growth parameters i.e., shoot and root fresh weights (g), and shoot and root lengths (cm) were recorded.

Root galling index was scored on a 0–5 scale (**Taylor, and Sasser, 1978**) where 0 = no galls, 1 = slight infection (1-10), 2 = moderate infection (11-30), 3 = moderately severe, 4 = severe (31-100), 5 = very severe <100. All experiments were replicated twice in replicated three times.

2.4. Effect of Bee products on soil microorganisms:

Duplicate samples each 200 gm from treated soil after 60 days post-treatment were taken for microbiological analysis and determine the chemical compounds of the soil compared with control treatments. The colony count method was used for determining the total count of soil fungi using martin's media 1950. Five plates for each appropriate dilution from each dilution were prepared and incubated at 27C° for 7 days during which developing colonies were identified in Fungi Identified Center Plant science at Faculty of science, Minia university(Domsch et al. 1980) for counted and related to one gram oven dry soil. The actual reduction % in soil fungi was calculated according to **Abd Elmonem et al.1989**. Also Yeast extract agar medium was used for determine the total counts of soil bacteria spore forms and actinomycetes. The dilution frequency method was used for determine the numbers of aerobic non-symbiotic nitrogen fixing bacteria (**Dobereiner et al.1976**). In order to determine the selective toxic action of the tested products Scheme of **Metcalf 1973** was adopted. Also the data were subjected to achieve the specific diversity of soil microorganisms.

A commonly used index of diversity is (H`) known as the **Shannon- winner index, (1959).**

$$H` = \sum_{n=1}^{1-i} (pi \log e pi)$$

H`=diversity index, Pi= n\N where, n= number of individuals of one species, N= number of individuals of all species. To express the way of individuals distribution in various microorganism species co- existing the tested variant, the second structure index, i.e. the equitability (E) was used and calculated according to **Lioyd and Gheraldi, (1964**) as follows:

Equitability= ((S`)/S)*100

E= size of equitability, S= number of observed microrganisms, S`= theoretical number of species.

2.4.Statistical analyses

The data were subjected to a completely randomized design with Costat software and means were compared by using Duncan's multiple range test.

REULTS AND DISCUSSION

3.1. Effect of honeybee products on Root-knot nematodes Meloidogyne javanica under greenhouse conditions

The applied tested honeybee products (propolis, bee venom, and drone milk) with a concentration of 0.5 ppm significantly reduced nematode parameters compared with the infected control (Table 1 &2). The reduction % in JV2 instars larvae at laboratory tests (in vivo) ranged between 75.92 to 84.25%. Drone milk was the most effective product with a mean reduction % in the two treatments (81.71%) followed by propolis (80.97) and the least product was bee venom (79.26%). Also, the tested honeybee products (in vitro) significantly reduced all variable nematode examined compared to infected treatment control as shown in Tables, 1 and 2. The percentage of reduction in gall numbers/root ranged between 67.5-89.52 %. Treating the plants with drone milk with a concentration of 0.5 ppm led to the highest reduction in the mean number of galls/roots system 89.52 % compared to infected nematode alone followed by propolis (83.01%). The lowest one was obtained with bee venom at 0.5 ppm by 67.5% as shown in Table (1). Application of drone milk at 0.5ppm showed also a significant reduction in the total number of eggs/ root and the percentage of reduction recorded at 95.44% followed by propolis (93.95% with no significant differences between honeybee products and highly significant when compared with the treatment of infected with nematodes. The lowest one obtained with bee venom was recorded by 87.67%. Number of developmental stages/root system; females; eggs/egg masses, and numbers of juveniles in 250 gm soil; nematode final population as well as calculated reproduction factor were also significantly reduced with all the applied honeybee products treatments compared to infected treatment with nematode alone (Table, 1and 2). Application of drone milk at 0.5 ppm showed also a significant reduction in the number of egg masses and the percentage of reduction recorded by 89.28 %, followed by propolis 77.94 % whereas the lowest one obtained with bee venom was recorded 71.95 %.

