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FORMULATION AND EVALUATION THE NEMATICIDAL ACTIVITY OF CERTAIN PLANT OILS AGAINST CITRUS NEMATODE *Tylenculus semi-penetrans*

[30]

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ABSTRACT

Four plant oils namely Barka, Sesam, Garlic and Almond were prepared as emulsifiable concentrate (EC). Polyethylene glycol 600 dioleate (PEG 600 DO), Toximol R and Toximol H were used as emulsifiers while xylene and toluene used as solvents. Four formulations only passed successfully (F1, D2, C3 and E5) in the Physico-chemical properties according to the standards of WHO. The results indicated that the successful prepared formulations showed different degrees of effectiveness against second stage juveniles of *Tylenculus semipenetrans* under laboratory conditions. Second stage juveniles seem as paralyze at 24 hrs, whereas this effect disappears completely after 72 hrs in case of Almond and Barka. On the other hand, the effect of Sesam and Garlic showed a slight effect at 24 hrs and increased gradually to give highly effectiveness after 72 hours. According to EC_{50} values at 72 hrs, Garlic was more effective than Sesam. The respective EC_{50} values were 0.6 and 2 mg/ml. These results emphasized the promising effect of certain plant oil formulations including Garlic and Sesam oil against pathogenic nematode, and that such formulation might be used for nematode control in small areas, as gardens and plant nursery.

INTRODUCTION

Agrochemicals have had a major role in improving in food production. However, concern has arisen about the negative impact that such chemicals have on human health and the environment. Some pesticides have active ingredients that act as hormone disruptors, and may cause loss of fertility, carcinogenesis and mutagenesis. The widespread application of agrochemicals to most cash crops has meant that pesticides are in the present ecosystem, aquifers and water systems of most agricultural areas. In the long term, this could have repercussions on both the environment and human health **(Dinham, 1933)**. There is therefore an urgent need to replace pesticides with biocontrol agents that are less toxic and more environmentally friendly **(Ibrahim *et al* 2006)**. The oily materials obtained by pressing and / or solvent extraction of oil seeds of fruits are called oils or fats in industry and commerce but lipids in biochemical and medical research. No strict definition of the term lipids seems to exist but it is normally used one of two ways. The wider frame relates to solubility properties where lipids considered as those substances, which are insoluble in water but soluble in certain organic solvents including hexane, diethyl ether and chloroform or binary mixtures. In more narrow sense, lipids are defined chemically as derivatives of long chain fatty acids and poly isoprenoids **(Appelqvist, 1989)**.

Essential oils are composed mainly of terpenes and phenilpropens, but the majority of essential oils contain predominantly (90%) terpenes. These terpenes contain mono terpenes with ten carbons, sesquiterpenes with fifteen carbons and diterpenes with twenty carbons **(Hay and Waterman, 1993; Zakaria 1991)**. Among alternative plant oils that are low in cost and local in production.

Several plant oils (fixed or essentials) have been found to possess antimicrobial, insecticidal and nematicidal activity **(Butler *et al* 1991 and Okoko *et al* 1999)**.

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Phytoparasitic nematodes are the most difficult crops pests to control **(Chitwood, 2002)**. They severely damage a wide range of agricultural crops, causing serious yield losses worldwide, especially in the tropical and subtropical regions, where environmental factors favour their survival and dispersal **(Sikora and Fernandez, 2005)**.

The citrus nematode *Tylenculus semipenetrans* was first found on citrus rootsin 1913 and is now known to be worldwide in distribution on that crop. The presence of this nematode was for the first time reported from Egypt by **Oeteifa (1955)**. Since it is widespread in all governorates where is found associated with poor growth and low yield of various citrus species **(Doss *et al* 1967)**.

The purpose of this work is to formulate of plant oils Almond, Sesam, Barka and Garlic as EC formulation and testing the efficiency of those formulations against citrus nematode.

MATERIALS AND METHODS

A- Tested Chemicals

a- Plant oils

1- Fixed oils: Barka, Sesam and Almond
2- Essential oils: Garlic

All tested oils (fixed and essential) were supplied by Zamzam factory Pyramids, Giza, Egypt.

b- Solvents

Xylene and Toluene were supplied by EL-Nasr Pharmaceutical Chemical Co. Cairo – Egypt.

c- Emulsifiers

1- Polyethylene glycol 600 dioleate (PEG 600 DO) was provided by the Egyptian Company For Starch Yeast and Detergents, Alexandria.
2- Toximol R and Toximol H were provided by Kafer – El- Zayat Company for pesticides formulation – Tanta

B- Preliminary qualitative screening phytochemical constituents of tested oils

The preliminary phytochemical screening tests were carried out on the tested oils as follow:

Sterol and / or triterpenes were detected according to method adopted by **Wall *et al* (1964)**, tannins estimated by the method described by **Claus (1961)**, phenolic glycosides detected according to **Balbaa (1981)**, Cardic glycosides estimated according to **EL-Kady (1997)**, alkaloids estimated by the method described by **Romo (1966)** and flavonoides detected according to **Vankatarman (1962)**.

