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Efficacy of Mint Derivatives, *Mentha Spicata* L., Against Two Species of *Tetranychus* Spp. (Acari: Tetranychidae) and the Predator, *Neoseiulus* sp.

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ABSTRACT

The mint plant and its derivatives are common extracts used in the management of plant pests as an alternative to pesticides with saving effects on the environment, plants, animals and humans. The acaricidal activity of mint plant derivatives, (*Mentha spicata* L.,), (mint oil, mint extract and menthol) were studied under laboratory conditions against adult females of the carmine spider mite, *Tetranychus cinnabarinus* (Boisduval), the two-spotted spider mite, *Tetranychus urticae* Koch and the predacious mite, *Neoseiulus* sp. The results indicated the effectiveness of the mint derivatives especially menthol and mint extract against *T. cinnabarinus* and *T. urticae* with a low effect on the predator, *Neoseiulus* sp. LC_{50} was 5713.9, 9631.03 and 13782.6 ppm for menthol, mint extract and mint oil, respectively, for *T. cinnabarinus*. However, the LC_{50} was 5463.2, 7349.7 and 7463.4 ppm for tested compounds mentioned before, respectively, for *T. urticae*. This revealed that the mint derivatives were more effective on *T. urticae* than *T. cinnabarinus* and menthol was more save on the predator *Neoseiulus* sp. than the other mint derivatives.

INTRODUCTION

Tetranychus spp. (Acari: Tetranychidae) are important pests that infest many crops and cause a high reduction in productivity of plants or kill the host plants (Nachman & Zemek 2002).

The predatory mite *Neoseiulus californicus* (McGregor), belongs to the family Phytoseiidae, prefers Tetranychid mites as food, but it consumes other phytophagous mites and small insects, such as thrips. It is found on various crops and is considered one of the main predatory mites that are used in IPM in Egypt (El-Sharabasy, 2010). Chemical control is the famous way for managing spider mites (Pontes *et al*., 2007) as soon as chemical pesticides cause many problems like pest resurgence, secondary pest outbreak, environmental toxic residues, and pesticide resistance (Isman 1999). The high reproductive potential and the short life cycle of spider mites, combined with pesticide applications overuse under greenhouse conditions, resulting in more quick resistance to many miticides (Ambikadevi & Samarjit 1997).

Essential oils extracted from plants are known to have biological activity against eukaryotic organisms (Deans and Ritchie, 1987) and prokaryotic (Konstantopoulou *et al*., 1992). The main monoterpenes that characterize the essential oil composition of the different species and hybrids are either cyclic C-2 or C-3 compounds (Zhang 2003).

Mentha piperita is not only playing an important role in killing or inhibiting viruses and fungi (Schuhmacher *et al*., 2003) but also has toxicity to many pests such as bean weevil (Raja *et al*., 2001). Natural plant extracts play a prominent role as alternatives to synthetic acaricides because of increasing environmental pollution, negative effects on non-target organisms and health hazards (Sharma *et al*., 2006).

The present study aimed to evaluate the toxic effect of mint plant derivatives (extract, oil and the active ingredient) against two species of *Tetranychus* and one predator, *Neoseiulus spp.*.

MATERIALS AND METHODS

Rearing Mites:

The unsprayed castor plants that carried *T. cinnabarinus* and *T. urticae* were collected and the mites were reared at 60± 5%RH and 25± 2° C.

Predator Rearing:

The predatory mite *Neoseiulus* sp. was collected from different plants. The colony was maintained under laboratory conditions in large plastic boxes (70x30x40 cm). Castor leaves highly infested with *T. cinnabarinus* were provided every day as a food source for predatory mites.

Preparation of Mint Extract:

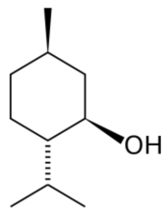
Leaves of the mint plant were left to dry at room temperature for a month, then ground into fine powder in an electric mill. This powder was soaked in a mixture of petroleum ether, ethanol, and acetone solvents of equal proportion (1:1:1) in a flask for about one week. Then, the flask was shaken in a shaker and finally, the contents were filtered. The used organic solvents were evaporated under reduced pressure; then the crude extract was weighed and kept in a deep freezer until use.

