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Nematicidal Activity of Honey Bee Propolis and Venom Against Root-Knot Nematode *Meloidogyne incognita* Infecting Strawberry Plants

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Abstract: Phytoparasitic nematodes are harmful agricultural pests, because the heavy yield losses caused to a large range of crops worldwide. Lack of effective nematode management products raised the demand of innovative nematode management tools. The efficacy of two types of bee propolis viz. Baladi and Chinese as well as bee venom on egg hatching inhibition and juveniles mortality of *Meloidogyne incognita* was conducted. All treatments were found to cause significant inhibition in hatching rate and juveniles mortality to various extents. However, a positive correlation was achieved among tested concentrations. The effect of two types of bee glue (propolis) applied at two concentrations viz. 1000 and 3000 ppm as well as venom at 2000 and 6000 ppm on the growth of strawberry cv. Festival infected with *M. incognita* under greenhouse conditions was tested. Results indicated that *M. incognita* infection caused an obvious reduction in plant growth parameters with a reduction percentage in total plant fresh weight and shoot dry weight reached 50.4 & 12.5 % for the first season and 64.3 & 50 % for the second season, respectively. The highest significantly improvement in total plant length was recorded with venom treatment at 2000 and 6000 ppm with a percentage of increase reached 37.5 and 52.7%, respectively. In addition, the best augmentation in total plant fresh weight (40.2%) and shoot dry weight (187.5%), was recorded with venom treatment at 6000 ppm followed by propolis Baladi (28.8% and 100.0%, respectively) at 3000 ppm. The best treatment in increasing peroxidase, polyphenol oxidase activity, proline, sucrose and TSS values was performed by bee venom. Bee venom recorded the highest significant reduction in root galling (55.3 & 66.1%) at the higher concentration (6000 ppm) at the two growing seasons. While, Propolis Baladi (100.0% at 2018) and bee venom (88.9 & 96.4%) recorded the highest significant reduction in the number of egg masses at higher concentrations in the two growing seasons. Bee venom can be using as natural agent in the IPM program for controlling *M. incognita*. Further studies need to assess the bee products effect under field conditions.

Key words: Root-knot nematode · *Meloidogyne incognita* · Strawberry · Propolis · Bee venom

INTRODUCTION

Phytoparasitic nematodes are harmful agricultural pests, because of heavy yield losses caused to a wide range of crops and their worldwide occurrence. Lack of effective nematode management products following the EU revision of pesticide use in agriculture has raised a large demand for new innovative nematode management tools combining the nematicidal effectiveness with environmental and human health safety.

Many economically important and highly valued fruits come from family Rosaceae including cherries, raspberries and strawberries. They are considered as an important source of nutrients, riching in fibers, vitamins, minerals and carbohydrates and poring of fats, proteins and calories. They are highly appreciated due to their delicious flavors and aromas. In addition, they are also rich in natural antioxidants that include vitamins, carotenoids and high content of phenolic compounds, especially anthocyanins and their consumption has been

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related to potential health benefits [1, 2]. The beneficial properties of phenolic compounds revealed to its ability to scavenge free radicals [3]. Strawberry is an important crop worldwide, which mainly produced in China, USA, Mexico, Egypt, Turkey and Spain [4]. Egypt is the fourth-largest producer of strawberries in the world [5]. However, several species of plant-parasitic nematodes have been estimated as causing damage to strawberries and the northern root-knot nematode (RKN) *Meloidogyne hapla* and the northern root-lesion nematode (RLN) *Pratylenchus penetrans* are the most important nematode pests worldwide [6, 7]. In addition, 12 % is the annual yield losses of strawberry due to damage by plant-parasitic nematodes in Egypt [8]. Honey bees *Apis mellifera* collect and produce propolis or "bee glue" from plant exudates and mixing with their saliva and beeswax and used by bees to provide thermal insulation, seal hive cracks as well as protect bees from predators and microorganisms [9, 10]. It is composed of resin (50%), wax (30%), essential oils (10%), pollen (5%) and other organic compounds (5%) [11, 12]. Furthermore, there are important organic compounds present in propolis such as phenolic compounds, esters, flavonoids, terpenes, beta-steroids, aromatic aldehydes and alcohols [13]. Different flavonoids, vitamins, minerals and enzymes also detected in propolis extracts [3, 14]. In addition, honey bee venom is a complex acidic mixture of proteins, peptides, enzymes and a variety of smaller molecules (amino acids, catecholamines, sugars and minerals) and more than 60 identifiable components in bee venom are found [15]. Melittin, which the most dominant substance in bee venom, appears to have antimicrobial, antitumor and anti-inflammatory properties and has indicated its ability to fight diseases [16]. Additionally, the proteins phospholipase A2 (PLA2), apamin and MCD peptide are the most toxic components [17].