C- Physico-chemical characteristics of the crude plant oils

1- Solubility: it was determined by measuring the volume of distilled water, Xylene, Toluene and acetone for complete solubility or miscibility of one gram of plant oil at 20ºC **(Nelson and Fiero, 1954)**. Then percentage of solubility was calculated according to the following equation.

% Solubility = w/v x 100
(where; W = weight of plant oil, V = volume of solvent required for complete solubility).

2- Viscosity: It was determined according to **ASTM D- 2196 (2005)** by using Brookfield viscometer model DVII + Pro where Centi poise is the unit of viscosity.
3- Surface tension: It was determined according to **ASTM D- 1331 (2001)** by using Cole – Parmer surface Tensiomate 21, where dyne/cm is the unit of surface tension measurements.

D- Formulation of the tested plant oils as emulsifiable concentrate

1- Screening the suitable emulsifier

Solubility of 5 ml of all tested emulsifiers in 100 ml plant oil was recorded. Then emulsion stability test was carried out after 24 hrs of preparing the mixture for clear solution according to specifications of **WHO (1979)**, by using 5% plant oil EC in Nile water, volume of any cream or oily separation was recorded, then the best emulsifier that achieved high emulsification of plant oil was recorded.

2- Improvement the emulsification of plant oil

It was carried out by increasing of the emulsifier to achieve high emulsification or by increasing both percentages of emulsifier and adding solvent, then emulsion test was carried out as mentioned before.

E- Physico-chemical properties of the locally formulated emulsifiable concentrate

1- Emulsion stability test: It was conducted according to WHO / M113 method **(WHO, 1979)**.
2- Cold stability test: It was determined according to **FAO / WHO (2002)**.
3- Accelerated Storage: It was determined according to **CIPAC M 46.1 (2002)**.

F- Physico-chemical properties of the spray solution of the locally formulated emulsifiable concentrate at the filled dilution rate

1- Surface tension: It was measured as mentioned before.
2- Viscosity: It was determined as mentioned before.
3- pH determination: It was measured using Cole Parmer 1484-44 pH / Conductivity meter.
4- Electrical Conductivity: It was determined by using Cole Parmer 1484-44 pH / Conductivity meter.
5- Emulsion stability test: It was determined according to Specifications of **WHO (1979)** as mentioned before.

G- Bioassay

Juveniles of *Tuenchulus semipenetrans* were collected by sieving infested soil directly. To evaluate the effectiveness of emulsifiable concentrate (EC) of tested oil, about 100 second stage Juveniles were used for every diluted oil. The final volume of diluted oil (EC) and nematode solution was 5 ml in 10 ml clean glass vials. Water was served as control and each treatment was replicated five times. The numbers of mobile and immobilized nematodes were counted after 24, 48 and 72 hrs.

H- Statistical analysis

Inhibition percentages were corrected using **Abbott's formula (1925)** and the inhibition regression lines were drawn according to the method of **Finney (1971)**.

RESULTS

Tested oils were selected depended on preliminary qualitative screening for their phytochemical constituents **Table (1)**. The most abundant compounds found in tested oils were cardic glycoside which is the main compound found in all tested oils, followed by phenolic glycoside, alkaloid, sterols and / or triterpenes and flavonoids which found in all tested oils except Garlic oil. While Antheoquion is only found slightly in Barka oil.

In the available current literature the phytochemicals that detected in tested oils are known as an effective biological agent against nematodes. **Sukul (1994)** indicated that, at the present more than hundred species of plants have been shown to possess nematicidial properties. The active principles present in some of these plants have already been isolated and identified. The effective pure compounds obtained from plants are glycosides, Quinones, unsaturated and saturated hydrocarbons, heterocyclic compounds, organic acids, aromatic compounds, esters, sulphur compounds and terpenes. **Gommers (1981)** reported that, one way of searching for such nematicidial compounds is to screen naturally occurring compounds e.g. alkaloid, phenol, sesquiterpenes, diterpenes, polyacetylenes and thienyl derivatives have nematicidial activity.

