

EFFECT OF SOME HONEYBEE PRODUCTS ON CONTROL OF ROOT-KNOT NEMATODE, *Meloidogyne javanica* ON TOMATO PLANTS

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ABSTRACT

This research work was carried out under greenhouse condition to evaluate three different honeybee products i.e. royal jelly, bee venom and propolis at different concentrations to control root-knot nematode, *Meloidogyne javanica*, on tomato plants. Results of this study revealed that all tested honeybee products at all concentrations significantly reduced all nematode parameters when compared with the treated plants with nematode alone. Applying the royal jelly at the rate of ١% was the highly effective in reducing all nematode parameters. The highest percentages of reduction in number of galls, egg-masses, developmental stages, females/root system, juveniles in soil, final nematode population (PF) as well as the reproduction factor (RF) were showed with this treatment by ٩٧; ٩٩; ١٠٠; ١٠٠; ٩٢; ٩٨ and ٩٨%, respectively. The treatment of royal jelly at the concentration of ٢% was the second effective one followed by bee venom in the third rank at the concentration of ٠,٠٠٥%. The lowest reduction obtained with the propolis at all tested concentration. Results also showed that all honey bee products at all concentrations enhanced markedly all plant growth parameters i.e. plant height; root length; fresh shoot and root weight. Results revealed that all applied treatments maintained on the cell wall and reduced the membrane permeability compared to plants treated with nematode alone. The lowest percentage membrane permeability (membrane leakage%) was found with the royal jelly at ١% as it reached ٢٦% compared to the treated plants with nematode alone (reached ٩٧%). The contents of the amino acid proline in plants was also affected with the applied treatments as the highest proline content was obtained with the same treatment of royal jelly at ١%.

Keywords: Tomato; Root-knot nematode; Honeybee products; Propolis; Bee venom; Royal jelly.

INTRODUCTION

Root-knot nematodes, *Meloidogyne* spp. cause conspicuous root galls and serious reductions in yield of several host plants. They are of major economic significance throughout the tropics and warmer regions of the world. Infected plants suffer from vascular damages which disturb water and mineral uptake (Luc *et al.*, ٢٠٠٥). Although, chemical nematicides hold major effective nematode control (Adegbite and Adesiyun, ٢٠٠١; Oyedunmade *et al.*, ١٩٩٢), the high cost, their non-availability at the time of need and the hazards they pose as environmental pollutants, discourage most potential users to search the cheaper and safer alternatives to the chemical nematicides.

Biological and cultural methods of control the plant pathogens i.e. fungi, bacteria, virus and nematodes were applied. Honeybee products and some of its therapeutic values were mentioned in the Holy Quran. The ancient Egyptians used honey in combination with other herbs and on its own, to treat wounds and diseases of the gut (Zumla and Lulata, 1989). Several authors have reported on the antimicrobial activity of propolis on fungi (Lindenfelser, 1967; Brumfit *et al.*, 1990 and Tosi *et al.*, 1996). Honey bee products i.e. pollen, propolis, bee venom and royal jelly are the promising materials that have antagonistic and medicinal properties against pathogens (Ghanem Nevine, 2011). Several researchers have been reported antimicrobial and antibiotic activities for honey bees and its constituents (Esin Basim *et al.*, 2006). Propolis as a one of honey bee products has a different biological effect such as: antibacterial (Christov *et al.*, 1999; Grange and Darvey, 1990; Menezes *et al.*, 1997); antifungal (Cafarchia *et al.*, 1999; Millertclerc *et al.*, 1987); antiviral (Amoros *et al.*, 1992).

This research work aimed to evaluate the different honeybee products i.e. royal jelly, bee venom and propolis at different concentrations against root-knot nematode, *M. javanica*, on tomato plants.

MATERIALS AND METHODS

In this research work, three different honeybee products i.e. royal jelly, bee venom and propolis were applied at three different concentrations 1, 2 and 10% (w/w), except bee venom which was used at two different concentrations i.e. 0.005 and 0.020% (v/w). The honeybee products were obtained by collecting from honeybee hives in special apiary at Diarb Negm region, Sharkia governorate. Both royal jelly and propolis were applied by incorporating both of them with the top 10 cm layer of soil pots at the rate of 1, 2 and 10% of soil weight at transplanting date. The bee venom concentrations were prepared by mixing the bee venom ampoule contents with tap water to prepare the concentrations of 0.005 and 0.020%.