Therefore, the main goal of this work was to characterize the nematicidal activity of bee propolis and venom against *Meloidogyne incognita* infecting strawberry.

MATERIALS AND METHODS

The experiments were conducted with collaboration of Plant Pathology Res. Inst. and Plant Protection Res. Inst., Agricultural Research Centre (ARC), Egypt.

Honey Bee Colonies and Study Area: Field experiments for collecting and producing honey bees propolis and venom were conducted at Research and Training Station Apiary, King Faisal University, Al-Ahsa Province, Eastern Region, Kingdom of Saudi Arabia during 2017. Honey bee colonies (*Apis mellifera yemenitica*) headed by newly queen and nearly equal strength in the brood, bees and food were used. Laboratory and greenhouse nematode experiments and chemical analysis were conducted at Department of Nematodes Diseases and Central Lab of Biotechnology, Plant Pathology Research Institute, Agriculture Research Center, Egypt.

Propolis (Resin) Sample Collection: At the end of the sidr honey season (November, 2017), propolis resin (Baladi propolis) was harvested by scraping propolis from the frame edges and rests, the bottom boards and insides of hive boxes. Scrapings may contain propolis from multiple seasons [18]. Chinese commercial propolis was used for comparing with Baladi propolis.

Extraction of Propolis: A methanol solvent was used to extract major plant secondary metabolites from any impurities, (i.e., beeswax) for further analysis or biotests. Propolis was kept overnight in a freezer (20°C) and then cut to small pieces. A sample of propolis was measured and Methanol solvent (1:30 w: v) was added and kept at room temperature for 24 h. Then, the suspension (propolis in Methanol solvent) sonicated in an ultrasonic bath at 20°C for 20 min. The obtained suspension was filtered using a filter paper at room temperature and the procedure was repeated with the part trapped in the filter, the residue was extracted again under the same conditions [19]. For further experiments, the obtained extract will be evaporated to dryness [20-22].

Bee Venom Collection: Bee venom was collected every 15 days for 20 minutes using the Bee Venom Collector Device. Such a device was put on top of the hives. The dry venom is collected using a sharp scraper. The fresh dried bee venom was carefully packed into a special container and stored in a dry and cool place until the experiment was done [23].

Pesticide: Oxamyl: (Vydate 24% E.C.) Methyle – N– N– dimethyl – (N (methyle) carbomycocyl) - 1- hioxamidate.

Laboratory Experiment: *In vitro* test was desioned to evaluate the effect of some honey bee products (propolis and bee venom) on egg hatching in addition to motility, viability and infectivity of nematode juveniles (J_2s). Egg masses (10 egg masses/dish) were exposed to different treatments for different incubation times and then subjected to a hatching test. Motility, viability and infectivity assays were carried out on J_2s in the same

conditions above reported (concentrations x exposure times) in pluronic F-127 plates. All observations were performed by stereomicroscope.

Methanol solvent used for two types of propolis (Baladi and Chinese) and bee venom extracts. Such extracts were tested against second-stage juveniles (J_2) of the root-knot nematode, *M. incognita*, comparing to nematodes alone in Petri dishes (5 cm-diameter) where there are five replicates for each extract. Three concentrations (0.0025, 0.050 & 0.1 µL) were taken from each solvent. Four mL of each treatment were added to one mL of *M. incognita* (100 J_2s) inocula per Petri dish. The dishes were examined under a microscope after 24, 48 and 72 hours to study the effect of such extracts on the activity of second-stage juveniles of root-knot nematode, whereas Petri dishes with egg masses were left for 7days to study the eggs hatchability under laboratory conditions.

