Nematicidal Efficiency of 10 % Emulsifiable Concentrate Formulation of Propolis on Root-Knot Nematode *Meloidogyne Spp*.

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ABSTRACT

Propolis is a red or brown resinous substance collected by honeybees from tree buds with a broad spectrum of biological properties. It was formulated as an emulsifiable concentrate (EC); the new formulation was subjected to all tests specified by pesticides specialized organizations like WHO and FAO. It passed successfully all tests and evaluated biologically against second stage larvae of Root-Knot nematode *Meloidogyne Spp.* under greenhouse conditions. The new formula inhibited markedly the ability of the 2nd stage larvae of Root-Knot nematode *Meloidogyne Spp.* to penetrate eggplant roots in addition to the marked decrease in the number of galls obtained from the calculations of root gall index (RGI). The obtained results showed that it could be possible to use propolis emulsifiable concentrate formulation in the field of nematode control after carrying out the complementary experiments in the future.

Keywords: Propolis, Emulsifiable Concentrate, Formulation and Root-Knot nematode.

1. INTRODUCTION

Propolis is a honey bee product with a broad spectrum of biological properties (Mello *et al.* 2012). As a resinous substance propolis is prepared by honey bees to seal the cracks, smooth walls and to keep moisture and temperature stable in the hive all year around. Raw propolis is typically composed of 50 % plant resins, 30 % waxes, 10 % essential and aromatic oils, 5 % pollens and 5 % other organic substances. It has been reported that propolis is collected from resins of poplars, conifers, birch, pine, alder, willow, palm, *Baccharis dracunculiforia* and *Dalbergia ecastaphyllum* (Kosalec *et al.* 2004).

The biological activities of propolis are attributed to a variety of major chemical constituents including phenolic acids, phenolic acid esters, flavonoids and terpenoids such as caffeic acid phenethyl ester (CAPE), artepillinic, caffeic acid, chrysin, galangin quercetin, apigenin, kaempferol, pinobanksin, 5-methyl ether, pinopanksin, pinocembrin and pinobanksin-3- acetate, over 500 compounds have been identified in propolis from many countries up to 2012 (Shuai *et al.* 2014).

The chemical composition of propolis is variable and depends on vegation 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).

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). Honey bee products i.e. pollen, propolis, bee venom and royal jelly are the promising materials that have antagonistic and medicinal properties against bacterial pathogens (Ghanem, 2011).

During the last decades, nematologists worldwide search the cheaper, safer and eco-friendly alternatives methods i.e. biological and cultural methods to control the plant-parasitic nematodes (El-Sayed and Mahdy, 2015). Ibrahim *et al*. 2015 reported that propolis suspension concentrate (SC) formulation showed good inhibition effect on the 2nd larval stage of Root-Knot nematode (*Meloidogyne Spp*.).

Root-knot nematodes (*Meloidogyne Spp.*) are one of the three most economically damaging genera of plant-parasitic nematodes on horticultural and field crops. Root-knot nematodes are distributed worldwide, and are obligate parasites of the roots of thousands of plant species, including monocotyledonous and dicotyledonous, herbaceous and woody plants. The genus includes more than 90 species (Moens *et al.* 2009). Four *Meloidogyne species* (*M-javanica*, *M-arenaria*, *M-incognite* and *M-hapla*) are major pests worldwide. *Meloidogyne* occurs in 23 of 43 crops listed as having plant-parasitic nematodes of major importance ranging from field crops, through pasture and grasses, to horticultural, ornamental and vegetable crops (Stirling *et al.* 1992). If Root-Knot nematodes become established in deep-rooted, perennial crops, control is difficult and options are limited. Root-knot nematode damage results in poor growth, a decline in quality and yield of the crop and reduced resistance to other stresses (e.g. drought other diseases). A high level of damage can lead to total crop loss. Nematode-damaged roots do not use water or fertilizers as effectively leading for additional losses for the grower.

Pesticide active ingredients by themselves may not mix well with water, may be chemically unstable, may be difficult to handle or store and may be difficult to apply for good pest control. To make an active ingredient useful, manufacturers add other ingredients (sometimes called inert ingredients to " formulate " the pesticide into the final product offered for sale (Private Pesticide Applicator Safety Education Manual, 2017).

Depending on the previously mentioned items and in continuation with our ongoing program to obtain new active ingredients. The main aim of this research paper is to formulate propolis in a suitable formulation type and evaluating the biological efficiency of the new formulation on the 2nd stage larvae of Root-Knot nematode (*Meloidogyne Spp.*) under greenhouse conditions, hoping the new formula to be used as a safer alternative nematicide in the field of nematode control after carrying out the necessary experiments in the future.

2. MATERIALS AND METHODS

2.1. Tested chemicals:

a) Propolis: Powder supplied by honey market.

b) Surface active agents: Tween 20 and poly ethylene glycol 600 dioleate (P.E.G 600 Do.) were supplied by EL-Gomhoria Co., Cairo, Egypt.

