Egyptian Journal of Crop Protection, 9 (2): 1-10.2014

EFFECT OF HONEY BEE PRODUCTS IN CONTROLLING ROOT-KNOT NEMATODE, *MELOIDOGYNE JAVANICA* ON TOMATO PLANTS

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ABSTRACT

This work was conducted to test three honey bee products i.e. royal jelly, bee venom and propolis at various concentrations to control root-knot nematode, *Meloidogyne javanica* infecting tomato plants under greenhouse condition. Results found that all tested honey bee products with all concentrations significantly reduced nematode parameters compared to nematode alone treatment. The highly effective treatments in reducing all nematode parameters were observed with royal jelly 1%, 2% and bee venom 0.005%. Numbers of galls, egg masses/root system, eggs/egg mass, females, developmental stages/root system, juveniles/250g soil, nematode final population in addition to nematode reproduction factor showed highly percentage of reduction with royal jelly at the rate of 1% by 98, 96, 93, 98, 88, 92, 99 and 99%, respectively. Propolis with various concentrations was the lowest effective one. Honey bee products with all concentrations had no significant effect on plant growth parameters i.e. fresh shoot and root weights, shoot and root length, proline content as well as the percentage of membrane leakage compared to treated plants with nematode alone. Results found that royal jelly at 1% had only significant effect on fresh shoots weight and proline content compared to nematode alone. Results confirmed also that all honey bee products reduced the percentage of membrane permeability and protected the root cell walls from disturbance compared to nematode alone.

Key words: Tomato; *Lycopersicon esculentum; Meloidogyne* spp.; Propolis; Bee venom; Royal jelly.

INTRODUCTION

Root-knot nematodes, *Meloidogyne* spp., are one of the most damaging plant-parasitic nematode groups worldwide as they cause serious severe yield losses to many economically important plant species in subtropical and tropical regions. Infected plants suffer from vascular damages, which disturb water and mineral uptake (Luc *et al*., 2005). They attack more than 2000 species of plants almost all cultivated plants such as vegetables, ornamentals and….etc (Agrios, 1997). Their infection on tomato is common in Egypt, Italy and worldwide and cause high crop damage especially in light soils (Netscher and Sikora, 1990; Abd-Elgawad and Aboul-Eid, 2001). Root-knot nematodes can be managed effectively by chemical nematicides but many of these nematicides are very expensive and pose human risk and environmental pollutants (Oyedunmade *et al*., 1992; Adegbite and Adesiyan, 2001; Abd-Elgawad, 2008).

Nematologists worldwide during the last decades search the cheaper and safer alternatives to the chemical nematicides i.e. biological and cultural methods to manage plant-parasitic nematodes. Honey bee products and its components were used as antimicrobial (Bogdanov, 2011). Several authors have reported 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

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antagonistic and medicinal properties against bacterial pathogens (Ghanem, 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).

The aim of this research work is to test different honey bee products i.e. royal jelly, bee venom and propolis at various concentrations against root-knot nematode, *M. javanica,* on tomato plants under greenhouse condition.

MATERIALS AND METHODS

Three different honey bee products were used in this work with three concentrations i.e. 1, 2 and 10% (w/w), except bee venom as it used at two concentrations of 0.0025 and 0.005 % (v/w). Honey bee products were obtained from the Experimental farm, Fac. of Agric., Minoufiya Univ., Shebin El-Kom. At transplanting date, royal jelly or propolis were mixed with soil in the top 10 cm layer of the pot. Bee venom was prepared by adding 5 ml tap water with the content of bee venom ampoule then applied as a soil drench around root zone.

This work was conducted at the Experimental Farm, Fac. of Agric., Minoufiya Univ., Shebin El-Kom, Minoufiya governorate, Egypt, under greenhouse condition. Experiment carried out in plastic pots (15 cm in diam.) filled with 2 kg non-sterilized clay-sand mixture soil (1:2, v/v). Three weeks-old tomato seedlings (*Lycopersicon esculentum* Mill.) cv. GS were transplanted into pots (one seedling/pot) and all treatments were applied at the same time of transplanting.

