Extraction, Formulation of Phenolic compounds from Morus Spp. and Canna Indica and Evaluation of Their Nemticidal activity against Root-Knot Nematodes Meloidogyne Spp.

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ABSTRACT: Total phenols were extracted from *Morus Spp.* and *Canna indica* and their nematicidal effect was evaluated against migration of Root-Knot nematodes under laboratory conditions. The obtained results showed that, the phenolic compounds extracted from *Morus Spp.* were more effective than phenols extracted from *Canna indica*. The EC_{50} values were 8.1 and 1214 ppm for *Morus Spp. and C. indica* ,respectively. So, phenolic compounds from *Morus Spp*. were formulated as soluble concentrate (SL). The formulated product passed successfully all physico-chemical properties for soluble concentrate formulations. The effectiveness of formulated product was revealed to be greater than its crude extract by 25.9 %.
Keywords: Root-Knot nematode, Phenol extraction, Migration technique and Formulation.

1.INTRODUCTION

Phytoparasitic nematodes are among the most difficult crop pests to control. They severally damage a wide range of agricultural crops, causing serious yield loss worldwide, especially in the tropical and subtropical regions, where environmental factors favor their survival and dispersal (**Javed et al., 2007**). Root-knot nematodes are obligate sedentary endoparasites of many plant species. Their wide host range encompasses more than 3000 plant species. It has been reported to cause yield loss up to 20 to 33 % (**Khan et al., 2011**).

Among different nematode management strategies chemical control has proved generally effective but majority of these chemicals are highly expensive and often hazardous. All kinds of nematicides are highly toxic not only to plants and livestock but also to soil micro-flora and fauna resulting ecological imbalance (**Jairajpuri et al., 1990**). There is an increasing interest in discovering nematicidal compounds in plant (**Chitwood, 2002**). Many compounds that have nematicidal or nematostatic activity such as thinyls, alkaloids, phenols, sesquiterpenes, diterpenes and poly acetylenes have been found in healthy plant tissues (**Gommers and Barker, 1988**). Different studies showed that induced resistance through the accumulation of various phenolic compounds and phytoalexins (**Cherif et al., 2007**). Most of phenolic compounds are poly phenols and they are categorized into 5 groups: - Simple phenols and phenolic acid, phenyl propanoids, flavnoids, tannins and quinine, large number of phenolic compounds have been found to have strong nematicidal activity (**Ohri and Pannu, 2010**). The purpose of formulating pesticide active ingredients for crop protection is uniformly spread a small amount of active chemical over large area or by helping the active ingredient remain at the site intended pesticidal action, improve uniform application, extend the stability of the active ingredient, provide a longer shelf-life, convenient packing and improve user safety there is an associated reduction in the need for re,application and reduction in the amount which enters the environment (**Mohamed, 2010**). The aim of this study is to extract the total phenols from two plants namely *Morus Spp*. and *Canna indica* and evaluating their nematicidal activity against Root-Knot nematodes, then formulating the most promising one in a suitable formulation form and testing the biological activity of local formulation under laboratory conditions in order to determine the role of formulation process in the enhance the efficiency of the end product.

2.MATERIALS AND METHODS

2.1.Phenol extraction:

200 gram of air dried powder leafs of *Morus Spp*. and *Canna indica* were defatted by extraction by petroleum ether (b.r 60-80 ^{o}C). The dried defatted powder was then extracted with ethanol (90 %) to complete the extraction. The alcoholic extract was concentrated to 50 ml under reduced pressure at 60^{o}C then diluted with 30 ml distilled water and then extracted with ethyl acetate (100 ml) the solvent extract was evaporated off and kept for investigation (**El khayaat, 1986**)

2.2. Bioassay:

Biological activity of total phenols extract or its formulation was carried out by using migration technique that used by (**El-Kady, 1997**) as follow:-