2.2. Physico-chemical properties of formulation basic ingredients:

2.2.1. Active ingredient:-

a) Solubility: It was determined by measuring the volume of distilled water, acetone, ethanol, DMF and xylene for complete solubility or miscibility of one gram of active ingredient at 20˚C (Nelson and Fiero, 1954). The % Solubility was calculated according to the following equation;

$$\% \text{ solubility} = W/V \times 100$$

[Where; W= active ingredient weight, V= volume of solvent required for complete solubility].

b) Free acidity or alkalinity: It was determined according to the method described by WHO specifications (1979).

2.2.2. Surface active agents:

a) Surface tension: It was determined by using Du-Nouy tensiometer for solutions containing 0.5 % (W/V) surfactant according to ASTMD-1331 (2001).

b) Hydrophilic-lipophilic balance (HLB): The solubility of surfactant in water is considered as approximate guide to its hydrophilic-lipophilic balance (Lynch and Griffin, 1974).

c) Critical micelle concentration (CMC): The concentration in which the surface tension of solution doesn't decrease with further increase in surfactant concentration, (CMC) of the tested surfactants was determined according to the method described by (Osipow, 1964).

d) Free acidity or alkalinity: It was determined as mentioned before.

2.2.3. Propolis local prepared emulsifiable concentrate (EC) formulation:

Depending on the physico-chemical properties of propolis, it was prepared as an emulsifiable concentrate formulation according to the method described by (Soliman, 2005) which includes the dissolution of propolis in enough amount of solvent followed by addition of an emulsifier and stirring for 1 hour. The solution was then poured in 100 ml flask and then completed to 100 ml by adding the same solvent, shaken vigorously to ensure homogeneity, filtered off and kept in a tightly closed vial, and the following physico-chemical properties for the formulation were determined:

a) Emulsion stability test: It was performed according to FAO/WHO MT 36.3. (2010).

b) Accelerated storage: It was determined according to CIPAC M46.1. (1995).

c) Cold stability test: It was determined according to FAO/WHO MT 39.3. (2010).

2.2.4. Spray solution at field dilution rate:

a) Surface tension: It was determined as mentioned before.

b) Viscosity: It was determined by using Brookfield viscometer model DVII+Pro, where Centipoise is the unit of measurement according to ASTM D-2196 (2005).

c) Electrical Conductivity: It was determined by using Cole-Parmer PH/Conductivity meter 1484-44, where µmhos is the unit of electrical conductivity measurements according to (Dobrat and Martijn, 1995).

d) PH: It was determined by using Cole-Parmer PH conductivity meter 1484-44 according to (Dobrat and Martijn, 1995).

2.3. Bioassay:

Plastic cups each of 6.5 cm in diameter was filled with 240 grams sterilized sandy soil. One eggplant seedling at the two leaf stage was transplanted to each cup. The bioassay experiment was carried out on two successive stages. The first stage; four concentrations from the 10 % emulsifiable concentrate formulation of propolis were prepared, and about

1000 2^{nd} stage larvae of root-knot nematode were soaked in each concentration for 48 hrs. in a completely sealed plastic test tube 3 cm under laboratory conditions. The second stage was done under green house conditions; the nematode suspension was pipette into holes around the base of each seedling. Inoculations were obtained from available pure culture. Propolis 10 % emulsifiable concentrate formulation (EC) was used at 10000, 1000, 100 and 10 ppm in 5 ml as soil infection treatment. Each treatment was replicated three times. Three cups were saved without treatment as control. The cups were irrigated regularly as needed. After seven weeks from treatment, the number of galls / gram root of the 2^{nd} stage larvae in 240 gram soil was determined (Feldmesser, 1972).

3. RESULTS AND DISCUSSION

3.1. Preparation of propolis as emulsifiable concentrate formulation (EC):

The biological activity of a pesticide, be chemical or biological in nature, is determined by its active ingredient (a.i also called the active substance). Pesticide products very rarely consist of pure chemical material. The active ingredient usually formulated with other materials and this is the product as sold, but it may be further diluted in use. Formulation improves the properties of a chemical for handling, storage, application and may substantially influence effectiveness and safety (Knowles, 2008**)**.

An emulsifiable concentrate formulation usually contains a liquid active ingredient, one or more petroleum-based solvents (which gives EC formulations their strong odor), and an agent that allows the formulation to be mixed with water to form an emulsion. ECs are among the most versatile formulations. They are used against agriculture, ornamental and turf, forestry, food processing, livestock and public health pests. They are adaptable to many types of application equipment, from small, portable sprayers to hydraulic sprayers, low-volume ground sprayers, mist blowers and low-volume aircraft sprayers (Private Pesticide Applicator Safety Education Manual, 2017).

Data in Table (1) declared that, propolis showed medium degree of solubility in dimethylformamide (DMF) and acetone, weak solubility in ethanol and no solubility in both xylene and water. On the other hand it recorded a slight free alkalinity 0.096. The solubility of propolis in DMF was 13.3 % which means that it could be prepared as an emulsifiable concentrate formulation.

Table (1): Physico-chemical properties of propolis as an active ingredient.

Solubility % in					Free alkalinity as NaOH
DMF	Acetone	Ethanol	Xylene	Water	0.096
13.3	5.7	traces	N.S*	N.S*	

N.S*: means insoluble.