The root-knot nematode, *M. javanica* inocula were obtained from pure culture of tomato heavily infected with *M. javanica* grown under greenhouse condition at 25±2^{o}C. Eggs of *M. javanica* were extracted from heavily galled roots using 0.5% sodium hypochlorite solution (NaOCl) method as described by Hussey and Barker (1973). Two thousand of nematode eggs were inoculated by pipetting into three holes made around the tomato root zone at the same time of transplanting. Each treatment replicated five times and the non-treated plants were served as a control treatment. Plants were arranged in a completely randomized block design in the greenhouse at approximately 25±2^{o}C. Plants were watered daily and fertilized weekly with a 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, number of galls, egg masses, females and developmental stages/root system, number of eggs/egg mass, number of juveniles (J_2)/250g soil, nematode final population (Pf) and reproduction factor (Rf) (Goodey, 1957) were recorded. Egg-masses were stained prior to 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), shoot and root lengths (cm) were recorded. Membrane leakage (ML %) was determined in fresh roots according to Leopold *et al*., (1981), and proline concentration was determined in fresh leaves followed the method of Bates *et al*., (1973).

- The percentage leakage of solutes was calculated as:

Membrane Leakage of substances (ML %) = Initial absorbance of bathing medium /Final absorbance at the bathing medium x 100.

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

P_f = (No. of egg masses/root system X No. of eggs/egg mass) + No. of females/root system + No. of developmental stages/root system + No. of juveniles in soil/250 g soil pot.

- Reproduction factor (Rf) was calculated according to the equation: $R_f = P_f/P_i$ (Norton, 1978) (P_i = initial population).

Females were collected by cutting the root system of each plant to small pieces of 2 cm and submerging in a beaker full of tap water for 4 days at room temperature until they became soft. The roots were then passed through 250 and 500 mesh sieves to separate the females from the root debris (Mahdy, 2002).

Statistical Analysis

Data were statistically analyzed according to standard analysis of variance by a one way ANOVA with the software statgraphics (Statistical Graphics. Crop., Rockville, MD), (1995). Variance homogeneity for all treatments was confirmed by the Bartlett test. The comparison between means was carried out using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS

All the applied honey bee products with all tested concentrations significantly reduced nematode parameters compared to nematode alone treatment (Table, 1). The percentage of reduction in nematode parameters ranged between 29-99%.

Treating the plants with royal jelly with the concentration of 1% led to high reduction in the mean number of galls/root system compared to nematode alone (Table 1). The highest reduction % of nematode galls/root system was recorded with royal jelly 1%, 2% and bee venom 0.005% with 91-98% reduction. The lowest one was obtained with propolis at 2% by 67% as shown in Fig. (1, A).

Application of royal jelly at 1% showed also a significant reduction in number of egg masses and the percentage of reduction recorded 96%, whereas the lowest one obtained with propolis at 1% as recorded 57% (Fig. 1,B). Numbers of eggs/egg masses; females; developmental stages/root system; juveniles in soil; nematode final population as well as reproduction factor were also significantly reduced with all the applied treatments compared to treated plants with nematode alone.

Table (1). Effect of honey bee products at different concentrations on controlling root-knot nematode *Meloidogyne javanica* on tomato plants.

Treatments	Concentration %	Nematode Parameters/ root system						*P_f	**Rf
		No. of galls	No. of egg masses	No. of eggs/ egg mass	No. of females	No. of develop. stages	No. of juveniles /250 g soil		
Royall Jelly	1	2.6 f	0.5 d	30 d	2 b	10 c	335 c	425 e	0.21 e
	2	10 ef	2 cd	60 c	8 b	10 c	336 c	1123 e	0.56 e
	10	16 de	6 bc	80 bc	18 b	60 ab	736 c	1774de	0.89 de
Bee Venom	.005	10 ef	2.5 cd	80 bc	8 b	20 bc	505 c	1164 e	0.58 e
	.0025	33 b	8 cd	110 bc	12 b	20 bc	620 c	4282 c	2.14 c
Propolis	1	31 bc	8.5 b	180 b	18 b	35 bc	805 c	6438 b	3.22 b
	2	21 cd	9 b	200 b	23 b	50 ab	2570 b	6843 b	3.42 b
	10	18 de	5 bcd	90 bc	23 b	20 bc	670 c	2333 d	1.17 d
Nematode alone		72 a	27 a	410 a	84 a	85 a	4010 a	33699a	16.85 a