3.2. Effect of honeybee products on Meloidogyne javanica under laboratory (in vivo) and its reproduction in greenhouse conditions

The least population final numbers were observed with drone milk (100.04) followed by propolis (138.98) and the highest was observed by bee venom (176.344 compared with untreated control (1726.3). The calculated reproduction factors also showed the least value in drone milk treatments. This value ranged from (0.14 to 0.65 in the two replicated sprays with a mean (0.20 to 0.35) compared with 3.45 in the control treatment.

3.3. Effect of honeybee products on plant growth

Results showed that all applied honeybee products had a no-significant effect on root weights compared to treated plants with nematode alone (Table 3). A significant increase was observed in plant high, shoot length, the number of branches, the number of leaflets, and shoot weight in the two experiments. The means results were 105.33, 42.33, 8.83,157.3, and 5.34 in the drone milk treatment respectively, while it was 95.15, 39.5, 8.83, 130.98, and 5.04 in the treatment of propolis the least values but not significant in the treatment of bee venom.

As shown in Fig 3 drone milk increased the plant growth parameters by 20.23, 1.6, 20.46,4.75 and 11.25 percentages than the uninfected control in plant high, shoot length, no. of branches, the number of leaflets, and shoot weight. Treatments with Propolis showed an increase of 9.35, 20.47and 5.00% in plant high, number of branches, and shoot weight while bee venom showed increasing in the number of branches and shoot weight with 9.00 and 10.43% respectively. The infection of nematodes without any treatment caused a reduction in plant growth parameters with -54.58, -37.99, -36.42, -91.3, and -74.79% in plant high, shoot length, no. of branches, number of leaflets and shoot weight, respectively.

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3.4. Effect on soil microorganisms and soil health

Results of the quantity effects of bee products on soil microorganisms counted total and different fungal and bacterial species are presented in Table (4). A slight reduction in counts of soil fungal population was observed in Dron milk treatments and B.v. The calculated reduction % in the number of soil microorganisms was reduced to different degrees depending on the tested product and species of microorganisms. From data presented in table (4) it could be concluded that not only inhibition but also stimulation of soil microorganism species can be observed depending on the product as well as the species of soil microorganisms. The interspecific diversity values indicate the qualitative relationship between the number of species and the number of individuals within them it was in all treatments like maximum diversity in control treatments. Also, the concentrations of different elements increased and do not change significantly.

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All tested honeybee products with a concentration of 0.5ppm significantly reduced

all nematode parameters i.e., number of galls; egg masses/root system; total no. of eggs; females/root system, number of juveniles in soil, final nematode population (Pf) as well as the reproduction factor (Rf) compared to infected nematode treatments. Results concluded that applying the drone milk at 0.5 ppm and propolis at 0.5% were effective products in reducing all nematode parameters in vivo or in vitro tests and improving certain plant growth parameters as shown in Table 3. Bee venom appears less effective than other products. It is not widely known that, similarly to the queen, drone honeybees have their special food. In a similar manner to RJ, drone milk (DM) is secreted by the hypopharyngeal and mandibular glands of worker honeybees (*Apis mellifera* L.). Drone milk is the main component of drone brood, which also contains larvae and pupae of drones in the comb. Drone milk is separated from the drone brood by extraction to eliminate the larvae and pupae during the harvest. The main components of drone