Greenhouse Experiments: A greenhouse experiment was conducted to evaluate the efficacy of above mentioned treatments against *M. incognita* infecting strawberry. Plastic pots (15cm diameter) filled with loamy-sandy soil were planted with strawbery seedlings (25 days old) cv. Festival. 1000 juveniles (J2s) of pure *M. incognita* were inoculated into holes nearby the root hairs of every seedling after 7 days of plantation. Two concentrations of methanolic extracts of each type of propolis ; Baladi and Chinese & bee venom extracts were used as follows: 1000 and 3000 ppm & 2000 and 6000 ppm, respectively. One week later, plants were treated with the selective materials as soil drench with rates as 1, 3 mL/pot for both propolis type treatments and 2, 6 mL for bee venom treatment in soil condition during two seasonal growing seasons. Five pots were treated with Oxamyl (10%G) as a standard nematicide @ the rate of 0.3g /pot. However, five pots were left free of nematode infection and any treatment to serve as control (Ck1). Another five pots were received nematode alone and served as control (Ck2). Sixty days after nematode inoculation, plants were carefully uprooted for further examination. At the end of the experiments, the different plant growth parameters as well as the different nematode parameters were assessed to determine the role of tested treatments in improving the quantity and quality of the target crop and depressing the nematode population to the level at which they can't cause disease. Nematode reproduction factor (Rf) was assessed 60 DAI. Treatments were compared using ANOVA.

Chemical Analysis: Plant phenotypic traits (proline content and total phénol) were determined in fresh leaves. Proline content was determined by the colorimetric acid ninhydrin method [24]. Total phenols determination was carried out according to Somerfield [25]. Peroxidase and polyphenol oxidase activities were evaluated according to Amako [26] and Coseteng [27]. Enzyme extracts were prepared, according to Melo [28]. Fruit characters (total sugar solids (TSS) with (°Brix) were determined through refractometry) and sucrose was also analyzed.

Statistical Analysis: The obtained data were tabulated to the analysis of variance program (ANOVA) according to Gómez-Caravaca [11] followed by the Multiple Range Test to compare means [29]. Median lethal concentration (LC_{50}) was estimated for each treatment.

RESULTS

Laboratory Experiment

Nematicidal Properties of Bee Glue and Venom Against Egg Hatching and Juveniles Survival of Root-knot Nematode *Meloidogyne incognita* under Laboratory Conditions: Data in the Table (1) represent the impact of two types of bee glue viz. propolis Baladi and propolis Chinese as well as bee venom at different concentrations (0.0025, 0.050 & 0.1 µL) on egg hatching inhibition and juveniles mortality of *Meloidogyne incognita*. Irrespective of tested concentrations, all treatments were found to cause significant inhibition in hatching rate and juveniles mortality to various extents. Among all treatments, bee venom concentrations showed better results than did those of both of propolis. However, a positive correlation was achieved among tested concentrations.

Meanwhile, the higher the concentrations the greater inhibition in egg hatching and juveniles mortality was recovered. The previously mentioned treatments at two tested concentrations showed nematicidal activity against newly hatched juveniles of *M. incognita* survival after 72h of exposure (Table 1). A positive correlation among all treatments at different concentrations was observed.

Greenhouse Experiments: Impact of bee glue and venom on the growth of strawberry infected with *Meloidogyne incognita* and nematode reproduction:

Data in Table (2) summarize the effect of two types of bee glue applied at two concentrations viz. 1000 and 3000 ppm as well as venom at 2000 and 6000 ppm on the

Table 1: Impact of two types of propolis bee glue and venom on *Meloidogyne incognita in vitro*

