

Study the Nematicidal Efficiency of *Corchorus olitorius*, *Cinnamomum camphora*, *Portulace oleraceae* and *Lantana camara* Extracted Saponins and Their Formulations on Root-Knot Nematodes *Meloidogyne Spp.*

Hala S. Ibrahim¹, S.E.S. Hamouda² A. M. A. El-kady² and H. I. Abd-Alla²

¹Fungicide, Bactericide and Nematicide Research Department, Agriculture Research Center (ARC), Cairo, Egypt.

²Formulation Research Department, Central Agricultural Pesticides Lab. (CAPL), Agriculture Research Center (ARC), Cairo, Egypt.

Saad_capl@yahoo.com

Abstract: Total saponin was extracted from plants namely *Corchorus olitorius*, *Portulace oleraceae*, *Cinnamomum camphora* and *Lantana camara*. The nematicidal efficiency was evaluated against second stage larvae under laboratory conditions through dipping and migration techniques. Also the effect of the tested saponins on the penetration of second stage larvae to egg-plant roots was studied under green house conditions. Migration technique was found to be more accurate in evaluating the nematicidal efficiency than dipping technique. The saponin of *Portulace oleraceae* was more effective against the migration of second stage larvae than that of *Lantana camara*, the respective EC₅₀ values were 4526.04 and 4906.8 ppm. On the other hand there was no relationship found between the tested concentrations and the inhibition percentage of migration in case of *Cinnamomum camphora*. From another point of view no gall formation was recorded on roots of egg-plant that was infected by 330 second stage larvae when treated with 5000 and 10000 ppm, for the saponins of *Portulace oleraceae* and *Lantana camara* the respective percentage of inhibition in root galling formation was 98.9 and 100 %. Both saponins of *Portulace oleraceae* and *Lantana camara* were formulated as soluble concentrate (SL) and passed successfully all the tests determined by the organizations of pesticides. Both saponin formulations of *Portulace oleraceae* and *Lantana camara* increased the nematicidal efficiency than their active ingredient saponins by 18.36 and 97.9 % respectively. [Hala S. Ibrahim, S.E.S. Hamouda A. M. A. El-kady and H. I. Abd-Alla. **Study the Nematicidal Efficiency of *Corchorus olitorius*, *Cinnamomum camphora*, *Portulace oleraceae* and *Lantana camara* Extracted Saponins and Their Formulations on Root-Knot Nematodes *Meloidogyne Spp.*** *Nat Sci* 2014;12(11):40-45]. (ISSN: 1545-0740). <http://www.sciencepub.net/nature>. 7

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1. Introduction

Plant parasitic nematodes constitute one of the most important pest groups of the economic crops, especially in developed and developing countries of the world (Sultana *et al.*, 2011). Root-knot nematodes (*Meloidogyne Spp.*) occur worldwide and are responsible of large part of the annual yield losses attributed to nematodes (Trudgill, 2001).

In the past, root-knot nematode diseases had been effectively and mostly controlled by synthetic nematicides. But synthetic nematicides had been confirmed to be a source of water, food and environmental pollution. Agricultural crop consumers are poisoned and in most cases the price of synthetic nematicides are very high. All these problems have greatly limited the use of nematicides (Olabiyi, 2005).

Recently, one alternative used has been to screen naturally occurring plant secondary compounds for appropriate nematicidal activity. Various nematicidal substances of plant origin such as triglycerides, sesquiterpenes, alkaloids, steroids, diterpenes, and flavonoids, have been identified in this way. These compounds can be developed for use as nematicides

themselves, or can serve as model compounds for the development of chemically synthesized derivatives with enhanced activity and reduced environmental impacts (Chitwood, 2002 and Faizi *et al.*, 2011).

Among these naturally occurring substances are saponins. Saponins are a class of secondary plant metabolites with diverse biological properties. These molecules have an interesting pesticide potential. The best studied pesticide activity is the insecticidal activity. These substances cause several forms of toxicity against harmful insects (anti-feedency, disturbance of moult, growth regulation, etc.) the insecticides activity of saponins comes from their interaction with alimentary cholesterol causing disturbance of the synthesis of molting hormone. The fungi-toxic activity was also relatively well studied, saponins demonstrated to interact with membrane sterols, disturbing the balance of membrane exchanges, destruction of the structure of the membrane and consequently a mortality of the mycelium. The molluscicidal and nematicidal activities of certain saponins were discussed in several works (Chaieb, 2012).