The experiment was carried out under greenhouse condition at the Experimental farm of Fac. of Agric., Menoufia Univ., Shebin El-Kom, Egypt, in plastic pots (10 cm in diam.) filled with sandy-clay soil (2:1, v/v). All treatments were applied at the same time of three week-old tomato transplants (*Lycopersicon esculentum* Mill cv. GS) transplanting into pots (one plant/pot).

Inoculums of root-knot nematode, *M. javanica* was obtained from pure culture of tomato heavily infected roots grown under greenhouse conditions at 20±2°C. Eggs of *M. javanica* were extracted from heavily galled roots by using 1.0% sodium hypochlorite solution (NaOCl) method as described by Hussey and Barker (1973). At the same time of transplanting, two thousand of nematode eggs were inoculated by pipetting into three holes made around the tomato root zone. Each treatment was replicated five times and the non-treated plants were used as control. Plants were arranged in a completely randomized block design in the greenhouse at approximately

20±2°C. Plants were watered daily and fertilized weekly with a nutrient solution.

After eight weeks of plant inoculation with nematode, plant growth parameters i.e. fresh weights of shoot and root (g), plant height (cm) and root length (cm) as well as membrane integrity (membrane leakage) and the amino acid contents were determined. Nematode parameters i.e. number of galls, number of egg masses/root system, number of eggs/egg-mass, number of females/root system, number of developmental stages/root system, number of juveniles/200 g soil; nematode population and reproduction factor (Goodey, 1967) were also determined. Egg-masses, females and developmental stages were stained prior to counting by dipping the infected roots in 0.1% phloxine-B solution for 20 minutes as described by Daykin and Hussey (1980).

Membrane Leakage (ML %) was determined following the method of Leopold *et al.* (1981). The percentage leakage of solutes was calculated as:

$$\text{Leakage of substances (\%)} = \frac{\text{Initial absorbance of bathing medium}}{\text{Final absorbance at the bathing medium}} \times 100$$

Final nematode population (PF) was counted according to the equation:

$$*PF = (\text{No. of egg masses} \times \text{No. of eggs/egg mass}) + \text{No. of females} + \text{No. of developmental stages} + \text{No. of juveniles in soil pot.}$$

Rate of nematode reproduction (RF) was recorded according to Norton (1978) using the following equation:

$$*RF = PF/Pi \quad (Pi = \text{initial population})$$

Egg-masses, females and the developmental stages were stained prior to counting by dipping the infected roots in 0.1% phloxine-B solution for 20 minutes as described by Daykin and Hussey (1980). Females were collected by cutting the root system of each plant in 2 cm pieces and submerging the roots in a beaker full of tap water for 4 days at room temperature until they became soft. The roots were then washed through 200 and 250 µm sieves to separate the females from the root debris (Mahdy, 2002).

Data were statistical analyzed according to standard analysis of variance by a one way ANOVA with the software statgraphics (Statistical Graphics. Crop., Rockville, MD), Variance homogeneity for all treatments was confirmed by the Bartlett test. The comparison between means was carried out by Duncan's Multiple Range Test (Duncan, 1960) as given in the figures.

RESULTS

Results of this study revealed that all applied honeybee products at all concentrations significantly reduced all nematode parameters i.e. number of galls; egg masses, females/root system, number of juveniles in soil, final nematode population (PF) as well as the reproduction factor (RF) when compared to the plants treated with nematode alone. The reduction

percentage of nematode parameters ranged between 40-100%. The highest reduction in galls/root system obtained with the application of royal jelly at the concentration of 1% of soil weight compared to plants treated with nematode alone as shown in Table (1). The percentage of gall reduction was 97% followed by the royal jelly at 2% and bee venom at 100% by 87% for both of them as shown in Table (1). The lowest percentage of reduction was obtained with the application of bee venom at the rate of 100% as the reduction reached 0%.

