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Nematicidal effectiveness of propolis against the root-knot nematode *Meloidogyne* spp.

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ABSTRACT:

Plant parasitic nematodes are harmful agricultural pests, causing severe yield losses of a wide range of crops worldwide. The lack of effective nematode management products has increased demand for innovative nematode management tools. This work was conducted to test propolis (bee glue) at different concentrations to control the root-knot nematode (*Meloidogyne* spp.) under laboratory conditions. A sample of propolis was measured and ethanol solvent (95%) was added and kept at room temperature for 24 h. The nematode eggs were exposed to the propolis extract at different concentrations (i.e., 2000, 4000, and 6000 ppm) for 24, 48, and 72 h. Results showed that propolis extract caused significant decreases in egg hatching, but to varying degrees. There was a gradual decrease in egg hatching with increasing the extract concentration and the duration of exposure.

KEYWORDS: Propolic, Root-knot nematode, *Meloidogyne* spp., Egg hatchability.

1- INTRODUCTION:

Nematodes are found in a wide variety of habitats. free-living nematodes live in the soil, in freshwater, marine sands and muds. In soil, they are important components of nutrient turnover. Other nematodes are parasites of almost every species of animal, humans, plant and they cause enormous social and economic damage (**Perry, 2011**). Phytoparasitic nematodes parasitize plants to seek suitable food. This food source is basically planted cell contents. Thus a plant response to parasitism is the reaction to the cellular and growth. feeding of the nematode **(Ahmad *et al.*, 2010)**. Most phytoparasitic nematodes infect plant roots and some species have evolved sophisticated interactive relationships with host cells to sustain a sedentary parasitic habit **(Davis *et al.*, 2004)**. Plants carry a wide range of microorganisms in their phyllosphere and rhizosphere which not only cause a large variety of diseases but also control of pathogens **(Elekcioglu *et al.*, 1994)**. Nematodes have an important niche in agro-ecosystem, causing a reduction in plant productivity

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Root-knot nematodes (*Meloidogyne* spp.) are very common and the most important nematode species of greenhouse-growing plants. Indiscriminate use of chemical nematicides to control nematode causes great injuries to human being, animal, vegetation and to the environment as a whole due to their non-target effect, hazardous nature besides they are expensive. So with the increasing awareness of possible deleterious effects of the chemicals, biological controls of plants pathogen have received considerable attention **(Garima *et al.*, 2005).**

The management of these nematode-parasites has little chance of success and is uneconomical because they live in the soil and feed on the internal plant tissues. Preventing the introduction of nematodes with planting material, seeds, or soil, using rotation and mixed cropping with the poor host, using nematode resistant varieties or rootstocks, and lowering nematode populations through nematicides are some of the most frequently used strategies **(Ploeg, 2008)**. Until recently, methyl bromide was widely used to manage nematodes and other soil-borne pathogens in high-value horticultural crops. However, concerns on its impact on environment necessitate the ban or revoke of this methyl bromide in 2005 for its gas emission and global warming. Although nematicides are effective in nematode management, it discourages users because of their high costs, non-availability at the time of need, the hazards they pose on human as well as on non-target organisms **(Nagaraju *et al.*, 2010)**. Other options for the management of root-knot nematodes become imperative and there is an increasing interest in non-chemical nematode management strategies **(Kerry, 1990).**

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, 2011)**. Several researchers have been reported antimicrobial and antibiotic activities for honeys and their constituents **(Esin *et al.*, 2006)**. Propolis as a one of bee products has different biological effects; antibacterial **(Christov *et al.*, 1999; Grange and Darvey, 1990; Menezes *et al.*, 1997)**, antifungal **(Cafarchia *et al.*, 1999; Millertclerc *et al.*, 1987)**, and antiviral **(Amoros *et al.*, 1992)**.