Saponins are involved in resistance and play relevant role in defensive chemistry of insects from their known biological activities, the toxicity of phytophagous insects, either insecticidal or antifeedant and to root-knot nematodes show the potential of these compounds to develop plant pest control products (Carlos *et al.*, 2011). With current worldwide derive toward organic agriculture which will subsequently leads to the production of organic foods, it is envisaged that search should continue on natural plants (lotanicals) which could be used instead of synthetic insecticides in crop protection (Olabiyi *et al.*, 2008).

So that, depending on the previously mentioned reasons especially the disadvantages of the synthetic nematicides, the main aim of this work is to extract saponin from different plants, evaluating its nematicidal efficiency on root-knot nematode, formulating the candidates in a suitable formulation type, comparing between saponin as an active ingredient and its formulation in order to discover new nematicides to be used in the field of pest control.

2. Materials and Methods

1) Plant material:

The aerial parts from *Portulaca oleraceae*, *Lantana camara*, *Corchorus olitorius* and *Cinnamomum camphora* were collected from field in Kafr karshoum village, Tala city, Menoufia governorate and air dried, grounded through mall Farbion 300 and kept in a closed brown bottle until saponin extraction.

2) Saponin extraction:

Saponin was extracted from powder of each tested plant according to the method described by El Fiki (1980) as follow:

The defatted powder of each plant was completely exhausted with distilled water (till no froth was given on shaking 5 ml of the extract) water extract was concentrated under vacuum. The residue was dissolved in alcohol (50 %) and saponin was precipitated by the addition of large amount of acetone.

3) Bioassay:

All tests were carried out on root-knot nematode *Meloidogyne Spp.*, propagated in pure culture in Central Agricultural Pesticides Laboratory (CAPL):

A) Under laboratory conditions:

Two methods were carried out to evaluate the nematicidal efficiency of the tested saponins under laboratory conditions.

A₁) Dipping technique:

Dipping technique was carried out according to the method described by Abd-alla *et al.* (2013) as follow:

Tested saponins were prepared in water and each concentration was replicated three times, the final volume of replicate was 10 ml contained in clean glass vial of 20 ml capacity. Control treatment was done by using (10 ml) about 1000 newly hatched larvae for each replicate. Motility counts were tallied after 24, 48 and 72 hours from treatment.

A₂) Migration technique:

Migration technique was carried out depending on El- Kady method (1997) to evaluate the nematicidal efficiency of the tested saponins and their formulations. The bioassay unit consisted of polyethylene tube of 1.3 cm long and 2.4 cm diameter covered at one end with muslin cloth. It filled with washed sand and with particle size 250 micron and placed standing in a Petri dish 5 cm diameter. Serial concentrations from each saponin and its formulation were prepared in water. Each concentration was pipette on the surface of sand in each tube. Then one ml of water containing 100 second stage larvae of *Meloidogyne Spp* was pipette on the surface of sand in each tube.

Each bioassay unit was transferred to Petri dish 9 cm diam. containing filter paper saturated with 2 ml water to keep humidity of the room to prevent evaporation. After 24 hours, the bioassay unit was taken from the wet room and then 8 ml distilled water was added to Petri dish of the unit. The number of larvae that had migrated into each bioassay dish was recorded 72 hours later. Treatment values were expressed as percentage of control values and each treatment was replicated four times.

B) Under greenhouse conditions:

The effect of the tested saponin on penetrating second stage larvae of root-knot nematode was studied. Two concentrations 5000 and 10000 ppm was prepared (LC₅₀ and its double value). Each concentration was replicated three times; each replicate contains 300 newly hatched second stage larvae. Three replicates without treatment with saponin were used as control. All treatments were kept at 25°C for 72 hours then transferred on roots of egg-plant planted on cups 6.5 cm diameter filled with 240 grams sterilized soil. The cups were irrigated regularly as needed. After seven weeks from treatment the number of galls / root was determined and the percentage of inhibition was calculated.

4) Formulation part:

Several steps were carried out to formulate *Portulace oleraceae* and *Lantana camara* as soluble liquid formulations:

a) Study the physicochemical properties of both saponins of *Portulace oleraceae* and *Lantana camara* (solubility and free acidity or alkalinity).

b) Determining the suitable wetting agent that is compatible with the tested saponins through several trials and measurements.

c) Each trial to be successful was stored under tropical conditions at 54±1 for three days.

d) The physicochemical properties of spray solution (PH, viscosity, surface tension, conductivity and flash point were studied).

The Physicochemical properties of the materials used for the preparation of *Portulace oleraceae*.

