World Journal of Agricultural Sciences 16 (5): 368-374, 2020
ISSN 1817-3047

DOI: 10.5829/idosi.wjas.2020.368.374

The Toxic Effect of *Moringa* Oil and *Moringa* Aqueus Extract on Glassy Clover Land Snail, *Monacha cartusiana* and Red Spider Mite, *Tetranychus urticae* Koch

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Abstract: The glassy clover land snail, *Monacha cartusiana* (Müller) and the red spider mite, *Tetranychus urticae* Koch are dangerous pests because they cause serious damage to most crops. Scientists do their best to replace the chemical molluscicides and acaricides with alternative natural pesticides. *Moringa* essential oil and the aqueous *Moringa* extract were studied under laboratory conditions against adult glassy clover snail, *M. cartusiana* and adult female of *T. urticae.* Also, LC_{50} of each treatment was calculated and the obtained results revealed that the active essential oil of *Moringa* was more effective than the aqueous *Moringa* extract against *M. cartusiana* and adult female of *T. urticae*. LC_{50} value was recorded 13968.83 ppm and 17808.47 ppm, LC_{90} value was 92044.97 ppm and 104967.84 ppm for *Moringa* oil and aqueous extract against *M. cartusiana*, respectively. However, LC_{50} value was 35.31 ppm and 184.69 ppm, LC_{90} value was 788.78 ppm and 2793.29 ppm for *Moringa* oil and aqueous extract against *T. urticae*, respectively. Also, the chemical composition of extract of *Moringa* oil was characterized by GC/MS analysis which revealed the presence of different chemical groups including varieties of fatty acid, alkaloids, terpenoids steroids and hydrocarbon compounds, the major compound was oleic acid (28.32%).

Key words: Monacha cartusiana · Tetranychus spp. · *Moringa* oil · *Moringa* aqueous extract

INTRODUCTION

Terrestrial molluscs have greatly increased in economic importance and they are considered a group of the most serious pests attacking agricultural crops around the world [1]. In Egypt, land snails have been increased and distributed rapidly in most Governorates and caused considerable damage especially in most areas where they found the optimum conditions for survival and dispersion.

They cause great damage to vegetables, field crops, orchard trees as well as ornamental and medical plants [2, 3]. Economic damage caused by these molluscs is due to feeding and contamination with their bodies, feces or slime, leading to deterioration of the product quality, in addition to the financial loss [4]. The glassy clover snail, *Monacha cartusiana* (Müller) causes damage to vegetables and field crops [5]. Therefore, control of these snails is becoming very important. Phytophagous mites, such as *Tetranychus urticae* Koch (Acari: Tetranychidae), are pests of crops of economic importance, such as cotton, soybean, tomato, papaya, strawberry and others [6-9]. When not controlled, these organisms can cause damage to crops. For most crops, the chemical method has been the main tool to combat this mite. The products used generally have molecules of wide spectrum, eliminating even natural enemies, besides possessing high residual power [9, 10].

Moringa oleifera Lamarck (Family: Moringaceae) is widely distributed in tropical and subtropical regions [11]. It is an important medicinal plant referred as a miracle tree [12]. Flowers of *M. oleifera* Lam. contain bioactive compounds including antioxidants, antimicrobial alkaloids, lectin and trypsin inhibitor [13, 14]. It is a plant studied for several purposes, from biodiesel production, to insecticidal activity on disease vectors and even on *T. urticae* [15-19]. However, the form of action and effects of the substances present in the oil are still little explored on agricultural pests.

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The objective of this study is to evaluate the comparison between the toxic effect of *Moringa* oil and aqueus *Moringa* extract against *M. cartusiana* and *T. urticae* and make GC/ MS analysis to *Moringa* seeds oil.