Preparation and Isolation of Essential Mint Plant Oil:

Essential oil of mint was extracted from fresh leaves of mint plant and extracted by steam distillation apparatus. This apparatus was found in Plant Protection Institute, Mansoura, Egypt. The essential oil was separated and dried over anhydrous sodium sulfate then stored in dark glass bottles at 4° C in the refrigerator until used.

The Active Ingredient of The Plant:

Menthol (99%) is a crystalline material and was bought from (El- Gomhoria company-Mansoura).



Menthol formula (**OPENDER *et al.*, 2008**)

Preparing the Stock Solution, the Tested Plant Derivatives:

Convenient stock concentrations of the extract, oil and the active ingredient were prepared depending on basis of the tested weight of the plant and the volume of the distilled water (w/v) in the presence of tween 80 (0.1%) as an emulsifier. The stock concentrations of extract and oil were kept in tightly closed glass bottles and stored under refrigeration until use. The stock solutions were prepared periodically and four diluted concentrations for each extract, oil and the active ingredient were used to draw LC-P lines and three replicates were used for each concentration.

Toxicity Test of *T. urticae* and *T. cinnabarinus*:

The toxicity of mint oil, mint extract and menthol was tested against the adult females of *T. urticae* and *T. cinnabarinus*. Thirty newly emerged adult females were transferred to the lower surface of castor leave discs (that was 2.5 cm diameter) that placed separately on moist cotton wool in Petri dishes. Each petri dish contains three replicates and ten individuals in each replicate. Each acaricide had four concentrations that were sprayed on the individuals. Mortality was recorded for 7 days after treatment. Mortality percentage was estimated and corrected according to Abbott's formula, 1925. LC_{50} values were determined using the probit analysis statistical method of Finney, 1971. Equation: Sun, 1950 (to determine LC_{50} index).

$$\textbf{Toxicity index for LC50} = \frac{\text{LC50 of the most effective compound}}{\text{LC50 of the least effective compound}} \times 100$$

Toxicity Test of The Predator, *Neoseiulus* sp.:

After detecting LC_{50} of mint extract, mint oil and menthol that applied on *T. cinnabarinus*, LC_{50} was applied on *Neoseiulus* sp.; Three replicates were used and ten individuals in each replicate.

RESULTS AND DISCUSSION

The Toxic Effect Of Mint Extract, Mint Oil And Menthol On Adult Females of *T. cinnabarinus*:

The data obtained in Table (1) showed that menthol caused high mortality when applied on *T. cinnabarinus,* especially with 5000 ppm and 10000 ppm compared with 5000 and 10000 ppm of mint extract or mint oil. As mentioned in the table, the mortality rate was 36.67% and 73.33% at 5000 ppm and 10000 ppm of menthol, respectively. While, the mortality rate was 20& 30 % for concentration 5000 ppm and 26.67& 43.33 % for concentration 10000 ppm of mint oil and mint extract, respectively. It's worth noting that, the mint extract was more effective than mint oil at concentrations, 15000 and 20000 ppm, that the mortality was 66.67&53.33 % at concentration 15000 ppm of mint extract and mint oil, respectively; and 80& 70 % at concentration 20000 ppm of mint extract and mint oil, respectively. Habashy *et al*., 2015 proved the effectiveness of camphor, the active ingredient of basil plant, and the basil extract than the basil essential oil when applied on *T. cinnabarinus*.

Table 1: Mortality % of carmine spider mite, *Tetranychus cinnabarinus* treated with mint derivatives under laboratory conditions 25±2 °C and 60±5% RH.