milk are proteins, lipids, fatty acids, carbohydrates, sterols, and water, and it contains vitamins and minerals, too. (**Bogdanov, 2011**). Plant sterols are important materials for insects to synthesize their hormones. From phytosterols, honeybees can produce ecdysteroids that regulate molting, metamorphosis, and reproduction. These ecdysteroids are found in different organs of the insects and are also synthesized by the honeybee queen ovaries (**Yamazaki, et al., 2011 and Hartfelderet al.2002**). Honeybee products have been found to contain significant antioxidant compounds, but in lower concentrations: glucose oxidase, catalase, ascorbic acid, flavonoids, phenolic acids, carotenoid derivatives, organic acids, amino acids, and proteins (**Bogdanov, 2011**). **Ali and Abd El-Ghafar (2002**) evaluated royal jelly and propolis as well as sterilized and non-sterilized bee honey for controlling *Ascospherea apis and Aspergillus flavus* fungi that cause chalk and stone brood in honeybee colonies. They showed that royal jelly and propolis significantly inhibited the fungi growth area when compared with untreated check. **Bamford (1987)** stated that propolis exhibited a severe inhibition effect on the growth of the fungus A. apis. According to **Chu et al., (1992),** the presence of 10-hydroxy-2-decanoic acid (10-HDA) in drone milk plays an important role in inhibiting the growth or promoting sporulation of *A. apis*. The proteins secreted by honeybees into drone milk and other honeybee products have different roles in the functioning of a honeybee colony as a superorganism. The low-molecular-weight proteins and peptides of royal jelly and drone milk might play a host-defense role against *Sarcina lutea*, *Botrytis cinerea*, and Paenibacillus sp. as reported by **(Bilikova et al., 2001)**. The spectrum of biological activity of royalisin was broadened by discovering its antifungal activity against Botrytis cinerea. It is possible to suggest that royalisin exhibits antibacterial, antifungal, and antinematode properties.

Our finding corresponds with the data on the defense of insects against pathogens that were essentially based on the synthesis of cationic peptides/polypeptides exhibiting a broad spectrum of antimicrobial and antifungal activity (Bulet et al., 1999; Otves, 2000). Royal jelly and drone milk have antioxidant properties including scavenging activity of 1,1- diphenyl-2-picrylhydrazyl (DPPH) radicals, and inhibition of linoleic acid peroxidation. Noweer and Dawood (2009) found that soil drenched with some honeybee product extracts (propolis) increased the protein content of faba bean plants. The data revealed that drone milk, propolis, and bee venom extracts reduced the juvenile- Meloidogyne sp population density and the number of root galls per root.

Honeybee products were used as antimicrobials (Bogdanov, 2011). Several authors have reported the antimicrobial activity of propolis on fungi (Lindenfelser, 1967; Brumfit et al., 1990 Tosi et al., 1996). Honeybee products i.e. pollen, propolis, bee venom, and royal jelly are promising materials that have antagonistic and medicinal properties against bacterial pathogens (Ghanem, 2011). Several researchers

have reported antimicrobial and antibiotic activities for honeybees and their constituents (Esin Basim et al., 2006). Propolis has different biological effects such as antibacterial (Christov et al., 1999; Grange and Darvey, 1990; Menezes et al., 1997); antifungal (Cafarchia et al., 1999); antiviral (Amoros et al., 1992). Results of Noweer and Dawood (2009) may explain our finding they indicated that honeybee products extracts (propolis) proved that these extracts contain sterols, flavonoids, and phenolic compounds as well as a few numbers of phenolic acids i.e. coumaric, ferulic, salicylic and benzoic acid. They found that also, all treatments of propolis extract either as foliar or soil drench application increased total chlorophyll and carotenoid faba bean plants. Drone milk is rich in nutrients, and a little-known bee product that exhibits beneficial healing and antinematicidal effect, it can be used as a cheap, safe, and effective natural remedy against root-knot nematode and improve both plant growth parameters and improve soil health. Some of the biological efficiency of drone milk has been confirmed by in vivo and in vitro experiments. Meanwhile, due to its high degree of hormonal activity, drone milk should be thoroughly examined to be safely used as a component of plant growth regulators in the future.

The use of honey bee by-products i.e. propolis, drone milk, and venom represents a promising new approach for the control of nematodes infecting tomato plants within environmentally friendly integrated pest management program which also, able to enhance the resistance of the plant to nematode infection and improve plant growth parameters and soil health. Moreover, the importance of using natural product resources instead of synthetic nematocides has risen globally. Therefore, attempts to increase number of biological agents are needed for safe and effective management for plant parasitic nematodes in organic agricultures.

Table 1: Effect of honeybee products on Root-knot nematodes *Meloidogyne javanica* under greenhouse conditions 1st and 2nd experiment and their average.