MATERIALS AND METHODS

Tested Snails: Adults land snail *Monacha cartusiana* were handily collected from infested fields at Mansoura district, Dakahlia Governorate. The obtained snails were transferred to the laboratory and then kept in plastic containers filled with moist sterilized sandy loamy soil at 25°C ± 2°C and 75 % ± 5 % soil moisture. Snails were fed on fresh leaves of lettuce (*Lactuca sativa* L.) for 14 days to be laboratory acclimatized. Dead and unhealthy snails were removed and only healthy ones were used in the experiments.

Mites Rearing: *Tetranychus urticae* was collected from unsprayed castor bean plants and reared at 25± 2°C and 60± 5%RH.

***Moringa* Essential Oil Extraction:** *Moringa* oil was extracted by steam distillation apparatus in Plant Protection Research Institute, Mansoura, Egypt, using seeds collected from the farm of Faculty of Agriculture, Mansoura University. The oil was separated dried over anhydrous sodium sulfate and stored in dark glass bottles at 4°C in refrigerator until used.

***Moringa* Aqueous Extract:** *Moringa* aqueous extract was extracted as leaves of *Moringa* tree were collected from the farm of Agriculture Faculty farm, Mansoura University and soaked in water and the concentration was detected on the base of weight of dried leaves powder/ the volume of water.

Preparing the Stock Solution of the Tested Material: Convenient stock concentrations of material were prepared on basis of the tested material, (*Moringa* oil or *Moringa* leaves powder), weight and the volume of the distilled water (w/v) in the presence of tween 80 (0.1%) as emulsifier. The stock concentrations were kept in glass stoppered bottles and stored under refrigeration. Such stock solutions were prepared periodically. Four diluted concentrations for oil and plant extract were used to draw the LC-P lines. Three replicates were used for each concentration.

Toxicity Test

Toxicity Test of *Moringa* Oil and *Moringa* Aqueous Extract Against *Monacha cartusiana*: Four concentrations of *Moringa* oil and *Moringa* aqueous extract 5000, 10000, 15000 and 20000 ppm were used. For each concentration fresh lettuce leaves were dipped for one minute and left for dryness [20]. The treated leaves were placed inside plastic boxes filled with moist sterilized sandy loamy soil. Ten adult snails of *M. cartusiana* were placed into each box. Each box was covered with muslin cloth fixed with rubber bands to prevent snails from escaping. Each concentration had three replicates and untreated lettuce disks were used as a control treatment. Mortality percentage was recorded after 1, 3, 5 and 7 days post treatments.

Toxicity Test of *Moringa* Oil and *Moringa* Aqueous Extract Against *T. urticae*: The toxicity of *Moringa* oil and *Moringa* aqueous extract was evaluated against adult females of *T. urticae*. Thirty newly emerged adult females were transferred to the lower surface of castor leaf discs (2.5 cm diameter) placed separately on moist cotton wool in Petri dishes. Each petri dish contains three replicates, ten individuals in each replicate. *Moringa* oil acaricide and *Moringa* aqueous extract acaricide had four concentrations of 100, 250, 500 and 750 ppm which were sprayed on the individuals. Mortality was recorded for 7 days after treatment.

The mortality percentage was estimated and corrected according to the Abbott's [21]. LC_{50} values were determined using probit analysis statistical method of Finney, [22]. Toxicity index of the tested compounds was determined according to Sun equation Sun [23] as follows:

Toxicity index (LC_{50}) = 100

Chemical Analysis: GC/MS analyses were conducted in the Central Laboratory for Food and Feed, Agriculture Research Centre. The analysis was performed on Agilent Technologies 7890 A Gas Chromatography (GC) Systems coupled with Mass Spectrometry (MS) detector. 1µL/L of fatty acid methyl ester solution was injected into the system by using GC auto sampler. Helium was used as carrier gas at the flow rate of 1 mL/min injector temperature 280°C; auxiliary temperature: 290°C; and ion source temperature 280°C. The oven temperature was programmed from 50°C (isothermal for 1.0 min), with an increase of 40°C/min, to 170°C (isothermal for 4.0 min), then 10°C/min to 310°C (isothermal for 10 min) fragments

from 45 to 450 Da. Total GC running time is 32.02 min. The compounds are identified by GC–MS Library.Wiley and Wiley Nist mass spectral data base was used in the identification of the separated peaks.