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 **(Ghisalberti, 1979; Da Silva *et al.*, 2018)**. The chemical composition of propolis is variable and depends on vegetation of the geographical area, the time of year and bee species. The most important and the best known properties of propolis are its antibacterial, antiviral, and antifungal activities **(Katarzyna, 2013)**. It is composed of resin (50%), wax (30%), essential oils (10%), pollens (5%), and other organic compounds (5%) **(Gómez-Caravaca *et al.*, 2006; Toreti *et al.*, 2013)**. Furthermore, there are important organic compounds present in propolis such as phenolic compounds, esters, flavonoids, terpenes, beta-steroids, aromatic aldehydes and alcohols **(Huang *et al.*, 2014)**. Different flavonoids, vitamins, minerals and enzymes also detected in propolis extract **(Xing and White, 1996; Mahdy and Abdel-Aal, 2014)**.

The current study was designed to evaluate the potential beneficial effects of propolis extract (Baladi propolis) on the control of the root-knot nematode (*Meloidogyne* spp.) through the toxic effects on egg hatchability under laboratory condition.

2- MATERIAL AND METHODS :

Propolis (Resin) Sample Collection:

At the end of the honey season, 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 **(Bankova *et al.* 2006)**.

Extraction of Propolis:

An ethanol 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 ethanol solvent (1:30 w: v) was added and kept at room temperature for 24 h. Then, the suspension (propolis in ethanol 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. For further experiments, the obtained extract will be evaporated to dryness.

Extraction of nematode eggs:

Eggs were obtained from a culture of nematode infected roots of tomato; root pieces containing egg masses were cut into small pieces and placed in a container of 500 ml capacity with 200 ml of 0.5% Clorox (sodium hypochlorite, NaOCI) solution shaken vigorously by hand for 4 min **(Hussey and Barker, 1973)**. This was done in order to digest the gelatinous matrix encasing the eggs. The solution was then poured through two nested sieves, 200-mesh (75 µm) and 500 mesh (25 µm). Eggs in the 500 mesh sieve were washed free of NaOCI solution with a slow stream of cold tap water into a container previously marked to contain 1 L. The cut roots in the original container were washed twice with water to obtain additional eggs. The collected eggs were topped with water to obtain the egg-water suspension for *in vitro* studies.

Counting of root-knot nematodes eggs:

Number of eggs in aqueous suspension was determined by using a stereo microscope. One milliliters of the egg-water suspension was pipetted after bubbling air through the suspension for homogeneity and dispensed into a counting tray. Counting was done two times and the mean number of eggs/ml estimated.

Hatchability test:

Eggs were collected by the method of **Hussey and Barker (1973)**. A suspension of eggs in water was prepared. One ml of egg suspension (100±10 eggs/ml) and 5 ml of extract was transferred in Petri dishes and kept at room temperature. Each treatment was 3-time replicated. The Petri dishes containing 1 ml egg suspension and 5 ml water served as control. After 24, 48, and 72 hours of exposure, the number of hatching eggs was counted under an inverted microscope.

Nematicide:

Oxamyl (Vydate) 24% L. Methyl-N'N'- dimethyl-N [(methyl) carbamoyl-oxy]-1-thioxamidate was used at the rate of 1L/100L.

3- RESULTS AND DISCUSSION:

Effect of exposure time and Inhibition Concentration (IC):

Regarding the effect of propolis extract on egg hatching of root-knot nematode after 72h, data in Table 1 show that toxicity of extract IC50 (Inhibition Concentration, 50%), IC90 and slope value was calculated. It shows that the neem extract is highly effective against egg hatching being the IC50 scored 170.2 ppm. Consequently, propolis extract caused 81, 86, and 87% inhibition of egg hatching on root-knot nematode at the concentrations of 2000, 4000, and 6000 ppm, respectively at 72h.

Table 1: Effect of propolis extract on egg hatching (%) of root-knot nematode (*Meloidogyne* spp.) after 72 hours exposure to the extract.