A bioassay unit consists of polyethylene tube of 1.3 cm long and 2.4 cm diameter covered at one end with two layers the first one is paper handkerchief then muslin cloth. It filled with washed air-dried sand of particle size 250 um and placed upright in a Petri-dish, 5 cm diameter. Serial concentrations from total phenols extract or its formulation were prepared by dilution with water. One ml of each concentration was pipetted on the surface of sand in each tube. Then one ml of water containing 100 second stage larvae of Root-Knot Nematodes *Meloidogyne Spp* were pippeted on the surface of the sand in each tube. Each bioassay unit was transferred to Petri dish (9 cm diam.) containing filter paper saturated with 2 ml water to serve as humidity room to prevent evaporation. After 24 hours the bioassay unit took up from humidity room, 8 ml distilled water was added in the Petri dish of bioassay unit. The number of juveniles that had migrated into each bioassay unit was recorded 72 hours later. Treatment values were expressed as percentage of control values and each treatment was replicated four times.

2.3.Preparation of formulation:-

2.3.1. the physico-chemical properties of the basic formulation:-

a. Free acidity or alkalinity: It was determined according to (WHO) specification, 1979.

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

$$\% \text{ solubility} = w/v * 100$$

(Where; w: active ingredient weight V: volume of solvent required for complete solubility).

2.3.2. Surfactants:-

a. Hydrophilic-lypophilic balance (HLB): The solubility of surfactant in water was considered as approximate guide to its hydrophilic- lypophilic balance (HLB). HLB of the tested surfactants was measured according to standard method, (**Lynch *et al.*, 1974**).

b. Critical micelle concentration (CMC): CMC is the concentration at which the surface tension of the solution does not decrease with any further increase in surfactant concentration. CMC of the tested surfactants was measured according to (**Osipow 1964**).

c. Surface tension: It was measured by Du-Nouy tensiometer for solutions containing 0.5% (weight/volume) surface active agents where dyne/cm is the unit of surface tension measurement .

d. Free acidity or alkalinity: It was determined according to (WHO) specification, 1979.

2.4. Preparation of total extracts in soluble concentrate form:-

The promising total phenols were prepared as soluble concentrate according to the method described by (**Mohamed, 2010**).

2.4.1. The physico-chemical properties of the prepared local (SL) formulation: The following physico-chemical properties were determined for the local prepared formulation before and after heat storage at (54±1 ^{o}C) for three days.

a. Surface tension: It was measured by Du-Nouy tensiometer.

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

2.4.2. The physico-chemical properties of spray solution of local formulation: - The physico-chemical properties of spray solution of local formulation 0.5 % were determined as the following:-

a. pH value: It was determined by using Cole-Parmer PH/Conductivity meter 1484-44. pH (Dobrat and Marttjn, MT 75.3, 1995)

b. Viscosity: It was determined by using Ostwald viscometer where centipoises are the unit of viscosity. Viscosity was determined according to ASTM D-2196

c. Surface tension: It was measured by Du-Nouy tensiometer where dyne/cm is the unit of surface tension measurement. Surface tension was determined according to ASTM -1331

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. Conductivity (Dobrat and Marttjn, MT 32, 1995).

e. Heat Storage Test: Accelerated Storage at 54±3 ^{o}C for 3 days according to CIPAC MT 46.1.

3.RESULTS AND DISCUSSION

Depending on Fig. (1), total phenols extracted from *Morus Spp*. and *Canna indica* showed nematicidal activity against migration of 2nd stage larvae of Root-Knot nematodes. On the other hand, a regration relationship was found between tested concentrations and percentages of migration inhibition with total phenols of both tested plants.