RESULTS AND DISCUSSION

Effect of *Moringa* Oil and *Moringa* Leaves Aqueous Extract on Adult Land Snail, *Monacha cartusiana* and Adult Female of Red Spider Mite *Tetranychus urticae* Koch: Data presented in Table (1) indicated that *Moringa* oil caused high mortality percentage for *M. cartusiana* adult snails than *Moringa* leaves aqueous extract. However, the toxicity of such treatment was increased with increasing the concentration.

As shown in Table (2) and Fig. (1) LC_{50} value was recorded 13968.83 ppm and 17808.47 ppm, LC_{90} values were 92044.97 ppm and 104967.84 ppm for *Moringa* oil and aqueous extract against *M. cartusiana*, respectively. Ibrahim [24] showed that, the seed powder of *M. oleifera* had a molluscicidal activity against the snails *Biomphalaria glabrata* and *Physa marmorata* but had no effect against *M. tuberculatus*. Also, Silva [25] found that aqueous *M. oleifera* Lam seed extract was toxic to *Bulinus* adult snails in a dose dependent manner and the total lethal concentration (LC_{50} and LC_{90}) values determined after 24 hours exposure from the whole streams were 468 ppm and 813 ppm respectively.

Data in Table (1) indicated that, the *Moringa* oil caused high mortality proportion on the red spider mite, *T. urticae* Koch than the aqueous extract. However, Table (2) and Fig. (1) described that the *Moringa* oil was more effective than the aqueous extract, with LC_{50}:35.31 ppm and 184.69 ppm, respectively. LC_{90} values were 788.78 ppm and 2793.29 ppm for *Moringa* oil and aqueous extract, respectively. The toxicity index was 100% for *Moringa* oil while it was 19.12% for aqueous extract. The slope values were 0.95 and 1.09 for *Moringa* oil and aqueous extract, respectively. Anderson [26] concluded that the green seed extract of *M. oleifera* are more toxic to *T. urticae* showed LC_{50} (6.94%). *M. oleifera* seeds, at different stages of maturation, have acaricidal activity and can be considered as potential tools in the management and control of *T. urticae.* Holtz [19] showed that aqueous extracts of *Moringa* seeds presented high toxicity to *T. urticae*, resulting in a lethal concentration for 50% of the population around 12.39%, *M. oleifera* oil is toxic to different groups of insects and to the mite.

Chemical Analysis: GC/MS analysis of *Moringa* oil was detected and listed in Table (3) and Fig. (2) according to their retention times and percentage composition. These compounds comprise 46.8% of the total composition. These compounds included different chemical groups including varieties of fatty acid, alkaloids, terpenoids steroids and hydrocarbon compounds. Oleic acid was the

Table 1: Corrected mortality percentages of *Monacha cartusiana* and *Tetranychus urticae* treated with *Moringa* oil and *Moringa* aqueous extract under laboratory conditions:

			Mortality after treatments %				
Pests	Treatment	Conc.(ppm)	One day	Three days	Five days	Seven days	Total Mortality %
M. cartusiana	*Moringa* Essential Oil	5000	3.33	6.67	6.67	10	26.67
		10000	3.33	10	10	13.33	36.67
		15000	6.67	10	13.33	20	50
		20000	6.67	13.33	20	23.33	63.33
	Moringa Aqueous Extract	5000	-----	6.67	6.67	6.67	20
		10000	3.33	6.67	13.33	6.67	30
		15000	3.33	10	13.33	16.67	43.33
		20000	6.67	13.33	16.67	20	56.67
T. urticae	*Moringa* Essential Oil	100	10	20	20	16.67	66.67
		250	13.33	23.33	23.33	16.67	76.67
		500	20	30	13.33	30	93.33
		750	23.33	30	13.33	30	96.67
	Moringa Aqueous Extract	100	--------	6.67	10	26.67	43.34
		250	3.33	10	13.33	23.33	50
		500	13.33	10	13.33	23.33	60
		750	16.67	20	13.33	33.33	83.33