As known nematode is aquatic helminth and the living medium is water. On the other hand, oils don't soluble in water. So, it's not easy to evaluate tested oils on nematodes. There is therefore an urgent need to prepare the tested oils in suitable formulation.

2- Physical properties of the tested oils

The most important requirement in formulating pesticide is the solubility of active ingredient in solvent. Data in **Table (2)** show that, all tested oils are soluble completely in xylene and toluene but they do not dissolve in water and acetone. **Thomas (1964)** reported that molecular weight and the structure of the aromatic compounds of the solvent determined its solvency for insecticides. **Zaazo *et al* (1966)** demonstrated that local aromatic solvents such as Xylene, Toluene, Suzesol and Shelsol had higher solvency than aliphatic solvents such as Kerosene. The Barka oil is more viscous (698.3) than other tested oils followed by Garlic, Seasam and Almond oils (546.7, 470.8 and 440.8) respectively. As the highest values of surface tension of Garlic (39.3) followed by Seasam, Almond and Barka oils (38.4, 38.4 and 38), respectively.

According to the fore going results it could be conculated that, all tested oils did not soluble in water and it completely soluble in xylene and toluene therefore, it should be preparing as emulsifiable concentrate. Also it could be use xylene or toluene as co-solvent or as emulsification enhances for these oils.

Table 1. Preliminary Screening of Phytochemical Constituents in Tested Oils

Oils / Effective component	Garlic	Barka	Sesam	Almond
Sterol and / or triterpenoid	—	+ + +	+ + +	+ + +
Tannis:				
Catecol	—	—	—	—
Pyrogalllol	—	—	—	—
Glycosides:				
Phenolic	—	+ + +	+ + +	+ + +
Cardic	+ + +	+ + +	+ + +	+ + +
Saponine	—	—	—	—
Flavonoids	—	+ + +	+	+
Alkaloids	—	+ + +	+ + +	+ + +
Anthroqinon	—	+	—	—

-: absent
+++: high value
+: present

Table 2. Physical properties of tested oils

Plant oils	Solubility % (W/W)				Viscosity	Surface tension
	Water	Xylene	Toluene	Acetone		
Garlic	Nil	100%	100%	Nil	546.7	39.3
Barka	Nil	100%	100%	Nil	698.3	38
Sesam	Nil	100%	100%	Nil	470.8	38.4
Almond	Nil	100%	100%	Nil	440.8	38.4
Water	___	___	___	___	10	72

3- Screening the suitable emulsifier

Throughout the experiment that deals with the effect of different emulsifiers on tested oils. **Table (3)** show that, the Toximol H, R are not miscible with Garlic and Sesam oils but they are completely and partial miscible in Barka and Almond oils, respectively. Also they are not precipitated with all tested oils but they form oily separation in all oils except Barka oil. While the polyethelene glycol 600 dioleate is completely miscible with Barka, Sesam and Almond oils and partial miscible with Garlic oils. All tested emulsifiers are not form good emulsion with all tested oils.

On the other hand the mixture of emulsifier (Polyethylene glycol 600 Do) showed the best emulsification but require improving to reduce cream separation to level suitable with **WHO (1979)** specifications.

4- Improvement of emulsification

Emulsifiable concentrates are made from oily active ingredient which are soluble in non- polar solvent (Xylene or Toluene). The emulsifiers are added to these formulations to ensure spontaneous emulsification with good emulsion stability properties. The succeeded formulative oil should be has creamy separation not exceeds 2 ml F1, D2, C3 and E5 **(Table 4)**.

5- Physical properties of the locally formulated plant oils as EC's

The Judgment on the successful emulsifiable concentrate formulation depends on passing the emulsion stability test in soft and hard water where no oil separation occurred and precipitation or cream separation should not exceed than 2 ml at the rate of 5%.

Data in **Table (5)** demonstrated the physicochemical properties of the four oils prepared as emulsifiable concentrate. Generally all local prepared formulations passed successfully in emulsion stability test because their creamy layers did not exceed 2 ml in hard and soft water, where as the emulsion stability of Garlic and Sesam were improved as a result to hot storage. The creamy layer of Garlic (EC) changed from 0.5 ml in hard and soft water before storage to no cream layer after hot storage. Also the creamy layer of Sesam (EC) was changed from traces in hard and soft water before storage to no cream layer after hot storage. On contrast creamy layer of Barka (EC) was increased after hot storage from 0.5 ml in hard and soft water before hot storage to 2 ml in hard water and 1 ml in soft water. The same indication was noticed in case of Almond (EC) creamy layer that changed from zero in hard water after hot storage to 1 ml before hot storage. Finally all local prepared formulations did not show any separation or precipitation in glass after cold and heat test.