Treatments	Conc. (ppm)	Mortality after treatments %				Total Mortality %
		One day	Three days	Five days	Seven days	
Mint oil	5000	----	6.67	6.67	6.67	20
	10000	6.67	6.67	6.67	6.67	26.67
	15000	-----	10	20	23.33	53.33
	20000	30	16.67	6.67	16.67	70
Mint extract	5000	6.67	16.67	-----	6.67	30
	10000	23.33	10	3.33	6.67	43.33
	15000	26.67	16.67	3.33	20	66.67
	20000	30	16.67	13.33	20	80
Menthol	2500	-----	3.33	20	----	23.33
	5000	-----	20	6.67	10	36.67
	7500	30	20	10	3.33	63.33
	10000	33.33	26.67	10	3.33	73.33

Furthermore, Table (2) and Fig. (1) indicated that the active ingredient menthol was the most effective material than mint extract and mint oil with LC_{50}: 5713.9 ppm and LC_{90}: 20332.6 ppm; followed by a mint extract that had LC_{50}: 9631.03 ppm and LC_{90}: 35923.3 ppm then mint oil with LC_{50}: 13782.6 ppm and LC_{90}: 49051.9 ppm. The toxicity index was 100% for menthol while it was 59.3 & 41.5% for mint extract & mint oil, respectively. Slope values indicated that menthol and mint oil had the highest same value 2.32 while mint extract had a slope value: of 2.24. LC_{90}/ LC_{50} confirm the value of this criterion recorded 3.56, 3.56 and 3.37 for menthol, mint oil and mint extract, respectively. Thus, the lowest ratio LC_{90}/ LC_{50} or the highest slope value means the steepest toxicity line. The obtained results were in contrast with Badawy *et al.* (2010) who showed a limited effect of two monoterpenoids on the hatching rate of *T. urticae* eggs and mortality rate of *T. urticae*. While our results were in agreement with Khedr and El-Kawas, 2013; and Habashy *et al*., 2015 that proved the effectiveness of plant extracts on *T. cinnabarinus***.**

Table 2: Efficiency of Mint derivatives against *Tetranychus cinnabarinus*:

Treatments	Conc.	Corrected mortality %	LC_{50}	LC_{90}	Slope± S.D.	Toxicity index LC_{50}	LC_{90} / LC_{50}	R	P
Mint oil	5000	20	13782.6	49051.9	2.32± 0.31	41.5	3.56	0.938	0.02
	10000	26.67							
	15000	53.33							
	20000	70							
Mint extract	5000	30	9631.03	35923.3	2.24± 0.30	59.3	3.73	0.967	0.121
	10000	43.33							
	15000	66.67							
	20000	80							
Menthol	2500	23.33	5713.9	20332.6	2.32± 0.3	100	3.56	0.973	0.162
	5000	36.67							
	7500	63.33							
	1000	73.33							