		Exposure period % of juveniles Mortality			
Treatments	Conc. µL	24 H	48 H	72 H	% Hatchability after 7 days
Propolis Baladi	0.0025	16.0±2.30 fg	28.0±2.00 d-g	44.7±2.90 cd	38.0±4.35 e-h
	0.050	29.7±4.48 d-g	38.7±2.40 cd	52.7±1.76 c	44.0±3.05 ef
	0.1	36.7±3.33 de	68.0±11.13 b	75.4±2.90 b	52.7±5.69 de
LC 50		1.0434	0.0428	0.0081	0.1183
Propolis Chinese	0.0025	13.4±1.70 gh	25.0±4.35 gh	44.4±5.69 ef	26.0±3.60 gh
	0.050	20.0±2.30 e-g	27.0±1.73 f-h	45.4±2.90 e	27.0±3.46 f-h
	0.1	34.0±5.29 de	39.4±5.66 e-g	64.7±6.35 cd	37.4±3.92 e-h
LC 50		2.3535	11.5998	0.0185	171.0766
Bee venom	0.0025	27.0±1.73 f-h	33.0±2.51 de	93.4±6.66 a	64.7±6.35 cd
	0.050	39.4±5.66 e-g	44.7±2.90 cd	97.0±3.00 a	86.7±13.33 ab
	0.1	73.7±10.80 bc	84.0±12.22 ab	100.0±0.00 a	100.0±0.00 a
LC 50		0.0346	0.0165	0.0001	0.001
Nematode alone		0.0±0.00 h	0.0±0.00 h	0.0±0.00 h	16.0±5.33 i

Each value is the mean of five replicates ± SE = Standard Error

Means in each column followed by the same letter (s) did not differ at P< 0.05 according to Duncan's multiple- range test

growth of strawberry cv. Festival infected with *M. incognita* under greenhouse conditions at 26±5. The experiments were repeated twice during 2018 and 2019.

Results indicated that *M. incognita* infection caused an obvious reduction in plant growth parameters with a reduction percentage in total plant fresh weight and shoot dry weight reached 50.4 & 12.5 % for the first season and 64.3 & 50.0 % for the second season, respectively. Irrespective of tested concentrations, most treatments, showed a moderate increase in shoot and root lengths and plant biomass exceeded that of untreated inoculated plants with various degrees. However, a positive correlation between tested concentration and strawberry growth parameters was recorded in most treatments. Bee venom treatments showed better results than did bee glue. Meanwhile, the highest improvement in strawberry total plant length was significantly recorded with venom treatment at 2000 and 6000 ppm with a percentage of increase reached 37.5 and 52.7%, respectively. On the other hand, the best augmentation in total plant fresh weight (40.2%) and shoot dry weight (187.5%), was recorded with venom treatment at 6000 ppm followed by propolis Baladi 28.8% and 100.0%, respectively at 3000 ppm. Propolis Chinese treatments, exhibited the highest performance in total plant fresh weight (13.9%) and shoot dry weight (25.0%) at the same concentration. Conversely, venom treatment at the highest concentration showed phytotoxicity as expressed in the second season of growing. It is worth noting the significant differences between the two seasons of strawberry growth (Table 2).

Means in Each Column Followed by the Same Letter (S) Did Not Differ at P< 0.05 According to Duncan's Multiple- Range Test: Results shown in Table (3) revealed that the total nematode population was significantly suppressed by all tested treatments with reproduction factor (Rf) ranged from 0.56 to 1.19 and 1.7 to 3.6 for the two seasons, respectively. Among tested treatments, bee venom performed the best and significantly suppressed the total root-knot nematode population with the percentage of reduction ranged from 65.1 to 69.7% and 69.7 and 72.6 % for both seasons, respectively. Apparently, the higher the concentration of treatments the greater suppression in the nematode population was observed. Propolis Baladi (66.4 & 64.1 %) gave greater suppression in the nematode population whether in soil or roots at the concentration of 3000 ppm than did other ones for the two seasons.

Moreover, between treatments, pots receiving Propolis Chinese showed no significant differences ($P \leq 0.05$) in the nematode population in the soil at higher concentrations (3000 ppm) at the two growing seasons. Root galling and the number of egg masses were significantly reduced by all treatments with % reduction ranged from 2.6 to 65.8 & 2.6 to 66.1 %, respectively and from 0.0 to 100 % at the two growing seasons, respectively. Bee venom recorded the highest significant reduction in root galling (55.3& 66.1%) (Table 3) at higher concentrations (6000 ppm) at the two growing seasons whilst Propolis Baladi (100.0% at 2018) and bee venom (88.9 & 96.4%) recorded the highest significant reduction in the number of egg masses at higher concentrations in the two growing seasons (Table 3). Oxamyl as a standard nematicide was superior and showed the highest reduction percentages in total nematode population (72.6 & 66.4 %), the number of galls (65.8 & 53.9 %) and egg masses (100 %) in the two growing seasons, respectively.