Physical properties of the spray solution concentration 0.5% of the local formulation

Data in **Table (6)** demonstrated the physical properties of local formulations. According to obtained data, no oily separation or creamy layer were detected as resulting to mix the promising local formulation with water at 0.5% concentration. For PH test, spray solution of Sesam EC. (76%) recorded the highest pH value 6.94 followed by Barka EC. (80%), Almond EC. (82%) and Garlic EC. (76%) the pH values were 6.55, 6.52 and 4.93 respectively. On the other hand the conductivity of spray solutions of tested oil EC's showed the same values 400 m mhos. Increase of electrical conductivity and decrease in pH values of insecticidal spray solution would lead to deionization of insecticides and increase its deposit and penetration in the tested surface then will increase the insecticidal efficiency **(Tawfik and EL-Sisi, 1987)**. As the highest value of surface tension of Almond formulated oil followed Garlic, Sesam then Barka the respective surface tension values were 72, 67.6, 64.1 and 59.2 D/Cm. Decrease of surface tension of pesticide spray solution give a prediction of increasing wettability and spreading on the treated surface then increasing pesticidal efficiency **(Osipow, 1964)**. The spray solutions viscosity of Barka formulated oil gave the maximum value 10.30 cm poise followed by 9.84, 980 and 9.40 for Almond, Sesam and Garlic. **Sherman, (1950)** reported that, viscosity of emulsion was function of type and concentration of emulsifying agent and pH. The same author observed a marked increase in viscosity occurred often when crude emulsions are homogenized. With another point of view **Richardson, (1974)** stated that, increasing viscosity of spray solution cause reduction drift and increasing the retention sticking and insecticidal efficiency.

The promising local formulations were evaluated under laboratory conditions against second stage juveniles of *Tylenculus semipenetrans* **Table (7)**, five concentrations were used for each tested oil formulation over time 24, 48 and 72 hrs. Generally there are a positive relationship were found between tested concentrations and percentages of inhibition with all tested oils under all

Table 3. Solubility of the tested emulsifiers (5%) in 95 oil

Tested oil	Emulsifiers	Solubility in plant oil as			Emulsification stability in Nil water
		Miscible	Precipitation	Separation	
Garlic	P.E.G. 600 DL	Partial miscible	-	-	4ml creamy layer
	Toximol R	Not miscible	-	Oily separation	8ml creamy layer
	Toximol H	Not miscible	-	Oily separation	8ml creamy layer
Barka	P.E.G. 600 DL	completemiscible	-	-	4ml creamy layer
	Toximol R	completemiscible	-	-	3ml creamy layer
	Toximol H	completemiscible	-	-	8ml creamy layer
Sesam	P.E.G. 600 DL	completemiscible	-	-	4ml creamy layer
	Toximol R	Not miscible	-	Oily separation	8ml creamy layer
	Toximol H	Not miscible	-	Oily separation	8ml creamy layer
Almond	P.E.G. 600 DL	completemiscible	-	-	8ml creamy layer
	Toximol R	Partial miscible	-	Partially Oily separation	8ml creamy layer
	Toximol H	Partial miscible	-	Partially Oily separation	8ml creamy layer

Table 4. Preparation steps of plant oils as emulsifiable concentrates

Oils	Formulation code	Emulsifier + solvent	Thickness of creamy layer	
			Hard water	Soft water
Garlic	**A1**	P.E.G. 600 DL+ toluen	6	6
	B1	P.E.G. 600 DL+ toluen	3	4
	C1	P.E.G. 600 DL+ toluen	3	3
	D1	P.E.G. 600 DL+ toluen	2	2
	E1	P.E.G. 600 DL+ toluen	1	1
	F1	P.E.G. 600 DL+ xylene	0.5	0.5
Almond	**A2**	P.E.G. 600 DL+ toluen	4	2
	B2	P.E.G. 600 DL+ toluen	2	2
	C2	P.E.G. 600 DL+ toluen	2	2
	D2	P.E.G. 600 DL+ xylene	Zero	1
Sesam	**A3**	P.E.G. 600 DL+ toluen	2	1
	B3	P.E.G. 600 DL+ toluen	-	1
	C3	P.E.G. 600 DL+ xylene	trace	trace
Barka	**A4**	P.E.G. 600 DL+ toluen	8	2
	B4	P.E.G. 600 DL+ toluen	4	1
	C4	P.E.G. 600 DL+ toluen	2	6
	D5	P.E.G. 600 DL+ xylene	1	1
	E5	P.E.G. 600 DL+ xylene	0.5	0.5

Table 5. **Physical properties of the prepared oils formulated under storage conditions**.

