Preparation of Propolis as Suspension Concentrate and Evaluation of Its Nemticidal Efficiency on Root-Knot Nematode *Meloidogyne Spp*.

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ABSTRACT: Propolis is a complex resinous material with many pharmacological and biological properties. So that, propolis was prepared as suspension concentrate (SC) formulation. The new formula passed successfully all tests related to suspension concentrates according to the international organizations that work on pesticides. Propolis suspension concentrate was then tested biologically on second larval stage of Root-Knot nematode *Meloidogyne Spp*. by dipping technique. The formulation showed good inhibition effect on the second larval stage of Root-Knot nematode as its EC_{50} was 120.8 ppm

Keywords: Propolis, Formulation, Root-Knot nematode and Dipping technique.

1. INTRODUCTION

The primary objectives of formulation technology are to optimize the biological activity of pesticide and to give a product which is safe and convenient for use (**Knowles, 2008**). Propolis is resin-like material from the buds of poplar and cone bearing trees. It is the dark brown or black sticky plant derived (glue) found around wounds on plant and sometimes around buds and is used by bees for sealing, ling, strengthening for their hives and serve as repellent materials inside the hives and around the entrance (**Banskota et al., 2001**). Propolis is a complex resinous material with many pharmacological and biological properties attributed to the presences of many volatile and non volatile phytochemical compounds (**Usman and Mohamed, 2015**), Most substances or compounds isolated from propolis include flavonoids, diterpenoids, caffeic acid esters, isoflavonoids (**Trusheva et al., 2010**). The pesticidal activity of propolis as insecticide, fungicide and nematicide was reported by several authors (**Noweer and Dawood, 2009; Adeyemi and Osipitan, 2014; Ahmed et al, 2008**). Propolis cannot be used as a raw material so the aim of this work is to formulate propolis in a suitable form and evaluating its nematicidal efficiency against Root-Knot nematodes under laboratory conditions.

2. MATERIALS AND METHODS

2.1. Chemicals

a) Propolis: powder supplied by honey market.
b) Surface active agents: Span 20, Tween 80 and tween 20 supplied by EL-Gomhoria Co., Cairo, Egypt.

2.2. The physico-chemical properties of the basic formulation:

2.2.1. Active ingredient:-

a) Solubility: It was determined by measuring the volume of distilled water, acetone, dimethyl formamide (DMF)and xylene for complete solubility or miscibility of one gram of propolis at 20 ^{0}C (**Nelson and Fiero, 1954**). The % solubility was calculated according to the following equation:

% solubility = w/v * 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 specification (1979).

2.2.2. Surface active agents:

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

b) Hydrophilic-Lipophilic balance (HLB):
The solubility of surfactant in water is considered as approximate guide to its hydrophilic-lipophilic balance (HLB) (**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 concentrations, (CMC) of the tested surfactants was determined according to **Osipow (1964)**.

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

2.3. Preparation of propolis as suspension concentrate (SC) formulation:

Several trials were carried out to prepare propolis as suspension concentrate SC 10 % according to the method described by (**Knowles, 2008**), suspensibility, foam and free acidity or alkalinity were studied before and after storage at 54±3 ^{o}C for three days to determine the successful trial according to CIPAC MT 46.1(2002).

2.4. Determination of the physico-chemical properties of the spray solution of the local formulation 10 % SC at the field dilution rate:

a- Surface tension: It was determined as mentioned before.
b- Viscosity: It was determined according to ASTM D-2196 (2005) by using Brookfield viscometer model DV Π + Pro, where centipoise is the unit of viscosity.
c- pH value: It was determined by using Cole-Parmer pH/Conductivity meter1484-44.according to method of (Dobrat and Martijn,MT75.3,1995)
d- Electrical conductivity: It was determined by using Cole-Parmer pH/Conductivity meter 1484-44, where μ mohs is the unit of electrical conductivity measurement.according to(Dobrat and Martijn,MT32,1995)

2.5. Bioassay:

It was carried out according to the method described by (**Feldmesser, 1972**) as follow: Egg-masses of *Meloidogyne Spp* were isolated from infested tomato roots obtained from pure culture prepared and propagated in green house. The second larval stage was obtained by incubating egg-masses in distilled water. Newly hatched larvae were collected by using micropipette to evaluate the prepared **formulation, about 100 second larval stage were used for** each concentration of diluted formulation. The final volume of diluted formulation and nematode solution was 5 ml in 10 ml clean glass vial, water was served as control and each treatment was replicated 5 times. The number of mobile nematode was counted after 24, 48 and 72 hrs.

2.6. Statistical analysis:

Inhibition percentages were corrected using **Abbott formula (1925)**, and the concentration inhibition regression lines were drawn according to the method of **Finney (1952)**.

3.RESULTS AND DISCUSSION

A) Preparation of propolis as SC formulation:

The main purpose of any agrochemical formulation is to make handling and application of the active ingredient as easy as possible. This is achieved in most cases by controlling the physical characteristics of the formulation contents (**Mohamed, 2010**).

Table 1: The physico-chemical properties of propolis as active ingredient.

Solubility % in					Free alkalinity as NaOH
DMF	Acetone	Ethanol	Xylene	Water	0.019
13.3	5.7	traces	N.S*	N.S*	

*: means insoluble.

Data in table (1) indicated that, propolis showed a slight solubility in DMF and acetone; the respective solubility percentages were 13.3 and 5.7 whereas it did not soluble in xylene and water. On the other hand it recorded a slight free alkalinity 0.0910. Depending on the above results, propolis could be formulated as suspension concentrate form in water (SC). So we need in this respect surface active agent act as wetting and dispersing agents to reach to stable solid/liquid system. The use of surfactants as wetting and dispersing agents has also led to a great deal of research on the colloidal and surface chemistry aspects of dispersion and stabilization of solid/liquid dispersions (**Tadros, 198**8).