Propolis extract	Concentration (ppm)			IC_{50} (ppm)	95% Confidence limits		IC_{90} (ppm)	Slope ± SE
	2000	4000	6000		Lower	Upper		
	81*	86	87	170.2	0.0	771.0	23027.5	0.6 ± 0.28

*Inhibition of egg hatchability (%)

Effects of propolis extract concentrations and exposure time:

Table 2 and Figs. 1-2 show the mean performance of propolis extract concentrations and exposure time to the extract on hatching of root-knot nematode eggs. The egg hatching rate was gradually reduced as the extract concentration increased from 2000 to 6000 ppm. In contrast, the egg hatching percentage (%) was gradually increased as the exposure time increased from 24 to 72 h.

Table 2: Mean performance (± SE) of the effect of propolis extract concentration and time on egg hatching of *Meloidogyne* spp.

Conc. (ppm)	Means ± SE	Time (h)	Means ± SE
0^{-}	52.44 ± 6.0 a	24	14.13 ± 2.4 c
0^{+}	5.89 ± 0.2 e	48	19.67 ± 4.4 b
2000	16.56 ± 1.0 b	72	25.33 ± 6.5 a
4000	12.89 ± 0.5 c	-	-
6000	10.78 ± 0.2 d	-	-

Negative control (0^{-}; nematode + water) and possative (0^{+}; nematode + nematicide)

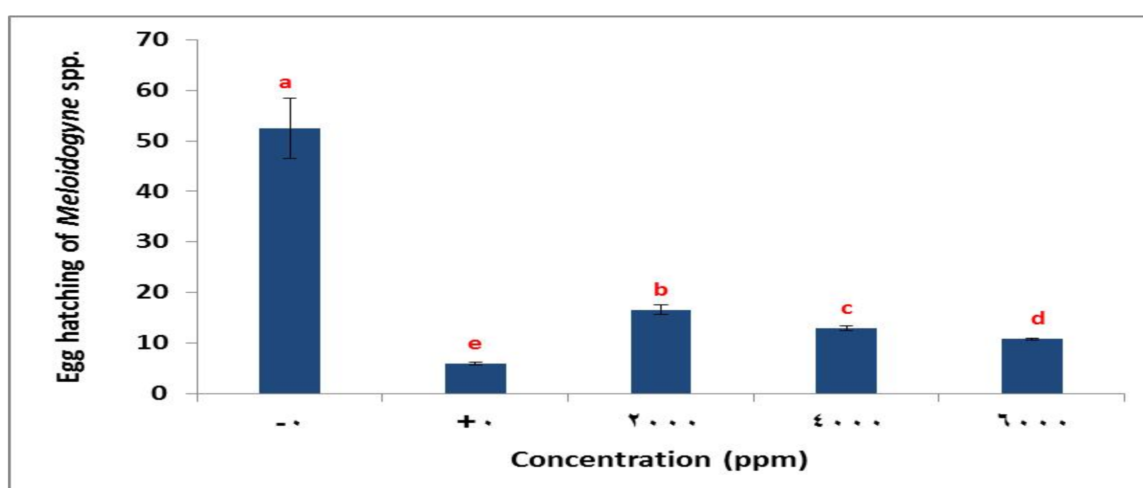

Fig. 1: Mean performance (± SE) of propolis extract concentrations on egg hatching of *Meloidogyne* spp.