Columns followed by different letters are significantly different according to Duncan's Multiple Range Test ($p < 0.05$). *P_f = Final population. **R_f = Reproduction factor

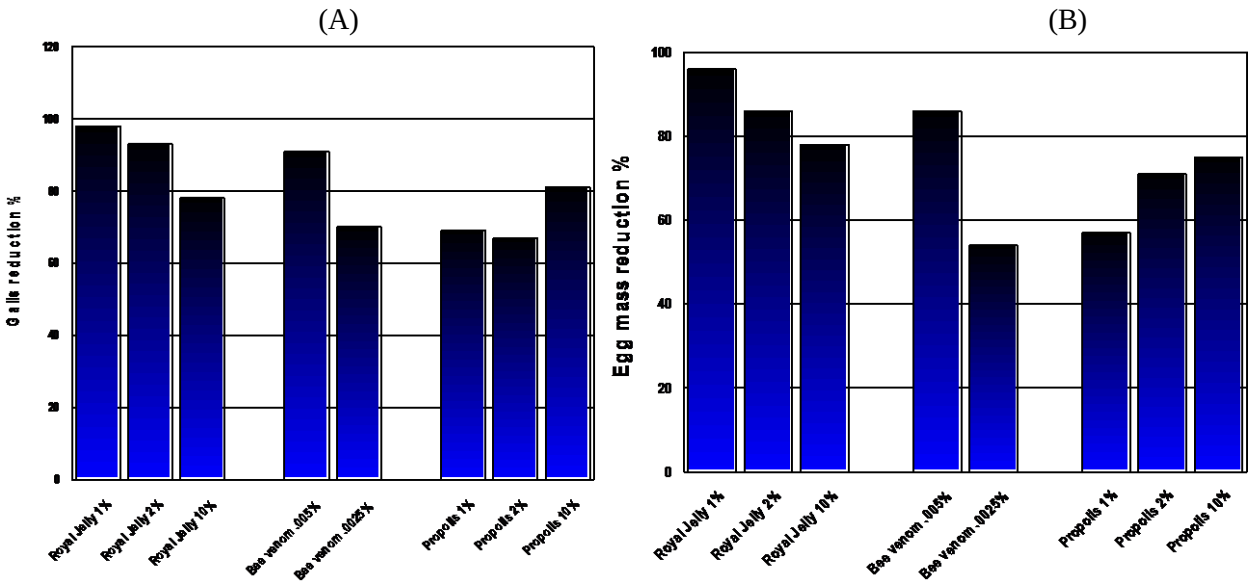

Figure (1): Reduction % of galls (A) and egg masses/root system (B) as affected by honey bee products application at different concentrations.

Reduction percentages of eggs/egg mass, females, developmental stages/root system, juveniles/250 g soil, final nematode population (P_f) in addition to reproduction factor (R_f) were recorded 93, 98, 88, 92, 99 and 99%, respectively with royal jelly application at 1%. Reduction % was recorded 85, 90, 88, 92, 97 and 97%, respectively with royal jelly application at 2% as shown in Figs. (2, A, B, C, D, E&F). The lowest effective one was recorded with propolis at 2%.

Results found that, all applied honey bee products had no significant effect on shoot and root weights and length compared to treated plants with nematode alone (Fig. 3&4). The significant increase was observed only in shoot and root fresh weights with royal jelly application at 1% compared to treated plants with nematode alone (Fig. 3).

Results also revealed that the proline content was affected by all applied honey bee products compared to treated plants with nematode alone as shown in Fig. (5). Royal jelly treatment at 1% recorded the highest significant enhancement of proline content followed by royal jelly at 2% compared to the other treatments. The lowest effect was observed with bee venom at 0.005%.

Results illustrated in Fig. (6) confirmed that the percentage of membrane leakage (ML%) was reduced with all honey bee products treatments at all used concentrations compared to nematode alone treatment. The lowest ML% was recorded with royal jelly treatment at 1%.

DISCUSSION

All evaluated honey bee products with all tested concentrations significantly reduced all nematode parameters i.e. number of galls; egg masses/root system; eggs/egg mass; developmental stages, females/root system, number of juveniles in soil, final nematode population (Pf) as well as the reproduction factor (Rf) compared to nematode alone treatments. Results confirmed that applying the royal jelly at 1%, 2% and bee venom .005% was the effective one in reducing root galls and egg masses nematode parameters.