Lantana camara soluble liquid formulations:

a) Solubility: It was determined by measuring the volume of distilled water 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:

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

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

b) Free acidity or alkalinity: It was determined according to CIPAC MT 31.1 (2002).

c) Surface tension: It was determined by using Cole-Parmer surface tension meter 21, where dyne/cm is the unit of surface tension measurements.

d) Viscosity: It was determined by using Brookfield viscometer model DV II + Pro, where centipoises are the unit of viscosity.

e) PH value: It was determined by using Cole-Parmer PH/Conductivity meter 1484-44.

f) Electrical conductivity: It was determined by using Cole-Parmer PH/Conductivity meter 1484-44, where μ mohs is the unit of electrical conductivity measurement.

g) Flash point: It was tested for both formulations by fisher/tag Cleveland open cup flash tester USA. The sample was gently heated and a flame was passed from the center of the opening of the cup, the flash point was recorded as the temperature at the thermometer when flash appears (Dobrat and Martign, 1995).

3. Results and Discussion

Evaluation of the nematocidal effect of *Corchorus olitorius*, *Portulace oleraceae*, *Cinnamomum camphora* and *Lantana camara* saponins was studied under laboratory conditions against second stage larvae of *Meloidogyne Spp* through dipping technique table (1). No nematocidal effect was noticed for the saponin of *Corchorus olitorius* against second stage larvae of *Meloidogyne Spp*, their inhibition effect was zero with all tested concentrations at 24, 48 and 72 hours. On the other hand a slight inhibition was recorded with other tested saponins (*Portulace oleraceae*, *Cinnamomum camphora* and *Lantana camara*). These indications started as low effect at 24 hours and showed a little increase at 48 hours and decreased at 72 hours.

Table (1): Evaluation of *Corchorus olitorius*, *Portulace oleraceae*, *Cinnamomum camphora*, *Lantana camara* saponins against second stage larvae of root-knot nematode through dipping technique under laboratory conditions.

Concentration ppm	Inhibition percentages of saponins of											
	<i>Corchorus olitorius</i>			<i>Portulace oleraceae</i>			<i>Cinnamomum camphora</i>			<i>Lantana camara</i>		
	24	48	72	24	48	72	24	48	72	24	48	72
1000	0	0	0	2.1	6.1	5.2	0.8	12.5	2.1	2.8	2.7	2.4
100	0	0	0	4.1	6.9	4.3	0.6	4.1	3.4	2.03	2.3	0.6
10	0	0	0	1.5	6.4	4.3	2.1	9.3	1.5	0	0	0

From another point of view there is no relationship between the tested concentrations and the percentages of inhibition. So, the three tested saponins of *Portulace oleraceae*, *Cinnamomum camphora* and *Lantana camara* were reevaluated as nematocides through migration technique table (2) generally there is a positive relationship between the tested concentrations and the percentage of inhibition in case of *Portulace oleraceae* and *Lantana camara* only. According to the EC₅₀ values, *Portulace oleraceae* was

more effective than *Lantana camera*, their EC₅₀ values were 4526.5 and 4906.8 ppm respectively. On contrast the slope values of *Portulace Oleraceae* (0.29) was sharper than the slope value of *Lantana camara* (0.25). From the above data it could be concluded that, the role of saponin as nematocide was to disturb the nematode sensors that orient the second stage larvae, So that the second stage larvae on treatment with saponins it did not succeed to migrate against the light.

Table (2): Evaluation of saponin of *Portulace oleraceae*, *Cinnamomum camphora* and *Lantana camara* against second stage larvae of root-knot nematode through migration technique under laboratory conditions.

Concentration ppm	Percentage of inhibition of saponins of		
	<i>Lantana camara</i>	<i>Portulace oleraceae</i>	<i>Cinnamomum camphora</i>
10000	53.1	53.9	25.5
1000	43.0	42.5	24.1
100	33.4	31.7	22.9
10	Non calculated	22.2	21.7
EC ₅₀	4906.8	4526.5	Non calculated
Slope	0.25336	0.2876	Non calculated

El-Kady (1997) indicated that, immersion test failed to evaluate the nematicidal efficacy of plant extracts. This may be due to the differences between the mode of action of plant extracts, and the mode of action of traditional acute insecticides. So the need to use another technique to screen plant extracts is necessary. From the above data, migration technique was more accurate in the determination of the nematicidal effect of saponins, to confirm this suggestion; the effect of saponin on the penetration of the second stage larvae to the roots of egg-plant was studied under green house conditions table (3).