Table 2: The phsico-chemical properties of the suggested surface active agents.

Surfactants	Appearance	HLB	CMC %	Surface tension Dyne/cm as cmc value	Free alkalinity as %NaOH	Free acidity as % H_2SO_4
Span 20	Emulsion	6-8	0.01	58	0.224	-
Tween 80	Clear solution	≥ 13	0.5	48	-	0.6
Tween 20	Clear solution	≥ 13	0.2	43.2	-	0.049

As shown in table 2, the physico-chemical properties of three nonionic surface active agents namely span 20, tween 80 and tween 20 was studied to determine if it was compatible with the physico-chemical properties of active ingredient or not. According to HLB values tween 20 and tween 80 were considered as dispersing agents, their HLB values were more than 13 whereas the HLB of span 20 was 6-8 so it was considered as wetting agent. On the other hand all tested surface active agents decreased surface tension of water from 72 for water to 58, 48, 43.2 dyne/cm in case of span 20, tween 80 and tween 20,respectively. Span 20 recorded free alkalinity as % NaOH 0.224 whereas tween 80 and tween 20 recorded free acidity as % H_2SO_4 0.6 and 0.049 respectively. From the above results, it could be concluded that, the tested surface active agents were suitable to prepare propolis as SC formulation because it was act as wetting agent in case of span 20 and as dispersing agent in case of tween 20. In most cases, suspension concentrates are made by dispersing the active ingredient powder in aqueous solution of wetting and dispersing agent using high shear mixer to give concentrated peremix(**Knowls, 2008**).

Table 3: The physico-chemical properties of local SC formulation of propolis.

Type of water	Before stoarge			After storage		
	Foam cm^3	suspensibility	Free alkalinity as 0.2 normal NaOH	Foam cm^3	Spontaneous emulsification	Free alkalinity as% NaOH
Hard water	0.4	100 %	0.576	traces	70.4 %	0.480
Soft water	0.2	100 %	0.144	traces	97.3 %	0.12

Hard water (342ppm as $CaCO_3$): Soft water (57ppm)

According to data presented in table (3), the local formulation passed successfully the hot storage test because it was alkaline before and after storage in hard and soft water, also it recorded a slight change in suspensibility in case of soft water from 100 % before storage to 97.3 % after storage, whereas it recorded decrease in suspensibility percentage in case of hard water from 100 % before storage to 70.4 % after storage, this change is accepted because it was more than 60 % according to FAO and WHO (2002).

Table 4: The physico-chemical properties of propolis 10 % SC local formulation spray solution at 0.5 % concentration.

	Surface tension(dyne/cm)	Viscosity (cm poise)	Conductivity (µ mohs)	pH
Sc.at 0.5%	49.09	7.34	100	7.88
water	72	1.0	400	8.7

According to data presented in table (4), spray solution of propolis 10% SC local formulation possessed low surface tension value 49.09 dyne/cm and high viscosity value 7.34 cm poise whereas it was a slight alkaline pH value was 7.88,than water alone. **(Spanoghe et al, 2007)**, stated that, increase in viscosity of spray solution, reduction drift and increased the retention sticking and pesticidal efficiency. Also **(Ryckaert et al., 2007**), concluded that, the reduction of surface tension of spray solution cause a good wettability, spreading and depositing of the particles of that solution surface. Therefore the pesticidal efficiency was increased.

Table 5: The efficiency of propolis 10 % SC formulation on second larval stage of Root-Knot nematodes under laboratory conditions.

EC_{50} ppm	EC_{90} ppm	Lower limit	Upper limit	Slope
120.8	151.6	122.74	131.78	13.2

According to data presented in table (5) . The local formulation showed nematicidal effect against second larval stage of Root-Knot nematode *Meloidogyne spp*.It is EC50 value was 120.8 ppm, also it recorded high slope value 13.2. The above results may be due to the propolis components that possessed several biological activites against fungi-bacteria,virus and nematode.(**Usaman and Mohamed,2015**)indicated that, most of the substances or compounds isolated from propolis include flavonoids ,diterpenoides, caffeic acid, esters and isoflavonoids .The nematicidal effect of these compounds was reported by (**Gommers,1981**). On the other hand preparation of propolis as SC formulation solved the big problem against it application under field conditions.(**Usman and Mohamed,2015**) reported that propolis cannot used as raw material.

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الملخص العربى

تحضيرصمغ النحل على صورة معلق مركز وتقييم كفاءته النيماتودية على نيماتودا تعقد الجذور

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***قسم المبيدات الفطرية والبكتيرية والنيماتودية ،** قسم بحوث مستحضرات المبيدات المعمل المركزى للمبيدات - مركز البحوث الزراعية - الجيزة**

صمغ النحل مادة راتينجية معقدة التركيب لها خواص دوائية وبيولوجية لذلك تم تجهيزها فى صورة (معلق مركز) SC وقد اجتاز المستحضر الجديد كل الاختبارات الفيزيائية والكيمائيئية المرتبطة بهذا النوع من أنواع المستحضرات والتى أقرتها المنظمات الدولية المتخصصة فى مجال المبيدات ثم جرب المستحضر الجديد على الطور اليرقى الثانى لنيماتودا تعقد الجذور بطريقة الغمر. أوضح المستحضر الجديد تأثيرا جيدا على الطور اليرقى الثانى لنيماتودا تعقد الجذور حيث كانت قيمة التركيز النصفى المميت هى ١٢٠.٨ جزء فى المليون.