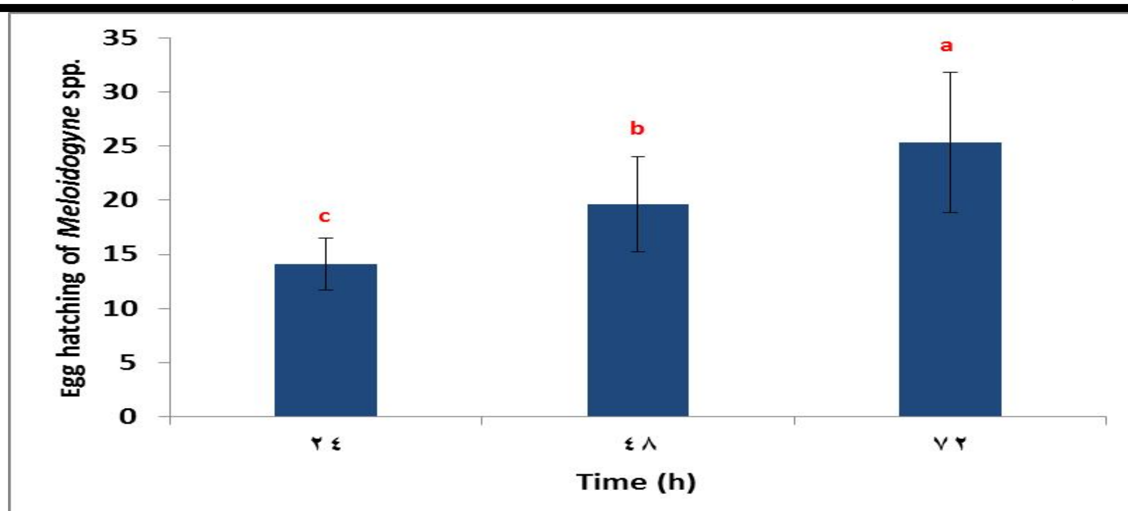

Fig. 2: Mean performance (± SE) of exposure time of propolis extract on egg hatching of *Meloidogyne* spp.

Interactive effects of propolis extract concentrations and exposure time:

Table 3 and Figs. 3-6 show the interactive effect of propolis extract concentrations and exposure time on the hatching of nematode eggs. Under the application of propolis extracts, the lowest effective concentration was 2000 ppm, where the egg hatching rate reached 13.67, 16.67, and 19.33% after 24, 48, and 72 h, respectively, as the least inhibition, followed by 4000 ppm. This concentration caused hatching percentage of 11.67, 12.67, and 14.33% after 24, 48, and 72 h, respectively. The highest effective concentration of the extract was 6000 ppm, which gave the lowest egg hatching rate, as the hatching percentage reached 8.00, 11.00, and 13.33% after 24, 48, and 72 h, respectively.

Table 3: Mean performance (± SE) of interaction between concentration and time on egg hatching of *Meloidogyne* spp.

Concentration (ppm)	Time (h)	Means ± SE
0^-	24	32.00^* ± 1.7 c
	48	52.00 ± 2.1 b
	72	73.33 ± 1.3 a
0^+	24	5.33 ± 0.3 h
	48	6.00 ± 0.0 h
	72	6.33 ± 0.3 h
2000	24	13.67 ± 1.2 ef
	48	16.67 ± 1.2 de
	72	19.33 ± 0.7 d
4000	24	11.67 ± 0.3 f
	48	12.67 ± 0.3 f
	72	14.33 ± 0.9 ef
6000	24	8.00 ± 1.0 gh
	48	11.00 ± 1.0 fg
	72	13.33 ± 1.5 ef

Data are means ± S.E. different lower or upper letters in a column indicate significant differences between the treatments at $P \leq 0.05$. Negative control (0^-; nematode + water) and possative (0^+; nematode + nematicide).