Table 2: Toxic effect (LC_{50} and LC_{90}) of *Moringa* oil and *Moringa* aqueous extract against *Monacha cartusiana* and *Tetranychus urticae.*

Pests	Treatments	Conc.	Corrected mortality%	LC_{50}	LC_{90}	Slope ± S.D.	Toxicity index LC_{50}	R	P
M. cartusiana	*Moringa* Essential Oil	5000	26.67	13968.83	92044.97	1.57±0.3	0.25	0.97	0.40
		10000	36.67						
		15000	50						
		20000	63.33						
	Moringa Aqueous Extract	5000	20	17808.47	104967.84	1.66±0.3	0.20	0.98	0.47
		10000	30						
		15000	43.33						
		20000	56.67						
T. urticae	*Moringa* Essential Oil	100	66.67	35.31	788.78	0.95±0.2	100	0.97	0.02
		250	76.67						
		500	93.33						
		750	96.67						
	Moringa Aqueous Extract	100	43.34	184.69	2793.29	1.09±0.2	19.12	0.88	0.01
		250	50						
		500	60						
		750	83.33						

R: Regression P: Probability

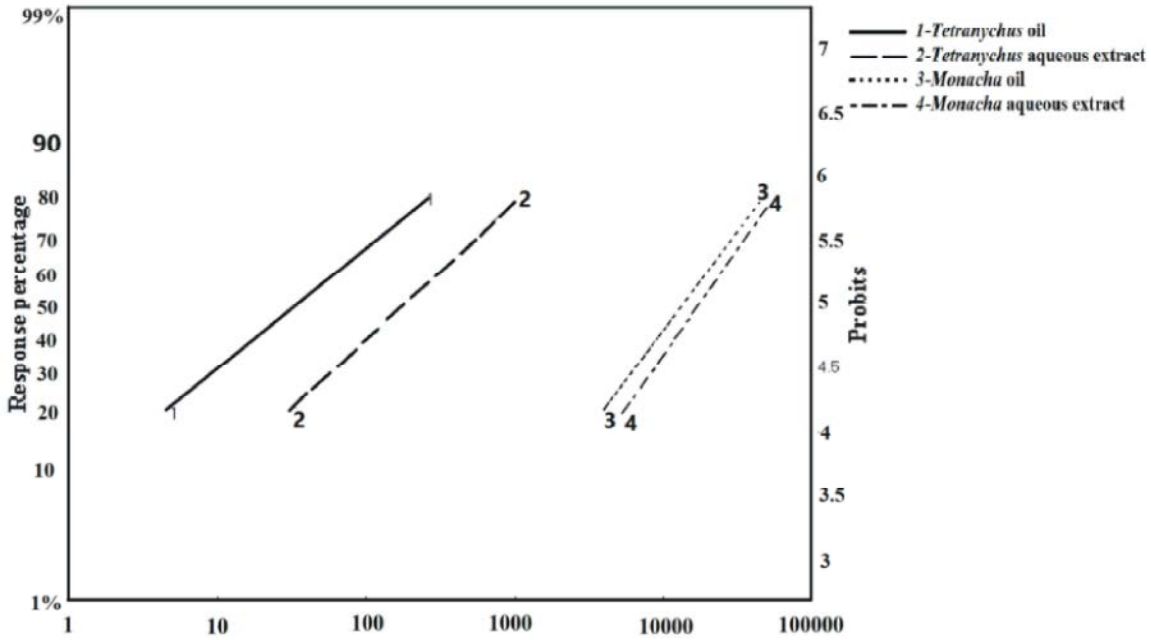

Fig. 1: Con/probit regression line of *Moringa* oil and *Moringa* leaves aqueous extract on *Monacha cartusiana* and *Tetranychus urticae*