Treatments	Conc.	Galls/ root	% R	Egg masses/	Eggs/ egg mass	No eggs/ Plant	R.% No eggs
1st experiment							
infected Cont.	0.00	30.33a	0b	24.66a	30.00a	799.8a	0b
Propolis	0.5ppm	7.66b	74.63a	5.33b	2.00b	10.6b	98.12a
Bee Venom	0.5ppm	10.66b	74.51a	4.33b	4.66b	20.17b	97.51a
Drone Milk	0.5ppm	3.33b	88.36a	3.33b	3.66b	12.18b	98.34a
F. value		64.97 ***	48.9 ***	44.05 **	155.36 **	1332.3***	7840 ***
2nd experiment							
infected Cont.	0.00	36.33a	0b	44.33a	37.66a	1669.47a	0.00c
Propolis	0.5ppm	6.66b	81.25a	14.66b	12.33b	180.75bc	89.06ab
Bee Venom	0.5ppm	11b	69.4a	17.33b	15.33b	265.66b	78.01b
Drone Milk	0.5ppm	3.66b	90.21a	9.33b	12.00b	111.96c	92.55a
F. values		63.58 **	70.33 *	31.17 **	79.11 **	776.7 **	250.87*
Avg. of two sprays							
infected cont.	0.0	33.33	0.00	34.49	33.83	1166.79	
Propolis	0.00	5.66	83.01	9.99	7.17	71.62	93.59
Bee Venom	0.5ppm	10.83	67.5	10.83	9.99	108.19	87.76
Drone Milk	0.5ppm	3.49	89.52	6.33	7.83	49.56	95.44

Table 2: Effect of honeybee products on *Meloidogyne javanica* under laboratory and greenhouse conditions

Treatments	Conc.	Larval mortality In Vivo	Female/ root	Larval /250 gm soils	Mean of Pf	(RF)Pf/Pi	Root gall index
First experiment							
Infected control		0	38a	516.66a	1384.7	2.76	3
propolis	0.5ppm	84.25a	5b	67.00b	90.26	0.18	2
Bee Venom	0.5ppm	84.25a	6.66b	56.66b	94.15	0.18	3
Drone Milk	0.5ppm	75.92a	6.66b	52.33b	74.50	0.14	2
F test		3.16ns	69.7***	99.99***			
2nd experiment							
Infected control		0.00	48a	449.8a	2203.5	4.40	4
propolis	0.5ppm	77.7b	4.3b	47.00b	238.71	0.47	2
Bee Venom	0.5ppm	75.00b	4.66b	46.66b	327.98	0.65	3
Drone Milk	0.5ppm	87.50a	2.66b	32.33b	150.61	0.30	2
F test		16.16*	56.30**	29.99*			
LSD		8.73	6.88	16.33			
Avg. of two spray							
Infected control		0.00	43.00	483.23	1726.3	3.45	4
propolis	0.5ppm	80.97	4.65 89.18	57.00	138.93	0.27	2
Bee Venom	0.5ppm	79.62	5.66 86.83	51.66	176.34	0.35	3
Drone Milk	0.5ppm	81.71	4.66 89.16	42.33	100.04	0.20	2

Pf = Population final

$RF = \frac{PF}{PI}$ (**Norton, 1978**) (Pi = initial population).

$$= \left((\text{No. of egg masses} * \text{No. of eggs}) + \text{No. of females per root} + \text{No. of juveniles in 250g soil} \frac{\square}{\text{pot}} \right)$$

Table 3: Effect of honeybee products on growth characteristic of tomato plants inoculated with *Meloidogyne javanica* under greenhouse conditions 1st and 2nd experiments and their average.