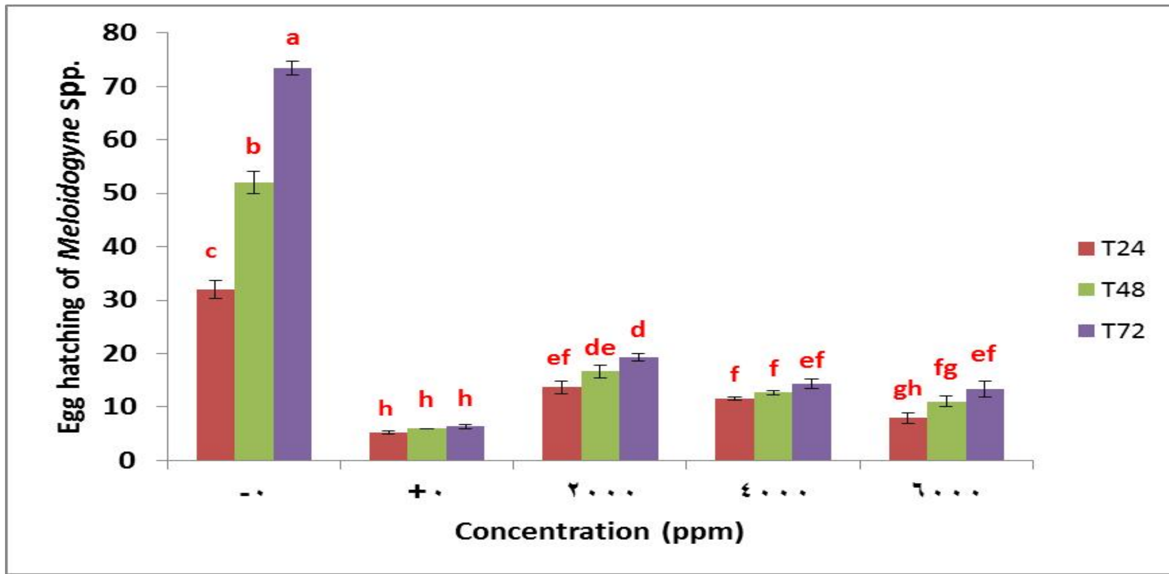

Fig. 3: Mean performance (± SE) of interaction between concentration and time on egg hatching of *Meloidogyne* spp.

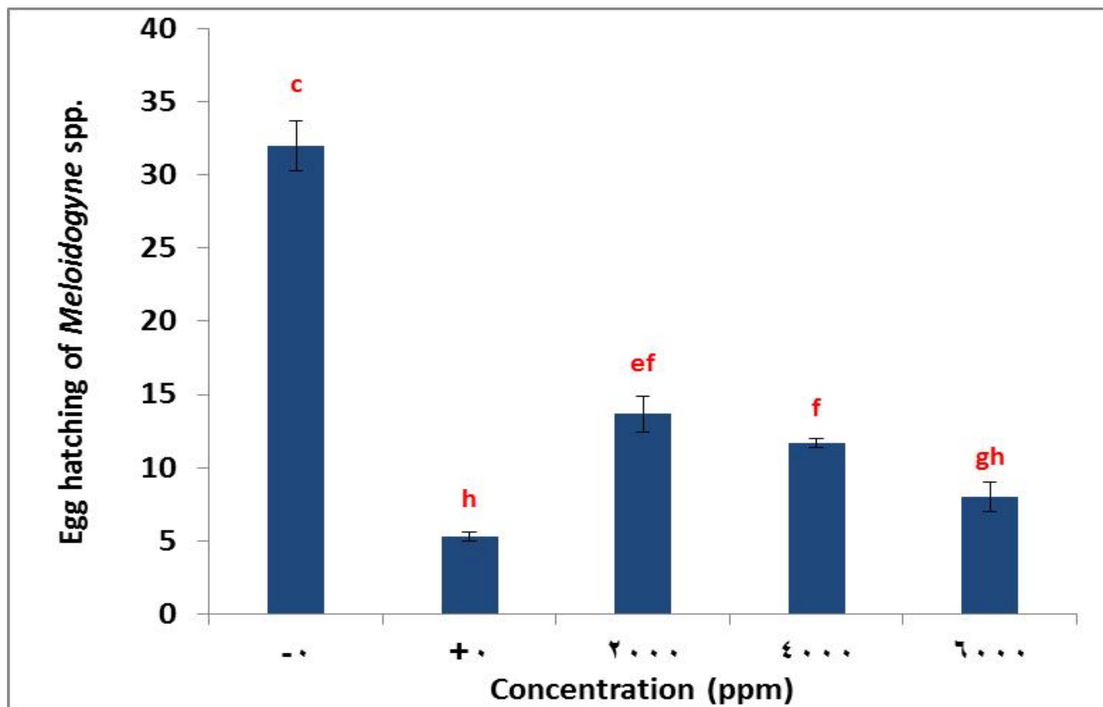

Fig. 4: Effect of propolis extract on egg hatching of root-knot nematode *Meloidogyne* spp. after 24h.