3.2. Physico-chemical properties of surface active agents:

Data in Table (2) showed the physico-chemical properties of surfactants used for the preparation of propolis as an emulsifiable concentrate. The properties of the chosen surfactants shall comply with that of the used active ingredient, both surface active agents showed low values of surface tension 35.8 and 41.5 dyne/cm for P.E.G 600 Do. and Tween 20 respectively. On considering HLB values, both surfactants showed HLB value between 8-16 which achieves one of the most important conditions for mixing components to formulate an active ingredient in the form of emulsifiable concentrate as reported by (Griffin, 1954). Tween 20 showed a slight alkaline property, while P.E.G 600 Do. showed a slight acidic property. About 20 trials were carried out to formulate propolis as an emulsifiable concentrate, two trials passed successfully all tests reported for emulsifiable concentrate formulations.

Table (2): Phsico-chemical properties of the suggested surface active agents.

Surfactants	HLB	CMC	Free alkalinity as NaOH	Free acidity as H2SO4	Surface tension Dyne/cm
Tween 20	16	0.2	1.53	-	41.5
P.E.G 600 Do.*	8-10	0.9	-	0.196	35.8

P.E.G 600 Do.*: poly ethylene glycol 600 dioleate.

3.3. Physico-chemical properties of propolis 10 % emulsifiable concentrate formulation before and after accelerated storage:

Table (3) showed the physico-chemical properties of the local prepared 10 % emulsifiable concentrate formulation under normal and accelerated storage conditions. The new formula passed successfully spontaneity and emulsion stability tests either under normal or accelerated storage conditions. Also there is no oil separation, precipitation or cream layer. There were no observable changes in the physico-chemical properties of the formulation before and after accelerated storage conditions, even for free alkalinity it showed alkaline properties in both cases.

Table (3): Physico-chemical properties of propolis 10 % emulsifiable concentrate formulation before and after accelerated storage.

Before storage							Cold storage	After storage						
Spontaneity %		Emulsion stability		Foam		Free alkalinity as NaOH		Spontaneity %		Emulsion stability		Foam		Free alkalinity as NaOH
Hard	Soft	Hard	Soft	Hard	Soft			Hard	Soft	Hard	Soft	Hard	Soft	
20	20	pass	pass	-	-	0.96	pass	60	60	pass	pass	-	-	1.6

3.4. Physico-chemical properties of spray solution at field dilution rate:

Table (4) showed properties of the spray solution at field dilution rate, it had the values of, 46.2 dyne/cm, 300 µ mhos, 7.2 cm/poise and 8.44 for surface tension, electrical conductivity, viscosity and PH respectively. These values could be viewed as high viscosity, conductivity and low surface tension. The decrease in surface tension can improve wettability and spreading on the treated surface then increasing deposit and activity of pesticide (Osipow, 1964). While the increase in electrical conductivity can result in an increase in retention and effectiveness of pesticides spray solution according to Tawfik et al, 1987. Also the increase in viscosity can result in increasing pesticide efficiency according to (Richardson, 1974), who stated that, increasing viscosity of spray solution caused a reduction in drift and an increase in the retention and sticking of spray solution on the surface of plant.

Table (4): Physico-chemical properties of propolis (EC) spray solution with 0.5 % concentration.

Surface tension dyne/cm	Viscosity cm/poise	Electrical conductivity µ mhos	PH
46.2	7.2	300	8.44

Data in Table (5) showed the effect of 10 % emulsifiable concentrate formulation of propolis by serial concentrations on the 2nd stage larvae of Root-Knot nematode under greenhouse conditions. The formulation showed inhibition effect that directly proportionate with the used concentrations, it inhibited the gall formation by 80, 70, 65 and 45 % corresponding to the concentrations of 10000, 1000, 100 and 10 ppm respectively, in addition the root gall index (RGI) refers to the ability of the formulation to inhibit the larval penetration to the roots with the observed decrease in the number of galls on roots. These results were consistent with that reported by Ibrahim et al., 2015 that propolis showed good inhibition effect on the second larval stage of Root-Knot nematode (*Meloidogyne Spp.*).

Table (5): The efficiency of propolis 10 % emulsifiable concentrate formulation on the ability of the 2nd stage larvae of Root-Knot nematode to penetrate eggplant roots under greenhouse conditions.

Concentration ppm	% of Inhibition	Root gall index*
10000	80	2
1000	70	2
100	65	2
10	45	2
control	0	3

*: Gall index 0 to 5. Where 0 = no galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, 5 = + 100 (root system completely galled) (Taylor and Sasser, 1978).

Conclusion:

Propolis was formulated as 10 % emulsifiable concentrate. The new formula passed successfully all physico-chemical properties of emulsifiable concentrate formulations and then tested against 2nd stage larvae of Root-Knot nematode *Meloidogyne Spp*. under greenhouse conditions. It showed good nematicidal effect on the 2nd stage larvae, which was deduced from the calculations of root gall index. The obtained results showed that propolis emulsifiable concentrate formulation could be used to control root-knot nematode after completing the other studies for the formulation in the future.

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