Oils	Type of Formulation	Emulsion Stability (ml. cream sep.)		Cold test	Accelerated storage Emulsion Stability (ml. cream sep.)	
		SW	HW		SW	HW
Garlic	EC 76%	0.5	0.5	Pass	Zero	Zero
Barka	EC 84%	0.5	0.5	pass	1	2
Sesam	EC 84%	traces	traces	Pass	Zero	Zero
Almond	EC 84%	1	Zero	pass	1	1

Table 6. Physico-Chemical properties of the spray solution concentration 0.5% of the prepared ormulation

Oils	Surface tension dyne/cm	PH	Conductivitym mhos	Viscositycm poise	Spontaneity		Emulsion Stability	
					HW	SW	HW	SW
Garlic	67.6	4.93	400	9.40	-	-	Zero	Zero
Almond	72	6.52	400	9.84	-	-	Zero	Zero
Sesam	64.1	6.94	400	9.80	-	-	Zero	Zero
Barka	59.2	6.55	400	10.30	-	-	Zero	Zero

Table 7. Nematicidal effect of the locally prepared plant oils as EC's against Juveniles of *Tylenculus semipenetrans* under laboratory conditions

Oils EC's	EC_{50} (mg/ml)			Slope		
	24h	48h	72h	24h	48h	72h
Garlic 76%	-	2.3	0.6	4.1	1.6	2
Almond 82%	0.719	0.794	n.c	2.1	1	0.04
Sesam 84%	1.6	5.0	2.0	1.3	1	1
Barka 84%	2.0	3.98	-	1.1	0.9	0.5

exposure periods. Second stage juveniles become paralyzed and seem to be dead when treated with Almond at 24 hours. This effect decrease with increasing the exposure periods to 48 hours whereas it disappears completely at 72 hrs. The same indication was found in case of Barka formulation which recorded highly effectiveness at 24 hours and their activity decreased gradually to disappear at 72 hours. This indication is agree with results that obtained by **EL-Kady *et al* (2006)**. On the other hand Sesam oil formulation showed a moderate activity at 24 hrs that increased gradually to give highly activity at 48 hours then it deflexed to recorded less activity at 72 hrs. On contrast the effectiveness was noticed as slight inhibition at 48 hours in case of Garlic and increased to give the highly effectiveness after 72 hrs. The refluxing in effectiveness that noticed in case of Almond, Barka and Sesam may be due to the nematode defense systems that could be degrade the toxicants products in tested oils.

According to EC_{50} values at 72 hrs Garlic was the most effective oil formulation followed by Sesam. The respective EC_{50} values were 0.6 and 2 mg/ml. the slope values of Garlic was steepest than Sesam that showed flat toxicity line depending in this indication tested nematode was more sensitive to Garlic than Sesam.

The effect of vegetable oils on nematodes was reported by **Miller, (1979)** which found that nematode populations were decreased by rates of lin seed oil, 0.5 and 1 ml of cotton seed or olive oils and 1 ml of corn, soybean, or safflower oil. These results suggest that vegetable oils might be used for nematode control in small areas such as gardens or lawns.

The synergistic effect of Sesam (*Sesamum indicum* L.) oil on the insecticidal activity of pyrethrins was noted years later in 1940 **(Crosby, 1995)** and responsible ingredient was shown to be Sesamin **(Haller *et al* 1942)**. The pesticidal effect of Garlic was reported by several workers, **Sukul *et al* (1974)** indicated that garlic extract is equally toxic to citrus and muchroom nematodes It has shelf life of more than six months.

Anter *et al* (1994) tested those different concentrations of smashed garlic *Allium sativum* to control *Meloidogyne incognita* infecting tomato, under green house conditions. A negative correlation was noticed between garlic dosages and each of nematode galling number of J2 in soil and number of eggs / plant.

The nematicidal effect of Garlic may be due to the volatile sulfur – based compounds which are effective as insect repellents and insecticides. Diallyl disulfide is one of such compounds which has a strong odor and acts as powerful insecticides **(Kaufmam *et al* 1999)**. Commercial preparations of Garlic are certified as insecticide against mites, nematode and mosquito larvae affecting a variety of crops **(Gamboa *et al* 2006)**.

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