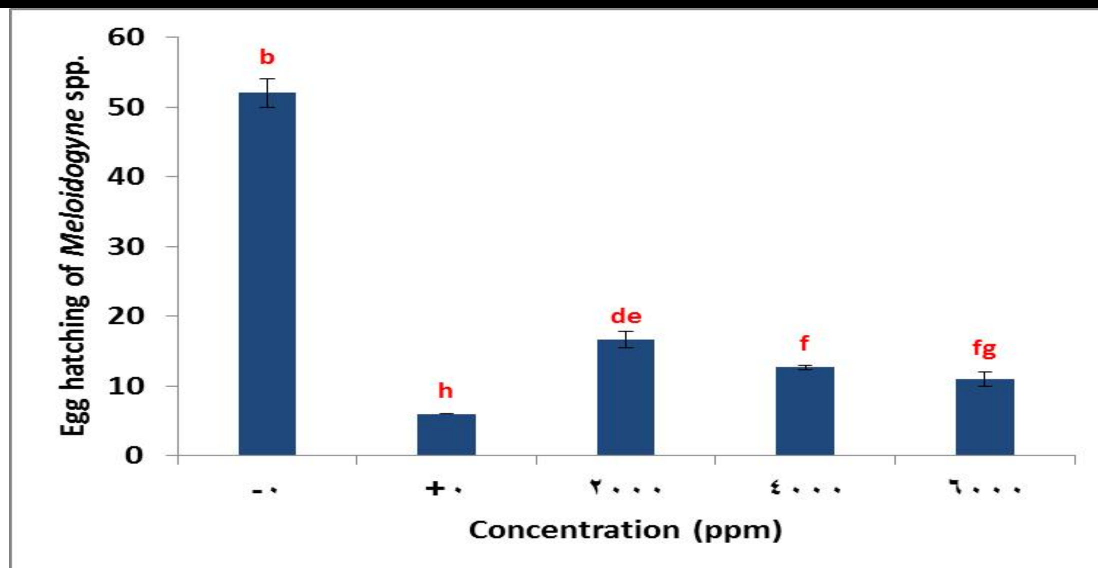

Fig. 5: Effect of propolis extract on egg hatching of root-knot nematode *Meloidogyne* spp. after 48h.

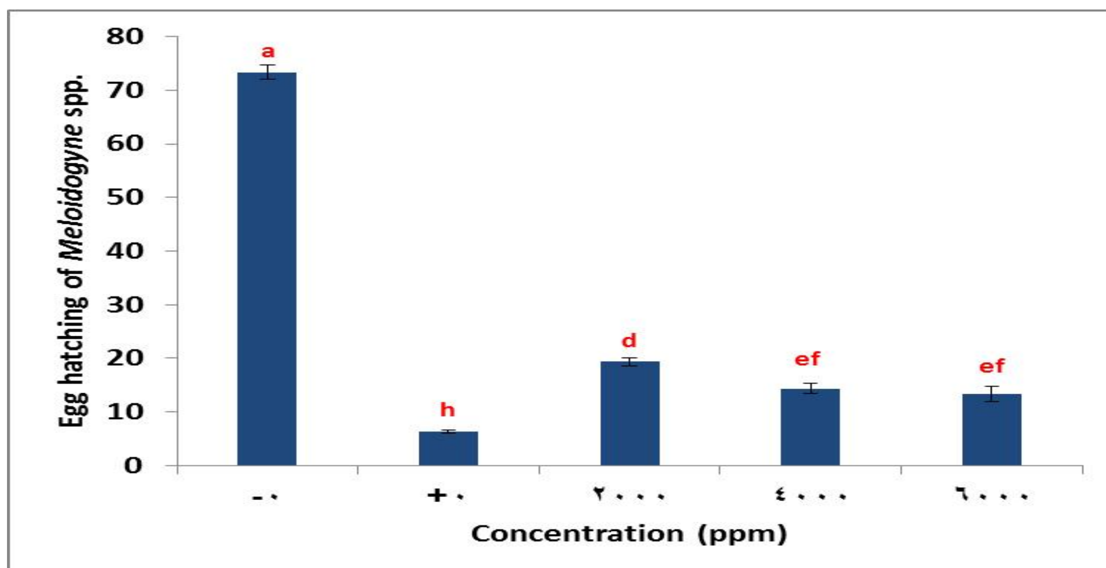

Fig. 6: Effect of propolis extract on egg hatching of root-knot nematode *Meloidogyne* spp. after 72h.