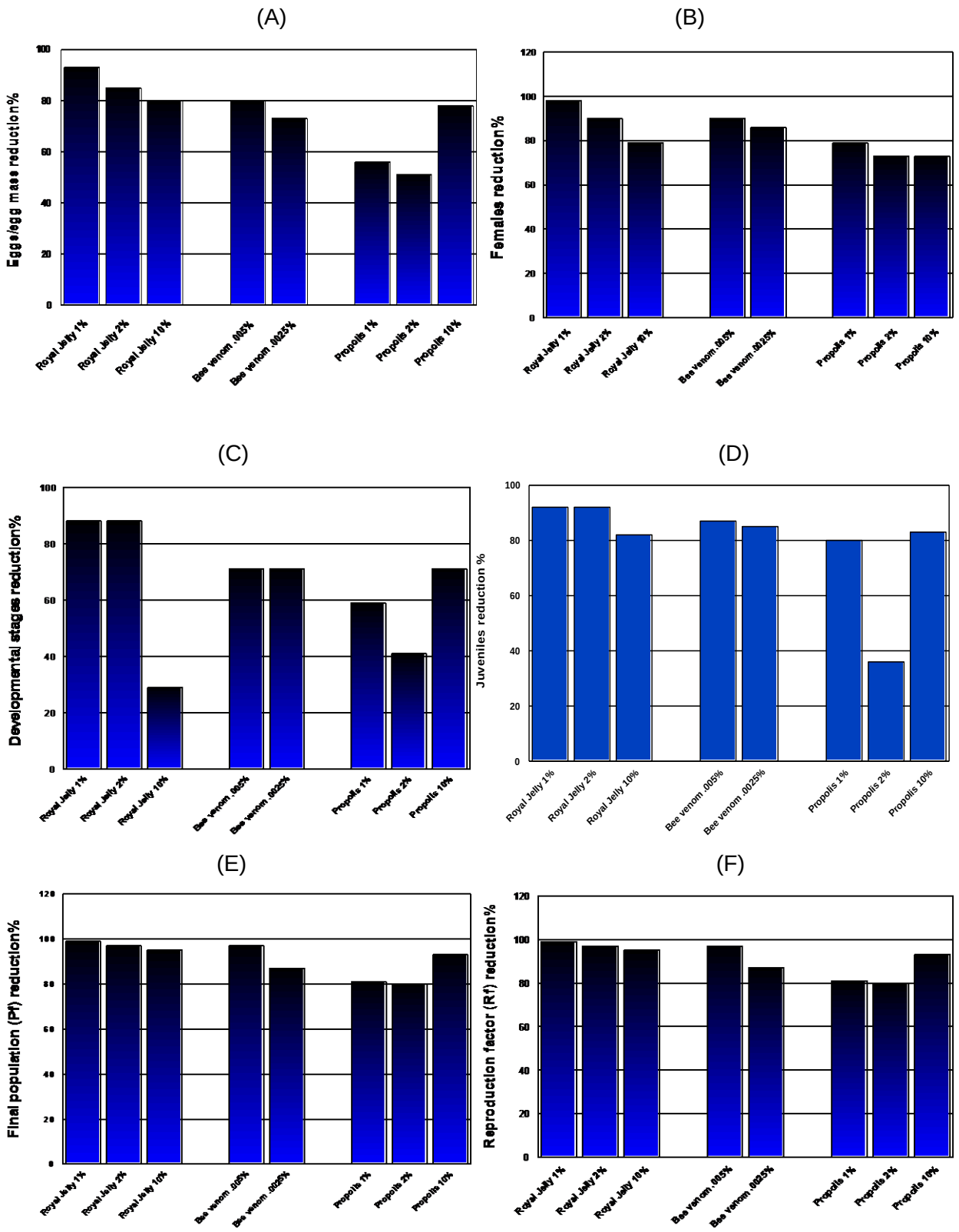

Figure (2): Reduction% of eggs/egg mass (A), females (B), developmental stages (C), juveniles (D), final population (E) and reproduction factor (F) as affected by honey bee products application at different concentrations.