Treatments	Concent.	Plant high (cm)	Shoot length (cm)	No. of branches/plant	No. of leaflets /plant	Fresh shoot weight (g)	Fresh root weight
1st experiment							
Non-infected Control	0.0	73.33b	39.66a	3.33bc	164.6 a	4.7a	0.29
infected Control	0.5ppm	40.66c	23.00c	1.66c	7.66b	1.09b	0.35
Propolis	0.5ppm	93.66a b	37abc	6.00a	145.3 a	4.97a	0.27
Bee Venom	0.5ppm	84.86a b	34.66b c	5.33ab	125.6 a	5.35a	0.26
Drone Milk	0.5ppm	100a	42.66a	5.66a	160.6 a	5.41a	.0.24
F. value		25.14**	5.49*	13.57**	22.2**	48.29**	1.97ns
2 nd experiment							
Non-infected Control	0.00	102.33 a	43.66a	11.33a	136.33 a	4.90	0.26a
infected Control	0.00	38.66b	28.66b	7.66b	17.66b	1.34b	0.34a
Propolis	0.5ppm	96.66b	42a	12.33a	116.66 a	5.1a	0.26a
Bee Venom	0.5ppm	71.66b	36.33a	10.66a	138a	5.25a	0.25a
Drone Milk	0.5ppm	110.66 b	42a	12a	154a	5.26	0.26a
F. values		11.66*	3.71*	11.91**	20.94*	35.29*	3.9ns
Avg. Of two sprays							
Non-infected Control		87.33	41.66	7.33	150.16	4.8	0.28
infected Control	0.00	39.66	25.83	4.66	12.66	1.21	0.34
Propolis	0.5ppm	95.15	39.5	8.83	130.98	5.04	0.26

Bee Venom	0.5ppm	78.26	35.45	7.99	131.8	5.3	0.264
Drone Milk	0.5ppm	105.33	42.33	8.83	157.3	5.34	0.251

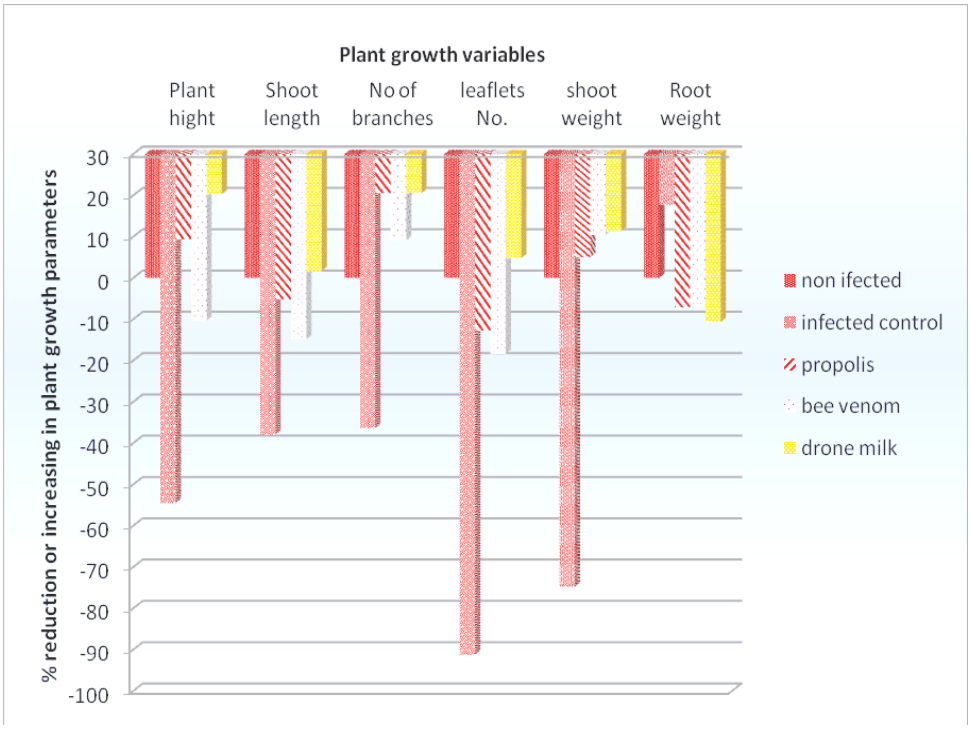

Fig. 1: Prcentage increasing or decreasing in plant growth parameters after treatment with honeybee products

Table 4: Effect of bee products on fungal and bacteria spectrum of clay loam soil (counts in 103/gm oven dried soil).