Table 2: Impact of two types of bee glue as well as venom at different concentrations on the growth of strawberry cv. Festival infected with *Meloidogyne incognita* under greenhouse conditions

		Plant Growth Response for 2018						Plant Growth Response for 2019					
Treatments	Conc. ppm	T. plant length	% Inc.	T. Fresh weight	% Inc.	Dry weight	% Inc.	T. plant length	% Inc.	T. Fresh weight	% Inc.	Dry weight	% Inc.
Propolis Baladi	1000	34.8^{c}	16.8	27.0^{e}	1.5	1.0^{cd}	25.0	55.3^{cd}	12.9	32.2^{efg}	15.0	2.0^{c}	45.3
	3000	38.8b^{c}	30.2	34.3^{bc}	28.8	1.6^{b}	100.0	56.0^{abc}	14.3	35.7^{de}	27.5	2.5^{b}	74.4
Propolis Chinese	1000	36.3^{c}	21.8	27.0^{e}	1.5	0.9^{d}	12.5	53.3^{bcd}	8.8	30.9^{fg}	10.4	1.1^{ef}	17.4
	3000	38.5^{bc}	29.1	30.3^{de}	13.9	1.0^{cd}	25.0	55.5^{bc}	13.3	33.0^{ef}	17.9	2.0^{c}	39.5
Bee venom	2000	41.0^{b}	37.5	33.3^{cd}	25.2	1.5^{b}	87.5	57.8^{ab}	18.0	37.9^{cd}	35.4	1.9^{cd}	61.6
	6000	45.5^{a}	52.7	37.3^{ab}	40.2	2.3^{a}	187.5	60.5^{a}	23.5	40.9^{bc}	42.9	3.0^{a}	109.3
Oxamyl		48.3^{a}	62.0	36.8^{abc}	38.3	1.2^{c}	5.0	51.3^{cd}	4.7	43.0^{ab}	53.6	3.0^{a}	132.6
Plant control		47.8^{a}	60.4	40.0^{a}	50.4	0.9^{d}	12.0	52.0^{cd}	6.1	46.0^{a}	64.3	1.5^{de}	150.0
Nematode alone		29.8^{d}	0.0	26.6^{e}	0.0	0.8^{d}	0.0	49.0^{d}	0.0	28.0^{g}	0.0	1.0^{f}	0.0
LSD 5%		4.099	---	3.708	---	0.2632	---	4.997	---	4.207	---	0.401	---

Each value is the mean of five replicates

Means in each column followed by the same letter (s) did not differ at P< 0.05 according to Duncan's multiple- range test.

Table 3: Impact of two types of bee glue as well as venom on development and reproduction as influenced by the addition of different concentrations of *Meloidogyne incognita* under greenhouse conditions

		Reproduction of nematode for 2018							Reproduction of nematode for 2019						
Treatments	Con. ppm	T. Population	% Red.	*Rf	Galls	% Red-	Egg masses	% Dec.	T. population	% Dec.	*Rf	Galls	% Dec.	Egg masses	% Dec.
Propolis Baladi	1000	909.0^{bc}	55.8	0.91	23.0^{ab}	39.5	3.0^{ab}	66.7	3573.0^{b}	42.0	3.6	70.0^{ab}	39.1	12.0^{ab}	57.1
	3000	690.0^{c}	66.4	0.69	17.0^{d}	55.3	0.0^{b}	100.0	2154.0^{bc}	65.1	2.2	71.0^{ab}	38.3	4.0^{ab}	85.7
Propolis Chinese	1000	1216.0^{b}	40.8	1.2	37.0^{ab}	2.6	9.0^{a}	0.0	2729.0^{bc}	55.7	2.7	112.0^{a}	2.6	28.0^{a}	0.0
	3000	1191.0^{b}	42.0	1.19	23.0^{ab}	39.5	4.0^{ab}	55.6	3648.0^{b}	40.8	3.6	93.0^{ab}	19.1	9.0^{ab}	67.9
Bee venom	2000	718.0^{c}	65.1	0.72	21.0^{c}	44.7	1.0^{ab}	88.9	1870.0^{c}	69.7	1.9	63.0^{ab}	45.2	4.0^{ab}	85.7
	6000	623.0^{c}	69.7	0.62	17.0^{d}	55.3	1.0^{ab}	88.9	1689.0^{c}	72.6	1.7	39.0^{bc}	66.1	1.0^{b}	96.4
Oxamyl		563.0 ^{c}	72.6	0.56	13.0^{c}	65.8	0.0^{b}	100.0	2070.0^{c}	66.4	2.1	53.0^{bc}	53.9	0.0^{b}	100
Nematode alone (N)		2055.0 ^{a}	0.0	2.1	38.0^{a}	0.0	9.0^{a}	0.0	6165.0^{a}	0.0	6.2	115.0^{a}	0.0	28.0^{a}	0.0
LSD 5%		498.29	---	---	18.18	---	8.4		1494.88	---	---	54.53	---	25.22	---