Egg masses shown in Table (1) gave the same trend as was found with the number galls as the application of royal jelly at 1% was the best one. The percentage of reduction in egg masses was recorded 99% (Table, 1). All the remaining nematode parameters i.e. females; developmental stages; juveniles in soil; nematode final population as well as the reproduction factor (Table, 1) were also significantly reduced with all treatments compared to the treated plants with nematode alone.

Application of royal jelly at 1% appeared the most effective one in reducing females; developmental stages; juveniles in soil; nematode final population as well as the reproduction factor compared to the others. The percentages of reductions were recorded 100; 100; 92; 98 and 98%, respectively as shown in Table (1).

Application the royal jelly at 2% came in the second rank as the percentage of reduction in females; developmental stages; juveniles in soil; nematode final population and the reproduction factor reached 92; 100; 88; 96 and 96, respectively. The lowest effect recorded with propolis at 2% as the reduction percentage was 76; 40; 36; 38 and 38%, respectively.

Results revealed also that all applied honeybee products enhanced markedly all plant growth characters i.e. fresh shoot and root weights, plant and root length (Table, 2), as well as the chemical component of the amino acid proline and at the same time maintained on the root cell wall and reduced its membrane permeability as shown in Table (3), compared to the treated plants with nematode only. Royal jelly product at all used doses showed promise results in this field when compared to the others.

Results confirmed that application royal jelly product at 1% of soil weight was also the effective one in enhancing all plant growth parameters compared with the others. Proline was also significantly enhanced and the percentage of membrane permeability was reduced with application royal jelly product at 1% of soil weight compared with the others (Table, 3).

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Table (٢): Effect of honeybee products on the plant growth characters of infected tomato plants with *Meloidogyne javanica*.

Treatment	Concentration (%)	Agronomic characters			
		Fresh shoot weight (g)	Fresh root weight (g)	Plant height (cm)	Root length (cm)
Royal Jelly	١	٢٣,٢ a	٢,٨٠ a	٦٢,٠ a	٢٠,٧ a
Royal Jelly	٢	١١,٧ c	١,٩٠ ab	٤٩,٣ ab	١٧,٠ a
Royal Jelly	١٠	١٥,٤ bc	٢,٠٢ ab	٦١,٣ a	١٥,٧ a
Bee venom	٠,٠٠٥	١٥,٤ bc	١,٨٠ ab	٥٤,٣ ab	١٦,٧ a
Bee venom	٠,٠٠٢٥	١٤,٢ bc	١,٩٠ ab	٥٨,٧ ab	١٧,٧ a
Propolis	١	١٧,٩ abc	٢,٣٠ ab	٤٥,٧ ab	٢١,٠ a
Propolis	٢	١٦,٢ bc	١,٤٠ b	٦١,٠ a	٢٠,٣ a
Propolis	١٠	١١,٩ c	١,٧٠ b	٦٠,٠ a	١٩,٧ a
Nematode alone (Control +)		١٦,٧ bc	١,٦٠ b	٥٣,٠ ab	١٣,٠ a
Control (-)		١٨,٥ ab	٢,٣٠ ab	٤٠,٣ b	١٨,٧ a

*Means followed by the same letters within a column are not significantly different by (P=٠.٠٥) according to Duncan's multiple range test.

Table (٣): Effect of honeybee products on the amino acid proline content and the membrane leakage % of infected tomato plants with *Meloidogyne javanica*.

Treatment	Concentration (%)	Proline content (µg/g dw)	Membrane leakage** (%)
Royal Jelly	١	٣,٣ a	٠,٢٦
Royal Jelly	٢	٢,٠ b	٠,٦٥
Royal Jelly	١٠	١,١ d	٠,٧٧
Bee venom	٠,٠٠٥	٠,٢ j	٠,٩١
Bee venom	٠,٠٠٢٥	٠,٣ i	٠,٦٣
Propolis	١	٠,٦ g	٠,٩٦
Propolis	٢	٠,٩ e	٠,٩٥
Propolis	١٠	١,٦ c	٠,٩٢
Nematode alone (Control +)		٠,٧ f	٠,٧٥
Control (treatment free)		٠,٥ h	٠,٩٧

*Means followed by the same letters within a column are not significantly different by (P=٠.٠٥) according to Duncan's multiple range test.