R: Regression **P: Probability**

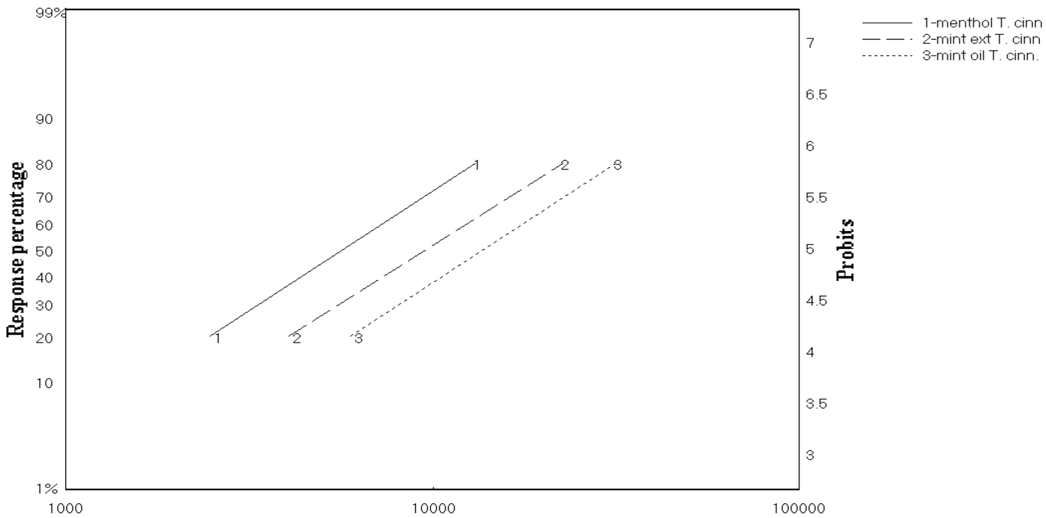


Fig. 1: LC-P lines for mint plant derivatives against adult female of *Tetranychus cinnabarinus.*

The Toxic Effect of Mint Extract, Mint Oil and Menthol on Adult Females of *T. urticae*:

Data obtained in Table (3) demonstrated that mint oil and mint extract caused high mortality when applied on *T. urticae* with 5000 ppm compared with menthol, the mortality rate was 40%, 40% and 36.67% when applied on mint oil, mint extract and menthol, respectively. However, the mortality rate was 83.33%, 53.33% and 53.33% for menthol, mint oil and mint extract, respectively, at 10000 ppm. As mentioned in Table (3), the mortality rate was 90% and 70% at 20000 ppm with mint extract and mint oil, respectively. So mint extract was more effective than mint oil at concentrations of 20000 ppm. Furthermore, the mortality rate was 20 % for 1000 ppm of mint oil and 73.33% for 15000 ppm of mint extract. It's worth noting that, menthol had a mortality rate of 23.33% and 60% at concentrations 2500ppm and 7500 ppm, respectively.

Table 3: Corrected mortality % of *Tetranychus urtica* treated with mint derivatives under laboratory conditions 25±2 ˚C and 60±5% RH.

Treatments	Conc. (ppm)	Mortality after treatments %				Total Mortality %
		One day	Three days	Five days	Seven days	
Mint oil	1000	----	6.67	10	3.33	20
	5000	3.33	10	16.67	10	40
	10000	10	6.67	20	16.67	53.33
	20000	26.67	10	13.33	20	70
Mint extract	5000	3.33	6.67	6.67	23.33	40
	10000	20	13.33	6.67	13.33	53.33
	15000	26.67	20	16.67	10	73.33
	20000	30	30	20	10	90
Menthol	2500	6.67	6.67	3.33	6.67	23.33
	5000	20	3.33	6.67	6.67	36.67
	7500	10	13.33	30	6.67	60
	10000	33.33	26.67	13.33	10	83.33

Habashy *et al*., 2015 proved the effectiveness of camphor, which was the active ingredient of basil plant, and the basil extract than the basil oil when applied on *T. urticae*.

Results showed in Table (4) and Fig. (2) demonstrated that the plant active ingredient, menthol had the lowest LC_{50} and LC_{90} that was 5463.2 ppm and 16585.3 ppm, respectively; then LC_{50} and LC_{90} were 7349.7 and 26656.9 ppm for mint extract, respectively; but LC_{50} and LC_{90} were 7463.4 and 26656.9 ppm for mint oil. Furthermore, the toxicity index was 100% for menthol while was 74.3 % and 73.2 % for mint extract and mint oil, respectively. The table, also, showed that the slope values were 2.66, 2.29 and 1.06 for menthol, mint extract and mint oil, respectively. While LC_{90}/ LC_{50} values were 3.03, 3.6 and 17.5 for menthol, mint extract and mint oil, respectively. From these results, we find that menthol and mint extract had the highest slope values and lowest LC_{90}/ LC_{50}, so they are the steepest materials.