Means in each column followed by the same letter(s) are not significantly different (P ≤ 0.05) by Duncan's multiple range test.

Each value is the mean of five replicates N = 1000 j_2 of root-knot nematode

*Reproudction factor (Rf) = No. of Nematode in soil + developmental stages + females + egg masses x eggs/ egg mass) in root No. of juveniles inocula

Table 4: Impact of two types of bee glue and venom on technological characters as well as peroxidase (PO) and polyphenol oxidase (PPO) activities in strawberry var. festival infected with *Meloidogyne incognita* under greenhouse conditions

		Technological characters for 2018						Technological characters for 2019					
Treatments	Con. ppm	Sucrose%	TSS%	Phenol	Proline mg/g	PO	PPO	Sucrose%	TSS %	Phenol	Proline mg/g	PO	PPO
Propolis Baladi	1000	28.2 abc	6.9 de	0.677b(29.0)*	1.3c(34.0)	0.58a(2.8)	0.072cd(27.3)	28.5 c	7.7 bc	0.622d(35.1)	1.6d(61.6)	1.384c(21.8)	0.199d(42.8)
	3000	29.9ab	7.7 bc	0.636be(33.3)	1.6b(64.9)	0.592a(1.2)	0.070d(29.3)	28.9 bc	7.9b	0.621d(35.3)	1.8cd(81.8)	1.207cd(31.8)	0.183e(47.4)
Propolis Chinese	1000	28.1bcg	6.7 e	0.611bcd(36.0)	0.97d(0.0)	0.593a(1.0)	0.087ab(12.1)	27.9 c	6.6 de	0.715c(25.4)	0.99e(0.0)	1.543b(12.8)	0.282c(19.0)
	3000	28.3abce	7.6 e	0.618bcd(35.2)	0.99d(2.1)	0.598a(0.17)	0.083bc(16.2)	27.9 c	6.9 cde	0.793b(17.3)	1.1e(11.1)	1.758a(0.62)	0.293b(15.8)
Bee venom	2000	29.7ab	8.3 ab	0.585cd(38.7)	1.2c(23.7)	0.502a(16.2)	0.053e(46.5)	29.4bc	8.1 ab	0.619d(35.5)	1.9bc(91.9)	1.306cd(26.2)	0.188e(46.0)
	6000	30.4a	8.6 a	0.574cd(39.8)	1.8b(85.6)	0.499a(16.7)	0.032f(67.7)	34.5a	8.9 a	0.536e(44.1)	2.1b(112.1)	0.977fg(44.8)	0.167f(52.0)
Oxamyl		29.5ab	7.0 cde	0.570cd(40.3)	2.1a(116.5)	0.303b(49.4)	0.029f(70.7)	31.0b	7.5 bcd	0.421f(56.1)	2.5a(152.5)	0.864g(51.2)	0.084g(75.9)
Plant control		28.0bc	6.5 e	0.518d(45.7)	0.28e(-71.1)	0.594a(0.83)	0.054e(45.5)	30.1bc	7.8 b	0.519e(45.9)	0.30f(-69.7)	1.094ef(38.2)	0.173f(50.3)
Nematode alone		26.0c	5.5 f	0.954a(0.0)	0.97d(0.0)	0.599a(0.0)	0.099a(0.0)	25.0d	6.5 e	0.959a(0.0)	0.99e(0.0)	1.769a(0.0)	0.348a(0.0)
LSD 5%		2.210	0.787	0.098	0.214	0.144	0.012	2.374	0.945	0.068	0.240	0.120	0.009

Each value is the mean of five replicates

Means in each column followed by the same letter(s) are not significantly different (P ≤ 0.05) by Duncan's multiple range tests.