most abundant compound (28.32%), followed by Stearic acid (9.25%), quinoline, 3-methyl and campesterol (1.18%), copaene (1.01%), lupenon and alpha-ergosterol (0.77%), cholesterol (0.59%), beta-amyrene (0.47%), piperazine, 1, 4-dimethyl (0.45%), squalaen (0.38%), coumaric acid (0.32%), pyrrolidine, 1-(1, 6- dioxooctadecyl)- (0.31%), arachidic acid and linoleic acid (0.27%), stigmasta-4, 22-dien-3.beta.-ol (0.25%), 6, 6-dimethoxypiperidin-2-one (0.22%), gama-sitosterol (0.2%), 4-ethoxy-6-piperidin-1-yl-[1, 3, 5]triazine-2-carboxylic acidamide (0.17%), 2-exo-hydroxy-5-ketobornane (0.15%), neocurdione (0.14%) and myristic acid (0.13%). The above results agree with findings of Abdulkarim [27] who showed that *Moringa* seeds contain a large amount of fatty acid like Arachidic, Behenic, Oleic and Palmitic acid. Also, Ragasa [28] proved the presence of higher concentration of compounds such as alkaloids in turn can negatively influence the feeding of insects. Augusto [29] reported that the aqueous extract of *M. oleifera* seeds contained bioactive molecules including saponins, volatile oils and lectins which were