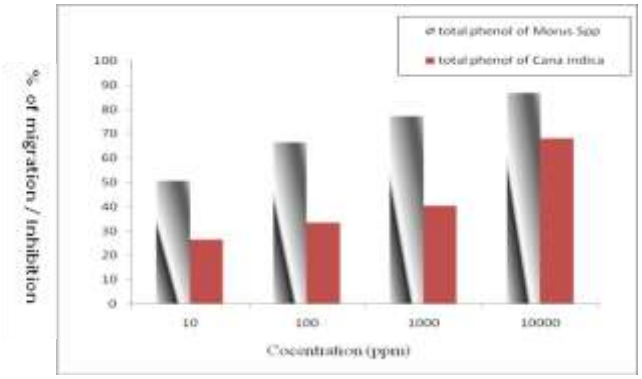

Fig 1: Effect of different concentrations of total phenols extracted from *Morus Spp.* and *Canna indica* against migration of 2nd stage larvae of *Meloidogyne Spp*.

According to table (1) total phenols of *Morus Spp*. was more effective than that of *Canna indica*, the respective EC_{50} values were 8.1 and 1214 ppm. Also both phenols recorded similar slope values 0.36 and 0.35. This indicated that, both phenols may be possessed the same mode of action, these results are agree with (**Mahajan et al., 1992**) who tested a wide range of phenolic compounds for their nematicidal activity against *Meloidogyne incognita*. Out of the 55 phenolic compounds tested, coumestrol, juglone, dihydroxy caffeic acid, quinine- 2,6-dihydroxy benzoic acid, gentislic acid , p-methoxy cinnamic acid, 3-phenyl phenol, 7-hydroxy coumarine, vanillic, syringic and pratacatechuic acid showed high nematicidal activity. Also (**Hung and Rohad, 1973**) reported that, the large number of larvae of *M. incognita* never penetrate the resistant variety of tomato due to some sort of inhibition and this inhibition provided by phenolic compounds.

Table 1: LCP line of total phenols extracted from *Morus Spp.* and *Canna indica* against migration of Root-Knot nematode.

Total phenols extracted from	EC_{50} (ppm)	Lower limit	Upper limit	Slope ± SE
Morus Spp.	8.1	1.1592	24.7305	0.363 ± 0.0629
Canna indica	1214	536.5192	3934.5738	0.348 ± 0.0592

The physico-chemical properties of total phenol of *Morus Spp*. as an active ingredient were set up in table (2), total phenols were soluble in water 33.3 % in contrast it was not soluble in xylene and acetone. Also it was acidic and their acidity value was 0.19616. Depending on the above data this active ingredient could be formulated as soluble concentrate (SL). Also it needs a slight acidic surface active agent according to FAO and WHO meeting (2002) on pesticide specifications; the pesticide which can be formulated is limited by solubility and hydrolytic ability.

Table 2: The physico-chemical properties of total phenols of *Morus Spp* as an active ingredient.

Material	water	acetone	xylene	Free acidity as H_2SO_4
Total phenols of *Morus Spp.*	33.3 %	NS*	NS*	0.19616

NS*: means not soluble

Data in table (3) showed the physico-chemical properties of tween 20 as a wetting agent. Tween 20 showed a clear solution when it is dissolved in water at 0.5 % whereas it recorded HLB value close to 13 and their CMC was 0.2. Also it recorded free acidity 0.186. On the other hand it decreased the surface tension of water from 72 to 43.2 dyne/cm. Depending on the above results it could be concluded that, there is an agreement between the free acidity of phenolic compound and tween 20. Also the HLB value of tween 20 was greater than 13, so it is considered as dispersing agent.

Table 3: The physico-chemical properties of tween 20 as wetting agent.

Surfactant	Type	Dispersability	HLB	CMC	Free acidity as H_2SO_4	Surface tension dyne/cm
Tween 20	Non ionic	Clear solution	> 13	0.2	0.186	43.2

Soluble phenols of *Morus Spp*. were prepared through blending tween 20 with three concentrations 2.5, 5, 10 %. The obtained data indicated that, surface tension was decreased by increasing the concentration of wetting agent whereas no sedimentation was found

Table 4: Effect of tween 20 percentage on the physico-chemical properties of local soluble concentrate formulation.