*Number between parenthesis represent% of ±Dec. or ±Inc. in parameters

The effect of various treatments at different concentrations on chemical components of the fresh leaves of strawberry infected with root-knot nematode *M. incognita* was tabulated in Table (4). It can be illustrated that all treatments understudying significantly increased Peroxidase compared to a healthy plant, but the treatments with nematode alone were recorded the highest increase in the enzyme activities after sixty days. The best treatment in increasing peroxidase and polyphenol oxidase activity was by the highest concentration of bee venom. Whenever the lowest increase in enzyme activities was obtained by the lowest concentration of Propolis Chinese at the two seasons.

Furthermore, all treatments increased significantly (P<0.005) the proline concentration compared to the healthy plant and infected ones. The best treatments for increasing proline values were bee venom followed by Propolis Baladi by the highest concentrations at the two

seasons. Total phenols in infected strawberry plant leaves was greater than that of untreated uninoculated plants with a percentage of increase amounted to 45.7 and 45.9 % for both seasons. All tested treatments exhibited lower levels in phenol content at the end of the experiment. However, the greater suppression in phenol content was recorded with venom. Technological characters in terms of sucrose and total soluble solids (TSS) in fruits of strawberry infected with *Meloidogyne incognita* and treated with mentioned treatments have been evaluated (Table 4). All treatments induced physiological changes in such parameters with various degrees. The higher concentrations showed better results than did lower ones. Out of all treatments, bee venom performed the best followed by Propolis Baladi with the percentage of increase in sucrose and TSS reached 30.4; 29.9, 8.6; 7.7 for both, respectively in the first season. The same trend was noticed in the second season with values 34.5; 28.9, 8.9; 7.9 for both, respectively.

DISCUSSION

This investigation was designed to demonstrate the effect of two types of bee glue applied at two concentrations viz. 1000 and 3000 ppm as well as venom at 2000 and 6000 ppm *in vitro* and *in vivo* on the growth of strawberry cv. Festival infected with *M. incognita* and chemical constituents changes as well. *In vitro* study revealed the antagonistic activity of tested honeybee products including two types of bee glue (Propolis Baladi and Chinese) and venom against egg hatching and second-stage juveniles (J_2) survival. Such treatments varied in their efficacy at different concentrations. Nematode mortality and egg hatching inhibition percentages increased proportionally to the concentrations of tested treatments. Venom significantly exhibited the greatest effect on nematode mortality and egg hatching followed by propolis Baladi with LC_{50} amounted to 0.001, 0.001 & 0.0081, 0.1183, respectively. These results support the findings that bee venom contains at least 18 pharmacologically active ingredients, including various enzymes, peptides and amines [30]. Sulfur thought to be the key component to cause cortisol release from the adrenal glands. Melittin, which is the major component of bee venom was found to suppress inflammation by inhibiting phospholipase (PLA) enzymatic activity [21]. This enzyme was abundantly released in extreme inflammatory disorders and actively found to cause organ and tissue deterioration. This leads to the loss of their functions [30]. Furthermore, melittin also found to block the production of neutrophil superoxide and analgesic because it blocks cyclooxygenase [31]. The apamine, melittin, phospholipase and hyaluronidase in bee venom can block or inhibit the nervous system. Phospholipase A2 contains 10-12% of peptides and is the most damaging portion of apitoxin. It is an enzyme which degrading the phospholipids that cellular membranes are made of fatty-acid-based lipids and proteins. Membrane lipids are principally of two types, phospholipids and sterols [30]. Moreover, propolis has demonstrated favorable effects such as antimicrobial, antifungal and antibacterial activities and may minimize the activity of free radicals (ROS) [32]. These effects are related to propolis components such as flavonoids, phenolic compounds, terpenes and enzymes. Phenolic compounds are bioactive organic compounds with an aromatic ring that are bonded chemically to one or more hydrogenated substituents in the presence of corresponding functional derivatives. That is illustrated the nematicidal efficacy of bee venom and propolis on J_2s and egg hatching directly *in vitro*.