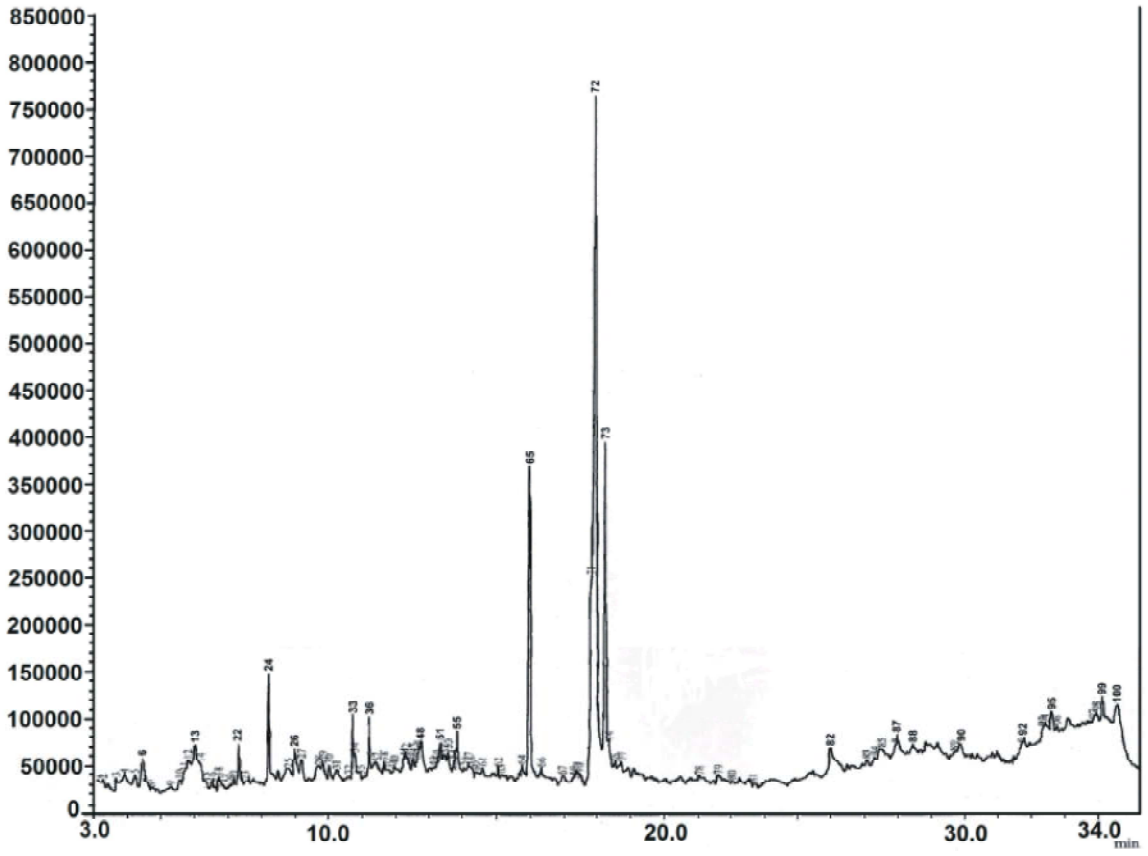

Fig. 2: GC/MS analysis of *Moringa oleifera* oil

Table 3: Main components of *Moringa oleifera* seeds identified by GC/MS

No.	Retention time (min.)	Components	Formula	Area (%)
1	4.67	4-Ethoxy-6-piperidin-1-yl-[1, 3, 5]triazine-2-carboxylic acidamide	$C_{11}H_{17}N_5O_2$	0.17
2	5.38	Neocurdione	$C_{15}H_{24}O_2$	0.14
3	6.77	2-Exo-hydroxy-5-ketobornane	$C_{10}H_{16}O_2$	0.15
4	6.84	Piperazine, 1, 4-dimethyl	$C_6H_{14}N_2$	0.45
5	7.19	6, 6-Dimethoxypiperidin-2-one	$C_7H_{13}NO_3$	0.22
6	10.94	Quinoline, 3-methyl	$C_{10}H_9N$	1.18
7	11.44	Copaene	$C_{15}H_{24}$	1.01
8	11.67	Campesterol	$C_{28}H_{48}O$	1.18
9	14.54	Myristic acid	$C_{14}H_{28}O_2$	0.13
10	14.69	Coumaric acid	$C_9H_8O_3$	0.32
11	17.77	Linoleic acid	$C_{19}H_{34}O_2$	0.27
12	18.34	Oleic acid	$C_{18}H_{34}O_2$	28.32
13	18.63	Stearic acid	$C_{18}H_{36}O_2$	9.25
14	19.14	Arachidic acid	$C_{20}H_{40}O_2$	0.27
15	20.125	Pyrrolidine, 1-(1, 6- dioxooctadecyl)	$C_{22}H_{41}NO_2$	0.31
16	20.358	Squalaen	$C_{30}H_{62}$	0.38
17	27.10	Alpha-ergosterol	$C_{28}H_{46}O$	0.77
18	29.49	Cholesterol	$C_{27}H_{46}O$	0.59
19	31.45	Stigmasta-4, 22-dien-3.beta.-ol	$C_{29}H_{48}O$	0.25
20	32.02	Beta-Amyrene	$C_{30}H_{50}$	0.47
21	32.10	Lupenon	$C_{30}H_{48}O$	0.77
22	33.57	Gama-sitosterol	$C_{29}H_{50}O$	0.2

known by its molluscicidal activity. Silva [25] showed that fatty acids, larvicidal and antinutritive activity of Oleic acid in *M. oleifera* seeds have been reported on *A. aegyptii*, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), *Lymantria dispar* L. (Lepidoptera: Lymantriidae) and *Culex quinquefasciatus* Say (Diptera: Culicidae). Silva [30] confirmed that the seed powder of *M. oleifera* had a molluscicidal activity against the snails *Biomphalaria glabrata* and *Physamar morata* and stated that the snails retracted into shell and suffered hemorrhage after treatment.

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