** Membrane leakage (%) = $\frac{\text{Initial absorbance of bathing medium}}{\text{Final absorbance at the bathing medium}} \times 100$ (Leopold et al., ١٩٨١).

DISCUSSION

Results of this study revealed that all tested honeybee products at all tested concentrations significantly reduced all related nematode parameters i.e. number of galls, number of egg-masses, number of developmental stages, number of females/root system, number of juveniles in soil, final nematode population (PF) as well as the reproduction factor (RF) when compared to the treated plants with nematode alone. Results confirmed that applying the royal jelly at the rate of ١% was the highly effective treatment in reducing all nematode parameters.

Honeybee products have been found to contain significant antioxidant compounds, but in lower concentration: glucose oxidase,

catalase, ascorbic acid, flavonoids, phenolic acids, carotenoid derivatives, organic acids, amino acids and proteins (Bogdanov, 2011). He found that also royal jelly have antibacterial, antifungal and antiviral antioxidative.

Fujiwara *et al.*, (1990) revealed that the peptide royalisin isolated from royal jelly have antibacterial activity against some gram positive bacteria. A special group of protective substances in the developing organism of honeybee is represented by proteins and peptides and by molecular weight compounds present in royal jelly. The antibacterial activity of 10-hydroxy-trans-2-decanoic acid, the natural component of royal jelly was described (Bonvehl and Jorda, 1991).

Ali and Abd El-Ghafar (2002) evaluated three concentrations 1, 5 and 10% from each of royal jelly and propolis as well as sterilized and non-sterilized bee honey for controlling *Ascospheera apis* and *Aspergillus flavus* fungi that cause chalk and stone brood in honeybee colonies. They found that royal jelly and propolis at 10% significantly inhibited the fungi growth area when compared with untreated check. Bamford (1987) stated that royal jelly exhibited a severe inhibition effect on all germination stages of the fungus *Ascospheera apis*.

According to Chu *et al.*, (1992) the presence of 10-hydroxy-2-decanoic acid (10-HAD) in royal jelly plays an important role in inhibiting growth or promoting sporulation of *Ascospheera apis*.

The proteins secreted by honeybees into royal jelly and other hive products have different roles in the functioning of a honeybee colony as a superorganism. The low-molecular weight proteins and peptides of royal jelly might play a host-defense role against pathogens 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 both antibacterial and antifungal properties. This finding corresponds with the data on defense of insects against pathogens that were essentially based on synthesis of cationic peptides/polypeptides exhibiting a broad spectrum of antimicrobial and antifungal activity (Bulet *et al.*, 1999; Otves, 2000).

Royal jelly have antioxidant properties including scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, inhibition of linoleic acid peroxidation. Royal jelly in addition has higher contents of proteins and polyphenolic compounds, which may be the major component for giving the antioxidant activities in royal jelly <http://www.indiamart.com/akash-international-mumbai/other-products.html>.

Noweer and Dawood (2009) found that soil drench with some honeybee product extracts (propolis) increased protein content. The data revealed that the propolis extract as soil drench reduced the juvenile-*Meloidogyne* sp. population density/one kg soil and number of root-galls/one gm roots

Noweer and Dawood (2009) found that the qualitative of some honeybee product 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, carotenoid and protein contents of faba bean plants. All propolis extracts enhanced plant growth characters i.e. shoot height; root dry weight; number of branches and pods/plant; number of seeds/pod as well as seed index.

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تأثير بعض منتجات نحل العسل على مكافحة نيماتودا تعقد الجذور المتواجدة على نباتات الطماطم