Total phenols of	tween 20 %	Surface tension of water 0.5 % dyne/cm	Sedmintation
Morus Spp.	2.5	58.9	Nil
	5.0	54	Nil
	10.0	49	Nil

According to data presented in table (5), the local formulation passed successfully hot storage test, because there are no changes were found between the solubility, sedimentation and free acidity of local formulation SL 10% before and after storage.

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Table 5: Effect of heat storage on the physico-chemical properties of locally prepared formulation SL 10%.

Before Storage			Heat Storage		
Solubility	Sedmentation	Free acidity as H_2SO_4	Solubility	Sedmentation	Free acidity as H_2SO_4
soluble	Nil	0.19615	soluble	Nil	0.19616

Data in table (6) showed that, spray solution of local formulation at 0.5 % recorded low surface tension value 47.65 dyne/cm; high viscosity value 7.4429 cm poise; low PH value 6.93 and high conductivity value 400 µ mohs. All these properties improved spreading, wettability, retention of sprayed solution, penetration, reduction drift and attraction between spray solutionand treated surface (**Osipow, 1964** and **Richardson, 1974**).

Table 6: The physico-chemical properties of spray solution of local formulation SL 10%.

Surface tension dyne/cm	Viscosity cm poise	Conductivity µ mohs	pH
47.65	7.4429	400	6.93

Data in table (7) compare between the biological activity of total phenols of *Morus Spp.* and its local formulation against migration of 2nd stage larvae of Root-Knot nematode under laboratory conditions. Generally, local formulation showed nematicidal activity against migration of 2nd stage larvae more than total phenols only, the EC_{50} decreased from 8.15 ppm in case of total phenols to 6.04 ppm in case of local formulation. Also the slope value of local formulation increased 4.4 times compared with slope value of total phenols only. The increasing in effectiveness of total phenols as a result to formulation was 25.9 %. The above indication may be due to the role of wetting agent that reduced the surface tension of spry droplet that spread on the body of nematode providing more covering for toxicant by decreasing contact angel of spray drops on body surface. From another point of view wetting agent may facilitate the penetration of active ingredient to reach its target and achieve its action (**El-Kady, 2008**).

Table 7: Comparison between the efficiency of total phenols extracted from *Morus Spp.* and its 10 % SL formulation on migration of 2nd stage larvae of Root-Knot nematode *Meloidogyne spp*.

Examined material	EC_{50}	Slope	Increasing in effectiveness
Morus Spp. total phenols	8.15	0.96	25.9 %
Formulation	6.04	4.25	

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الملخص العربى

استخلاص وتجهيز الفينولات الكلية من نباتى التوت و الكانا انديكا وتقييم كفاءتهما على نيماتودا تعقد الجذور

هشام ابراهيم عبد الله محمد .

قسم بحوث مستحضرات المبيدات - المعمل المركزى للمبيدات –

مركز البحوث الزراعية

تم استخلاص الفينولات الكلية من نباتى التوت والكانا انديكا . كما تم تقييمهما من الناحية البيولوجية على هجرة نيماتودا تعقد الجذور تحت ظروف المعمل. أوضحت النتائج ان الفينولات الكلية المستخلصة من التوت كانت أكثر فاعلية من تلك المستخلصة من نبات الكانا انديكا وهو ما ظهر واضحا من قيم التركيز النصفى المميت لكلا منهما حيث كانت ٨,١ و ١٤٤,١ جزء فى المليون لهما على الترتيب. لذلك فقد تم تجهيز الفينولات الكلية المستخلصة من التوت فى صورة مركز قابل للذوبان فى الماء. واجتاز المستحضر بنجاح كل الاختبارات الفيزوكيمائية المرتبطة بهذا النوع من أنواع مستحضرات المبيدات. كما جرب المستحضر على هجرة نيماتودا تعقد الجذور وأظهرت النتائج أن المستحضر كان أكثر تأثيرا من المادة الفعالة (الفينولات الكلية المستخلصة من التوت) كما زادت فاعليتة عن مادتة الفعالة بمقدار ٢٥,٩ %.