Treatments / Sp.	Prop-olis	± Reduction	Bee venom	± Reduction	Dron milk	± Reduction	check
Aspriglus niger	14	+233	8	+66.6	5	+116	6
A. eydowii	12	+350	16	200	6	+50	4
A. terreus	0	0.0	2	-----	0	-----	0
flavus	0	0.0	5	+33.3	2	+133	3
ustus	5	+125	2	+150	0	+200	4
achraceus	6	+120	0	+200	0	+200	5
nidulans	5	+150	6	-100	2	+100	2
Penicillum corylophilum	12	+300	10	-50	3	+125	4
P. chrysogenium	2	+200	0	----	2	----	0
P. nigricans	13	+325	11	-75	4	+100	4
Mucor racemosus	10	+333	5	+33.3	0	+200	3
Peacilmyces variotii	0	-100	0	+200	0	+200	1
Rhizopus stolnfer	11	+100	2	0	0	+200	1
Fusarium xysporum	21	+100	1	+150	1	+150	2
Stachbotrys atra	0	-100	0	+200	0	+200	1
Alternaria alternata	10	+301	3	+50	2	+100	2
Trichoderma amatum	7	+233	2	+133.	2	+133	3
Total fungi	128	+320	73	17.5	33	+117	40
Sporforming.b	38	+81.9	45	-4.28	44	-.52	21
Azotobacter	43	+134	47	+53.1	35	+90.	32
Azospirillum	29	+145	38	+10	41	-50	20
Total bacteria	120	+164	130	+21.9	120	+35.	73
Diversity	2.50		2.177	+199.	2.397		2.3
Theoretical number	12.2		8.32	+135.	9.03		12.
Equitability%	76.6		52.00		69.46	102.2	71.0

REFERENCES

Abbott WS 1925. A method of computing effectiveness of an insecticide. J Econ Entomol 18:265–267

Abdel-Aal, A. A. A. and Galal, Neveen M. (2013): Effect of some honeybee products on control of root-knot nematode, *Meloidogyne javanica* on tomato plants *J. Plant Prot. and Path., Mansoura Univ., Vol. 4 (4): 435 - 444, 2013*

Abd-Elgawad, M.M.M. (2008). The status of phytonematode management in Egypt with special reference to applicable nematicides. Egyptian Journal of Agronematology, 6:33-46.

Ali, M. A., and Abd El-Ghafar, N. Y. (2002): Laboratory evaluation of some honey bee products against *Ascospherea apis* and *Aspergillus flavus*. Research: 118-125.

Amoros, M.; Sauvager, F.; Gire, L. and Cormier, M. (1992). In vitro antiviral activity of propolis. Apidologie, 23: 231–240.

Bamford, S. (1987): Studies on the infection of honeybee larvae with *Ascospherea apis*. Bibliographic Citation: 200pp.

Bilikova, K.; Wu, G. and Simuth, J. (2001). Isolation of a peptide fraction from honeybee royal jelly as a potential anti-foulbrood factor. Apidologie, 32: 275-283.

Bogdanov, S. (2011). Functional and biological properties of the bee products: a review. Bee Product. Science: 1-12.

Brumfitt, W.; Hamilton-Miller, J.M. T; Franklin, I. and Hamilton-Miller, J. M. T. (1990). Antibiotic activity of natural products: 1. Propolis. Microbios, 62: 19-22.

Bulet, P.; Hetru, C.; Dimarcq, J. and Hoffmann, D. (1999). Antimicrobial peptides in insects; structure and function, Dev. Comp. Immunol., 23: 329- 344.

Bulet, P.; Hetru, C.; Dimarcq, J. and Hoffmann, D. (1999). Antimicrobial peptides in insects; structure and function, Dev. Comp. Immunol., 23: 329-344.

Cafarchia, C.; de Laurentis, N.; Milillo, M. A.; Losacco, V. and Puccini, V. (1999). Antifungal activity of Apulia region propolis. Parasitologia, 41: 587–590.

Christov, R.; Bankova, V.; Tsvetkova, I.; Kujumgiev, A. and Tejera, A. D. (1999). Antibacterial furofuran lignans from Canary Islands propolis. Fitoterapia, 70:89–92.

Chu, L.K.; Liu, T.M. and Ho, K. K.(1992). Growth inhibition of *Ascospherea apis* by royal jelly and 10-hydroxy-2-decanoic acid. Bull. Instit. Zool., Acad. Sinica 31(2): 73-79.