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أجري هذا البحث تحت ظروف الصوبة لتقييم ثلاثة منتجات من منتجات نحل العسل هي (غذاء ملكات النحل، سم النحل و البروبوليس) باستخدام تركيزات مختلفة لمكافحة نيماتودا تعقد الجذور على نباتات الطماطم. وأظهرت النتائج أن جميع منتجات نحل العسل التي تم اختبارها في جميع التركيزات أدت لانخفاض كبير في كل الصفات المرتبطة بالنيماتودا مقارنة بنباتات الكنترول. وقد أدى استخدام الغذاء الملكي بمعدل ١٪ إلى فاعلية كبيرة في الحد من جميع الصفات المرتبطة بالنيماتودا فقد أظهرت أعلى نسب تخفيض في عدد العقد النيماتودية، كتل البيض، الأطوار المختلفة للنيماتودا، عدد الإناث/لكل مجموع جذري وعدد يرقات الطور الثاني المعدي في التربة، والتعداد النهائي لعشيرة النيماتودا (PF) وكذلك عامل التكاثر (RF) مع هذا العلاج بنسبة ٩٧، ٩٩، ١٠٠، ١٠٠، ٩٢ و ٩٨ و ٩٨٪، على التوالي. كما أظهر استخدام الغذاء الملكي بتركيز ٢٪ في المرتبة الثانية يليه سم النحل في المرتبة الثالثة بتركيز ٠,٠٠٥٪. وكان اقل تخفيض عند استخدام البروبوليس بجميع تركيباته.

أوضحت النتائج أن جميع منتجات النحل العسل في جميع التركيزات عززت بشكل ملحوظ جميع الصفات لنمو النبات مثل ارتفاع النبات، طول الجذر؛ وزن المجموع ووزن الجذور. وكشفت النتائج أن جميع المعاملات المستخدمة أدت للحفاظ على جدار الخلية وخفض نفاذية الجدر مقارنة مع نباتات الكنترول. أعطى استخدام الغذاء الملكي بتركيز ١% أقل نسبة نفاذية للأغشية ٢٦٪ مقارنة مع نباتات الكنترول والتي وصلت إلى ٩٧٪. وقد تأثر محتوى الحمض الأميني البرولين في النباتات المعاملة وكان أعلى محتوى من البرولين عند استخدام الغذاء الملكي بنسبة ١٪. **الكلمات المفتاحية:** الطماطم، نيماتودا تعقد الجذور، منتجات نحل العسل، البروبوليس، سم النحل، الغذاء الملكي

قام بتحكيم البحث

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كلية الزراعة - جامعة المنوفية

Table (1): Effect of honeybee products on the nematode parameters of tomato plants infected with *Meloidogyne javanica*.

Treatment	Concentration (%)	No. of galls/ root system	% Reduction	Nematode Parameters / Population						Nematode final population (Pf)	% Reduction	Reproduction factor (build-up) (Pf/Pi)	%Reduction		
				No. of egg masses/ root system	% Reduction	No. of developmenta I stages/ root system	% Reduction	No. of females /root system	% Reduction					No. of juveniles/ 100 g soil	% Reduction
Royal Jelly	1	2.30 f	97	0.30 d	99	0.00 c	100	0.00 b	100	333.30 c	92	342.30	98	0.17	98
Royal Jelly	2	9.00 ef	87	1.00 cd	96	0.00 c	100	6.70 b	92	0.00,00 c	88	066.70	96	0.28	96
Royal Jelly	10	10.00 de	79	0.00 bc	81	0.00 ab	80	16.7 b	80	733.30 c	82	1200.00	92	0.60	92
Bee venom	0.0005	9.30 ef	87	2.30 cd	91	16.7 bc	80	6.70 b	92	333.30 c	92	080.70	96	0.27	96
Bee venom	0.0020	32.7 b	04	7.00 cd	74	16.7 bc	80	1.00 b	88	616.70 c	80	1413.40	91	0.71	91
Propolis	1	30.3 bc	07	8.30 b	69	33.3 bc	60	16.7 b	80	800.00 c	80	2344.00	83	1.17	80
Propolis	2	20.7 cd	71	8.60 b	68	0.00 ab	80	2.00 b	76	2066.7 b	36	9306.70	38	4.68	38
Propolis	10	17.3 de	76	4.30 bcd	84	16.7 bc	80	2.00 b	76	666.70 c	83	1090.40	93	0.04	93
Nematode alone (control +)		71.0 a		26.7 a		83.3 a		83.3 a		4000.0 a		10113.6		7.06	

Means followed by the same letters within a column are not significantly different by (P=0.05) according to Duncan's multiple range test.