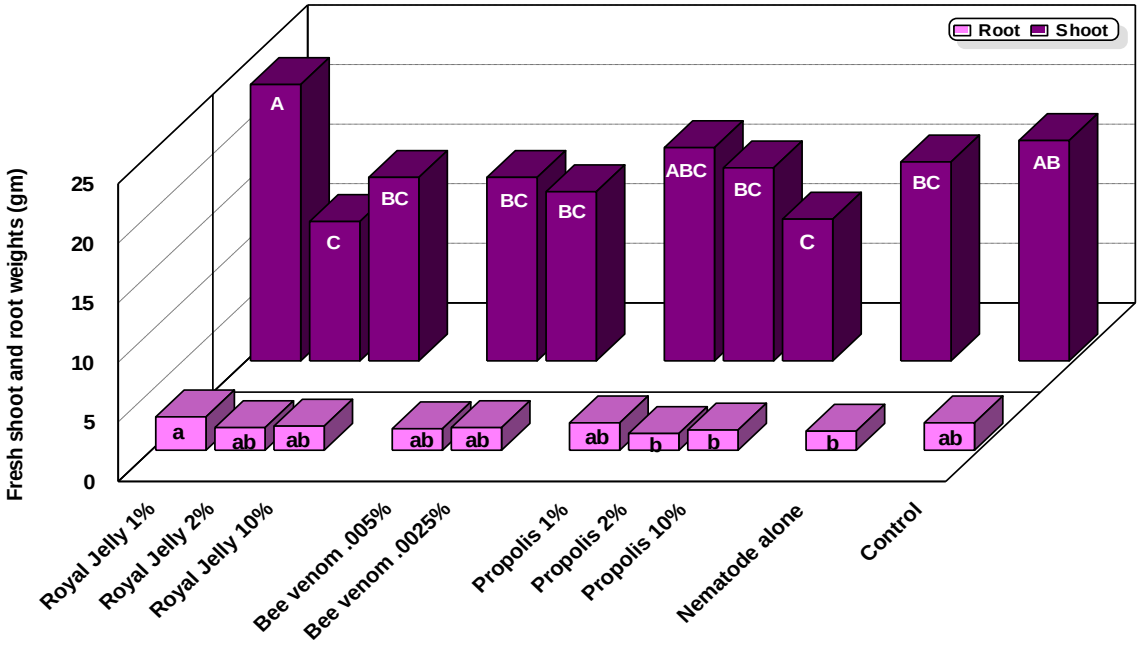

Figure (3): Effect of honey bee products on shoot and root fresh weights of tomato plants infected with root-knot nematode, *M. javanica*.