The results show a gradual decrease in egg hatching with increasing the concentration of each extract. The increase in exposure period and an increase of the concentration also decrease of egg hatching.

The recent approach in nematode control is direct strategy towards the possibility of reducing populations of plant-parasitic nematodes in soil by using natural substances extracted from some plants. Such methods don't lead to the disturbance of the biological balance of nature. Utilization of antagonistic plants or their byproducts is of common use all-over the world for avoiding hazards of the traditional chemical nematicides. The use of certain plant extracts for controlling plant-parasitic nematodes has been increased in

the recent years (**Pandey and Dwivedi 2000**; **Dias *et al.*, 2000**; **Insunza *et al.*, 2001**; **Rakesh *et al.*, 2001; Haroon *et al.*, 2018, Haroon *et al.*,2019**).

Worldwide, during the last decades, nematologists searched for the inexpensive and safer alternatives to the chemical nematicides, i.e. biological and cultural methods to manage plant-parasitic nematodes. Bee products and its components, including propolis, were used as antimicrobial **(Bogdanov, 2011)**. Several authors have reported the antimicrobial activity of propolis on fungi **(Lindenfelser, 1967; Brumfit *et al.*, 1990; Tosi *et al.*, 1996)**. Honey bee products, including pollens, bee venom, royal jelly, and propolis are the promising materials that have antagonistic and medicinal properties against bacterial pathogens **(Ghanem, 2011)**. Several researchers have been reported antimicrobial and antibiotic activities for honey bees products, including propolis **(Esin *et al.*, 2006)**. Propolis as a one of honey bee products has different biological effects such as: antibacterial **(Christov *et al.*, 1999; Grange and Darvey, 1990; Menezes *et al.*, 1997)**; antifungal **(Cafarchia *et al.*, 1999; MillertClerc *et al.*, 1987)**; and antiviral **(Amoros *et al.*, 1992)**.

The results of this study showed that the tested propolis at all tested concentrations (2000, 4000, and 6000 ppm) led to a significant reduction in egg hatching compared to nematodes without extract. The results confirmed that applying propolis as an extract at the highest concentration (6000 ppm) was a very effective treatment in reducing the rate of egg hatching although the nematicide (Oxamyl 24% L.) was more effective. As a positive result, the propolis extract is a natural product with its using; the environment is maintained against contamination.

It has been found that honey products contain important antioxidant compounds, including glucose oxidase, catalase, ascorbic acid, flavonoids, phenolic acids, carotenoid derivatives, organic acids, amino acids, and proteins **(Bogdanov, 2011; Bonvehl and Jorda, 1991)**. **Ali and Abd El-Ghafar (2002)** evaluated the concentrations of 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 propolis extract at 10% significantly inhibited the fungi growth area when compared with untreated check.

It has been also found that a soil drench with some honeybee products, including propolis extract, increased protein content in faba bean plants to strengthen them against nematode infection. The propolis extract applied as a soil drench reduced the juvenile-*Meloidogyne* spp. population density per one kg soil and number of root-galls per one gm of roots. It has been also found that the qualitative of some honeybee product extracts, including propolis extract, proved that these extracts contain a significant levels of sterols, flavonoids, and phenolic compounds, as well as a few numbers of phenolic acids, including coumaric, ferulic, salicylic, and benzoic acids. Propolis extracts applied either as a foliar or soil drench treatment increased total chlorophyll, carotenoid and protein contents of faba bean plants. In addition, 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. All of these positive results are in favor of plants against *Meloidogyne* spp. (**Noweer and Dawood, 2009**).

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