Cornara, Laura; Biagi, M.; Xiao, J. and Burlando, B. (2017) Therapeutic Properties of Bioactive Compounds from Different Honeybee Products Front. Pharmacol., 28 June 2017 | https://doi.org/10.3389/fphar.2017.00041200412

Daykin, M. E., and Hussey, R. S. (1985). Staining and histopathological techniques in nematology. Pp. 39- 48 in Barker, K. R.; Carter, C. C. and Sasser,J. N., Eds. An advanced treatise in Meloidogyne, Vol. II Methodology, Raleigh: North Carolina State University Graphics.

El Roby, S. M. H.; EL Awamy, I.O. and Soliman, Ahlam F. (2015). Monitoring of some carbamate and synthetic pyrethroid pesticides in certain fruits at the eastern area of Libya Fifth International Conference of Plant Protection Res. Institute Hurghada- Egypt 3-6 May 2015. (DOI 10.13140/RG.2.1.3109.8006

El Roby, A., & Darwish, M. (2018). Biological activity of certain natural products against *Varroa destructor* (Acari: Varroidae) and their selectivity against *Apis mellifera* (Hymenoptera: Apidae). *Journal of Phytopathology and Pest Management*, *5*(3), 67-76. Retrieved from http://ppmj.net/index.php/ppmj/article/view/158

Esin Basim; Hueseyin B. and Musa O. (2006). Antibacterial activities of Turkish pollen and propolis extract against plant bacterial pathogens. Journal of Food Engineering, 77: 992–996.

Freires, I. A., Queiroz, V. C., Furletti, V. F., Ikegaki, M., de Alencar, S. M., Duarte, M. C., et al. (2016). Chemical composition and antifungal potential of Brazilian propolis against Candida spp. *J. Mycol. Med.* 26, 122–132. doi: 10.1016/j.mycmed.2016.01.003

Ghanem Nevine, B. (2011). Study on the antimicrobial activity of honey products and some Saudi Folkloric substances. Research J. of Biotechnology, 6 (4): 38-43.

Goodey, J. B. (1957). Laboratory methods for work with plant and soil nematodes. Tech. Bull. No.2, Min. Agric. Fish, Ed.London pp 47.

Grange, J.M. and Darvey, R. W. (1990). Antibacterial properties of propolis (bee glue). Journal of the Royal Society of Medicine, 83: 159–160.

Hartfelder K, Bitondi MM, Santana WC, Simoes ZL: Ecdysteroid titer and reproduction in queens and workers of the honey bee and a stingless bee: loss of ecdysteroid function at increasing levels of sociality? Insect Biochem Mol Biol 2002; 32:211–216.

Hussey, R. S. and Bark er, K. R. (1973). A comparison of methods collecting inocula of Meloidogyne spp. including a new technique. Plant Disease Reporter,57: 025-028.

Lindenfelser, L. A. (1967). Antimicrobial activity of propolis. American Bee Journal, 107: 90-92.

Mahdy,M.E. and Abdel-Aal, A. A.(2014). Effect of honeybee products in controlling root-knot nematode, Meloidogyne javanica on tomato plants. Egyptian Journal of Plant Protection, 9 (2): 1-10.2014

Menezes, H.; Bacci, M. Jr.; Oliveria,S.D. and Pagnocca, F.C.(1997). Antibacterial roperties of propolis and products containing propolis from Brazil. Apidologie, 28: 71–76.

Millert-Clerc, J.; Michael, D.; Simeray, J. and Chaumont, J. P. (1987). Etude preliminaire des proprieties fongistatiques de la propolis com-parees a celles de quelques produitscommerciaux. Plantes mdicinales et Phytotherapie, 21:3–7.(English summary)

Norton, D. C. (1978). Ecology of plant parasitic nematode. John Willey and Sons, New York, p. 238.