In vivo studies revealed that the root-knot nematode, *M. incognita* caused a significant reduction in plant growth parameters (shoot and root length, shoot weight) with reduction percentage in total plant fresh weight and shoot dry weight reached 50.4 & 12.5 % for the first season and 64.3 & 50 % for the second season, respectively. Application of propolis Chinese exhibited the highest performance in total plant fresh weight (13.9%) followed by shoot dry weight (25.0%) at the same concentration. Propolis has recently been widely used in beverages and food to improve health and prevent diseases because of the wide range of its biological activities [33, 34]. However, the best augmentation in total plant fresh weight (40.2%) and shoot dry weight (187.5%) was recorded with venom treatment at 6000 ppm. Moreover, bee venom (65.1 to 69.7%; 69.7 to 72.6 %) performed the best and significantly suppressed the total root-knot nematode population for the two seasons, respectively. Apparently, the higher the concentration of treatments the greater suppression in the nematode population was observed. Propolis Baladi (66.4 & 64.1 %) gave greater suppression in the nematode population whether in soil or roots at the concentrate of 3000 ppm than did other ones for the two seasons. Findings of Maxwell, [35] confirmed the current evidence regarding total nematode population, root galling and the number of egg masses of *M. incognita* infecting strawberry. Moreover, soil drench with propolis increased the protein content of *faba* bean plants [7]. Data showed that

the propolis extract as soil drench reduced *Meloidogyne* sp. population density in soil and root galls in roots. As honey bee products have certain significant antioxidant compounds: glucose oxidase, catalase, ascorbic acid, flavonoids, phenolic acids, carotenoid derivatives, organic acids, amino acids and proteins [34-36]. Herein, total phenolic content increased significantly in leaves of strawberry plants infected with *M. incognita* compared to untreated uninoculated plants. Phenols may serve as defense compounds against pathogens [37]. Early increases in phenol caused by pathogen invasion trigger the transcription of mRNA that codes for phenylalanine ammonia-lyase (PAL); increasing amounts of PAL in the plant brings about the synthesis of phenolic compounds [38]. In the present study, the number of total phenols in infected strawberry plant leaves was greater than that of untreated uninoculated plants with a percentage of increase amounted to 45.7 and 45.9 % for the two seasons. The application of venom gave greater suppression in phenol content amounted to 39.8 and 44.1% for the two seasons. Conversely, all tested treatments exhibited lower levels in phenol content at the end of the experiment. Results suggest that, the lower level of phenols in later stages linked to the oxidation of phenols by polyphenol oxidase (PPO) [39]. Organic acids induce systemic resistance by activation of various defense-related enzymes viz. PO, PPO and PAL [40, 20, 41]. Obviously, the current investigation recorded the higher activity of peroxidase in plants treated with the highest concentration of bee venom generating the speculation of induced defense responses in strawberry infected with *M. incognita*. The trend of increasing PPO activity was similar to that of PO in all treatments.

Proline concentration of strawberry leaves can be used as a suitable marker for stress induced by both abiotic and biotic factors [13]. The increased proline in leaves due to the higher population of nematodes might indicate the adaptive osmoregulation or acclimation responses in plants to the nematodes biotic stress by increasing metabolites and solutes, which increase plant resistance [9]. Moreover, bee venom gave a highly significant increase in proline values (85.6 and 112.1) by the highest concentration at the two seasons, which may be increased strawberry resistance to nematode infection [35].

Technological characters in terms of sucrose (30.4, 34.5%) and TSS (8.6, 8.9%) significantly increased with the introduction of the highest concentration of venom in concomitant with oxamyl at the two seasons. Although we acknowledge that these are laboratory and in *vivo* studies and may not be directly applicable to a field setting, it highlights the need for further research into the off-target effects of biological pest control on root-knot nematodes.

CONCLUSION

The use of honey bee by-products i.e. propolis and venom represents a promising new approach for the control of root-knot nematode*, M. incognita* infecting strawberry within an environmentally friendly integrated pest management through enhancing the resistance of the plant to nematode infection for their significant antioxidant compounds. Moreover, the importance of using natural resources instead of synthetic antioxidants has risen globally. Therefore, attempts to increase active compounds are needed for safe and effective nematode management.

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