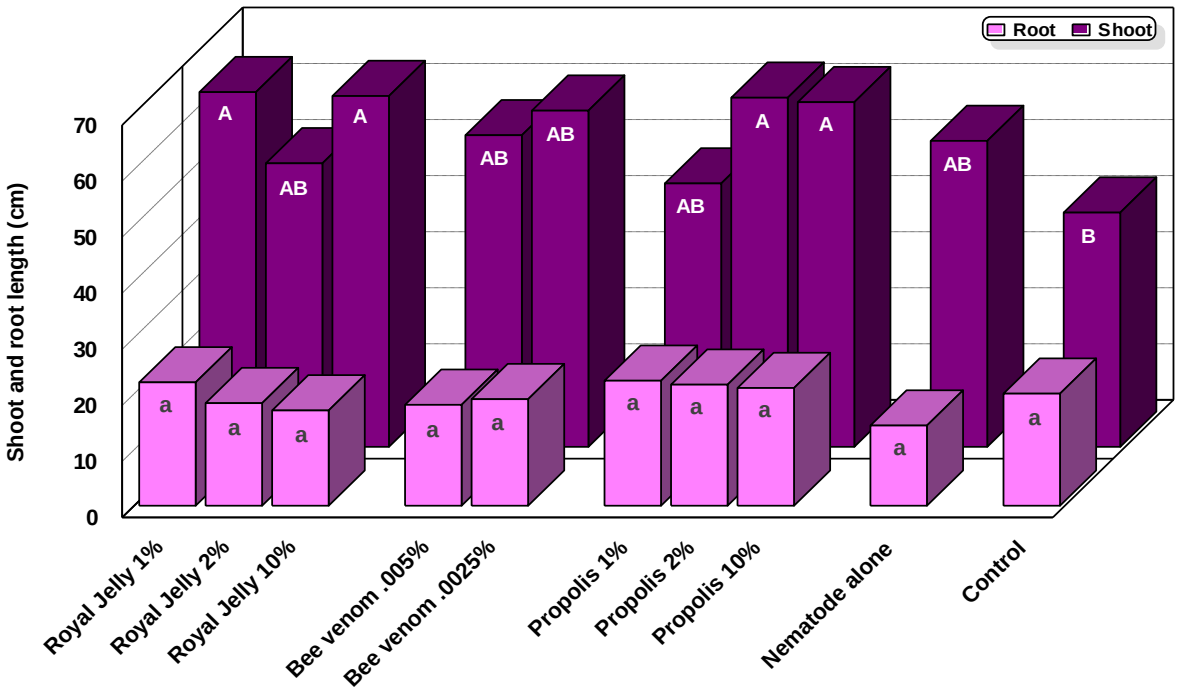

Figure (4): Effect of honey bee products on shoot and root length of tomato plants infected with root-knot nematode, *M. javanica*.

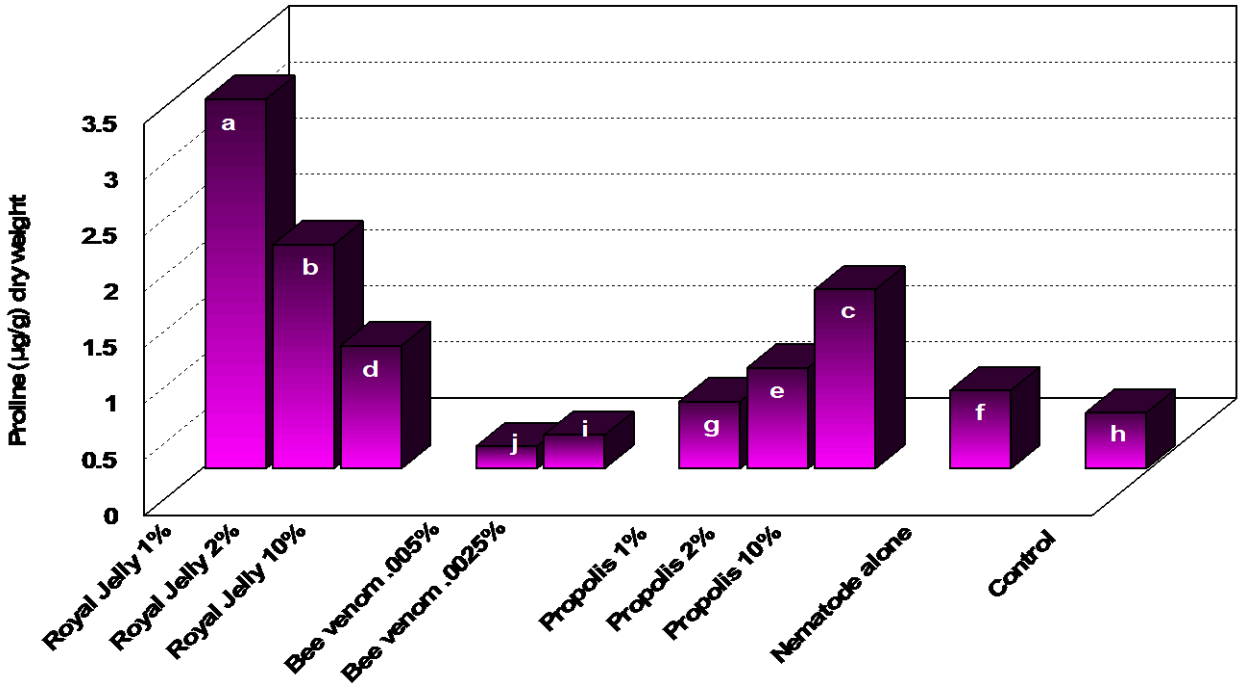

Figure (5): Effect of honey bee products on the contents of proline of tomato plants infected with root-knot nematode, *M. javanica*.