Noweer, E.M. and Dawood, M. G. (2009). The efficiency of propolis extract on faba bean plants and its role against nematode infection. Commun. Agric. Appl. Biol. Sci., 74(2): 593-603

Otvos, L. J. R. (2000). Antibacterial peptides isolated from insects. J.Peptide Sci., 6: 497–511

Strehl, E; Volpert, R; Elstner, E . F; (1994) Biochemical activities of propolis extracts: III Inhibition of dihydrofolate reductase, Z. *Naturforsch*. 49c, 39–43

Taha, A.A. and Ibrahim, Dina S.E.S. (2020). Nematocidal Activity of Honey Bee Propolis and Venom Against Root-Knot Nematode Meloidogyne incognita Infecting Strawberry Plants. Academic Journal of Entomology 13 (3): 67-75, 2020

Taylor, A. L., and Sasser, J. N. (1978): Biology identification and control of root-knot nematodes (*Meloidogyne* spp.) Coop. Pub. Dept. Plant Pathol. North Carolina State Univ. and U. S. Agency Int. Dev. Raleigh, N.C.111 pp.

Thorne, G. (1961): Principles of Nematology. Mcgrow –Hill Book Co., Inc. New York, 553pp.

Tosi, B.; Donini, A.; Romagnoli, C. and Bruni, A. (1996). Antimicrobial activity of some commercial extracts of propolis prepared with different solvents. Phytotherapy Research, 10: 335-336.

http://www.indiamart.com/akash-international-mumbai/other-products.html.

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الملخص العربي

استخدام منتجات نحل العسل لادارة مكافحة نيماتودا تعقد الجذور في الطماطم وتأثيرها على صحة التربة ومعايير نمو النبات.

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أجريت هذه الدراسة تحت ظروف المعمل والصوبة وذلك لتقييم ثلاثة منتجات مختلفة من نحل العسل ، وهي البروبوليس ، وسم النحل ، ومستخلص لبن الذكور بتركيز 0.5 جزء في المليون لإدارة مكافحة نيماتودا تعقد الجذور ، Meloidogyne javanica ، وتأثير اضافة هذة المركبات علي صحة التربة وخصائص نمو النبات . أوضحت النتائج أن جميع منتجات نحل العسل المختبرة احدثت خفضا معنويا في كل متغيرات الاصابة للنيماتودا عند مقارنتها بالنباتات المصابة دون معاملة. واظهر مستخلص لبن الذكور تأثيرا فعالا ومعنويا في تقليل جميع مؤشرات اختبار النيماتودا سواءا في المعمل او الصوبة يلية البروبوليس حيث كانت أعلى النسب المئوية للانخفاض في عدد اليرقات ، وكتل البيض ، والإناث / جذر ، وأعداد اليرقات المعدية / 250 جم من التربة ، ومجموع النيماتودا النهائي (PF) وكذلك عامل التكاثر (RF) مع لبن الذكور بنسبة 89.52 ؛ 80.68 ؛ 89.16 ؛ 91.24 ؛ 94.18 و 94.2٪ على التوالي. كان معاملة البروبوليس هي التالية في الفاعلية حيث أعطى 83.01 و 71.03 و 89.18 و 88.20 و 91.95 و 92.17 دون وجود فروق معنوية بينهم. تم الحصول على أقل انخفاض بسم النحل بتركيز 0.5 جزء في المليون. كما أظهرت النتائج أن جميع منتجات نحل العسل عند التركيز المستخدم حسنت بشكل ملحوظ جميع مؤشرات نمو النبات مثل ارتفاع النبات. طول الجذر عدد الافرع و الوزن الطازج ، ووزن الجذر. قمنا بأخذ عينات وتحديد الكائنات الحية الدقيقة في التربة عبر مجموعات تصنيفية متعددة لتحديد كيفية تأثر وفرة الأنواع وثرائها وتنوعها وتأثيراتها علي مكونات التربة كميا ونوعيا. تأثر التنوع الكمي والعددي بمعاملات المنتجات الثلاثة. ولكن معاملة لبن الذكور ادت الي زيادة قيمة Diversity للكائنات الحية القيقة في التربة ,وفقًا لذلك حدث تحسين في معايير نموالنبات. وخلصت النتائج الي انه

يمكن استخدام لبن ذكور النحل والبروبليس كوسيلة امنه لمكافحة نيماتودا تعقد الجذور مع تحسين خواص التربة ومحتواها من الكائنات الحية الدقيقة.