Depending on the percentages of inhibition of root galling formation, both tested concentrations 5000 and 10000 ppm prevented completely second stage larvae from penetrating egg-plant root. The percentage inhibition in root galling formation was 98.1 and 100 % at 5000 and 10000 ppm in case of *Portulace oleraceae* saponin and 100 % with the same concentrations in case of *Lantana camara* saponin. These results agreed with what was mentioned by Omar *et al.* (1994) that, *in vitro* and pot experiments, saponins were found to reduce total populations, number of eggs and juveniles motility and viability of root-knot nematodes.

Table (3): Evaluation of *Portulace oleraceae*, *Lantana camara* saponin penetration of root-knot nematode second stage larvae on egg-plant.

Concentration ppm	<i>Portulace oleraceae</i>	% Inhibition	<i>Lantana camera</i>	% Inhibition
Control	36.3	-	36.7	-
5000	0.7	98.1	0	100
10000	0	100	0	100

The simplest of all formulations to make is the solution concentrate, an aqueous solution of active ingredient, which merely requires dilution in the spray tank. The number of pesticides which can be formulated in this way is limited by water solubility and hydrolytic stability of active ingredient (Mohammed, 2010).

According to the physico-chemical properties of *Portulace oleraceae* and *Lantana camara* saponins, both tested the saponins were soluble in water and alkaline the respective free alkalinity were (1.5), (0.22) so they were formulated as soluble liquid formulations (SL) according to differences in wetting agent (Tween 20, Tween 40 and tween 80). As visual (SL) formulation of saponin, no change in color and precipitation after six months. The soluble concentrate formulation of saponin dissolved readily and, when

diluted at a ratio of (5:95) (V/V) soluble concentrate formulation: distilled water to produce stable solution with out precipitation. All the stable prepared formulations passed the recommended test of foam and then reevaluated through migration technique table (4). On comparison with table (2) the EC₅₀ value of both tested formulations decreased than their values of saponins as active ingredients. The EC₅₀ values of *Portulace oleraceae* saponin was 4526.5 ppm and became 3981.1ppm in case of formulation, also the EC₅₀ value of *Lantana camara* saponin was 4906.8 ppm and decreased to 116.77 ppm in case of formulation with a consequence increase in effectiveness by 18.36 and 97.6 % respectively. Also the slope values of both formulations became sharper than their saponins active ingredients.

Table (4): Comparison between EC₅₀, slope and the increase in effectiveness of both formulations of *Portulaca oleraceae* and *Lantana camara*.

The value of	<i>Lantana camara</i>	<i>Portulaca oleraceae</i>
EC ₅₀ ppm	116.77	3981.1
Slope	1.13	0.9715
Increase in effectiveness	97.9 %	18.36 %

The Physicochemical properties of the tested formulations spray solution were tabulated in table (5). The PH values of *Portulaca oleraceae* saponin was 6.77 whereas that of *Lantana camara* saponin formulation was 7.77 so *Portulaca oleraceae* spray solution showed little acidic character while *Lantana camara* spray solution showed little alkaline character. On the other hand the surface tension values of both tested formulations were 42.63 and 60 dyne/cm in case of *Portulaca oleraceae* and *Lantana camara* respectively, both formulations reduced surface tension of water in spray solution by 29.37 and 10 % for *Portulaca oleraceae* and *Lantana camara* respectively. The lower surface tension is desirable

property for most agricultural sprays because it facilitates the spreading of the droplets on the leaves or other target surface to increase the surface active area, improves penetration and up take of the product into the plant and can facilitate the retention of the material by the rain, ensuring improved fastness. Therefore the pesticidal efficacy was increased (Furmidge, 1962). Also both tested formulations showed low conductivity 10. From another point of view both tested formulations recorded high flash point value > 70 °C. According to WHO specifications, the liquid formulations must have flash points not less than 27.8°C.

Table (5): The physico-chemical properties of *Portulaca oleraceae* and *Lantana camara* spray solution by concentration of 0.5 %.

Component	Viscosity cm / poise	Surface tension dyne/cm	Conductivity μ mhos	pH
<i>Portulaca oleraceae</i>	10.89	42.63	10	6.77
<i>Lantana camara</i>	19.26	60	10	7.77

Conclusion

The saponins extracted from *Corchorus olivarius* and *Cinnamomum camphora* has no nematocidal efficiency on root-knot nematode whatever was the bioassay technique used, while the saponins of *Portulaca oleraceae* and *Lantana Camara* were effective on treatment by using migration technique rather than dipping technique. In addition to that formulating saponins as active ingredients increased its efficiency proving that the additions used to formulate any substance plays an important role in changing its efficiency.

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