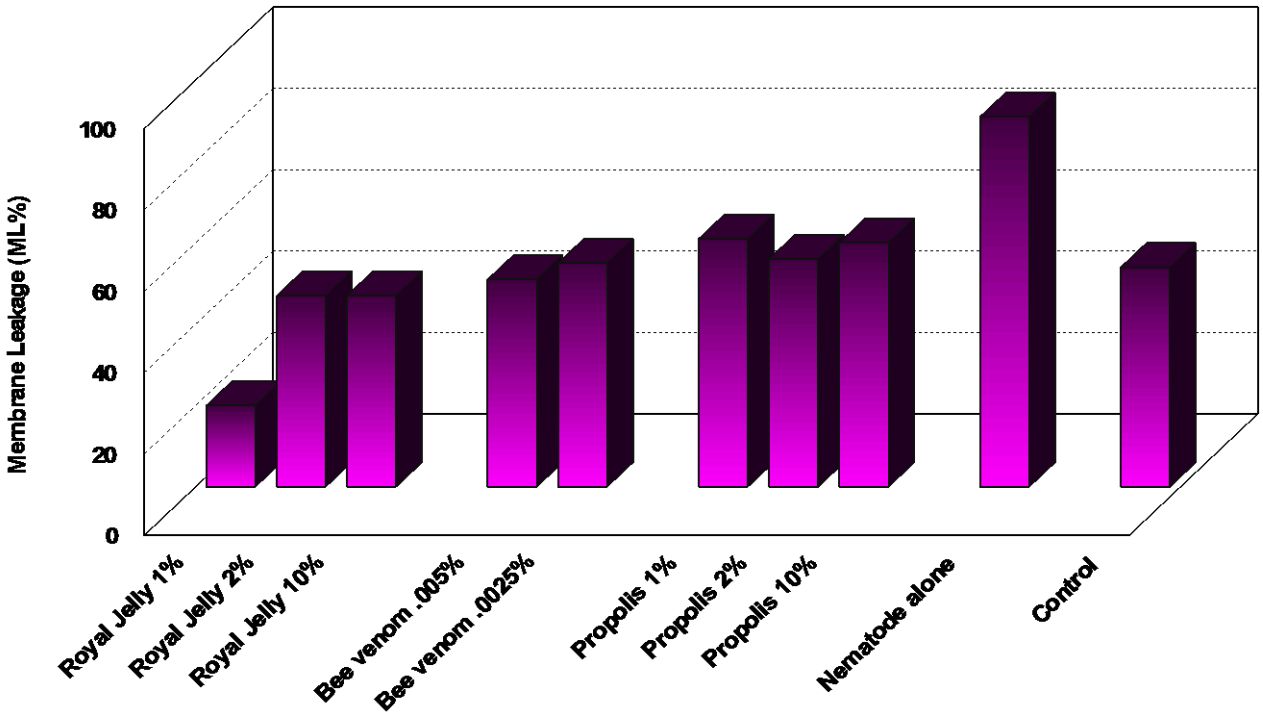

Figure (6): Effect of honey bee products on the percentage of root membrane leakage of tomato plants infected with root-knot nematode, *M. javanica*.

Honey bee 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 substances.

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 low molecular weight compounds present in royal jelly. The antibacterial activity of 10-*hydroxy-trans*-2-*decenoic 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 *Ascospherea 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 the growth of the fungus *A. apis*.

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

The proteins secreted by honey bees into royal jelly and other honey bees 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 *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 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. Moreover, this formation may help in understanding how royal jelly reduced membrane leakage and maintenance cell wall by inhibition of lipid peroxidation.

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

It was suggested that the proline concentration of tomato leaves was a suitable marker for stress induced by both abiotic and biotic factors (Grote and Claussen, 2001). Moreover, Ghasempour *et al*., (2007), reported that the increased proline in leaves due to the higher population of nematodes, might be indicate the adaptive osmoregulation or acclimations responses in plants to the nematodes biostress by increasing metabolites and solutes, which increase plant resistance. Moreover, it can be noticed that, royal jelly record a highly significant increases in proline concentration and a maximum value was by 1% royal jelly, which may be increased tomato resistance to nematode infection.

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 and carotenoid faba bean plants.

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