Table 4: Efficiency of Mint derivatives against *Tetranychus urticae*

Treatments	Conc.	Corrected mortality %	LC_{50}	LC_{90}	Slope ± S.D.	Toxicity indexLC_{50}	LC_{90}/ LC_{50}	R	P
Mint oil	5000	20	7463.4	130560.5	1.03± 0.14	73.2	17.5	0.991	0.594
	10000	40							
	15000	53.33							
	20000	70							
Mint extract	5000	40	7349.7	26656.9	2.29± 0.3	74.3	3.6	0.943	0.025
	10000	53.33							
	15000	73.33							
	20000	90							
Menthol	2500	23.33	5463.2	16585.3	2.66± 0.3	100	3.04	0.947	0.011
	5000	36.67							
	7500	60							
	1000	83.33							

R: Regression **P: Probability**

These results were in agreement with Momen *et al.* (2001); Suncica *et al.*, 2012; Mwandila *et al*., 2013; Elhalawany and Dewidar (2017) suggested that essential oils of some plants including menthol have the potential to be used for control of *T. urticae* without great effect on the two predacious mites *Neoseiulus californicus* and *Phytoseiulus persimilis.*

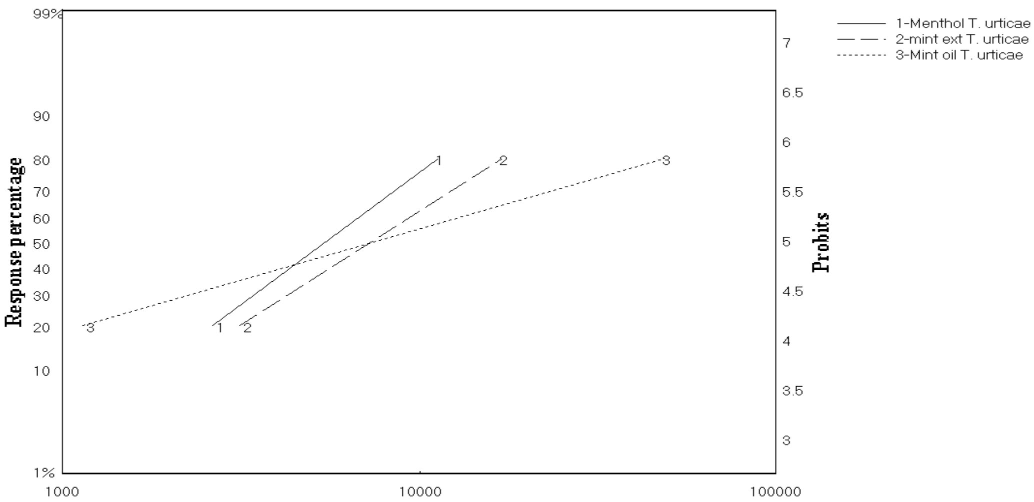


Fig. 2: LC-P lines for mint plant derivatives against the adult female of *Tetranychus urticae*.

Effect of LC_{50} of mint oil, mint extract and menthol on the predatory mite, *Neoseiulus* sp.

When mint oil, mint extract and menthol concentrations were applied on *T. cinnabarinus,* LC_{50} was calculated and applied on the predatory mite, *Neoseiulus* sp. to detect the total mortality percentage. Table (5) was indicated that menthol LC_{50} had the lowest effect on the predator and caused 13.33% after three days of treatment. The mint extract caused 20% mortality; however, mint oil caused 30% mortality after three days of treatment. From these results, we can conclude that the active ingredient, menthol, is safer than the other tested materials. The obtained results were in agreement with Momen *et al.* (2001) who suggested that the higher percentage of the hydrocarbons of *Mentha virdis* were responsible for the toxic effect on *T. urticae* and on the predacious mites namely *Typhlodromus athiase* Porath and Swirski, *Phytoseius finitimus* Ribaga and *Amblyseius barkeri* (Hughes). Sopp *et al*. (1990) demonstrated that peppermint oil was more toxic to the predacious mites tested than mint oil except for females of *Amblyseius zaheri*. Also, the obtained results agreed with Elhalawany and Dewidar (2017) who used some essential oils including menthol and concluded the lowest effect of them on the two predacious mites *Neoseiulus californicus* and *Phytoseiulus persimilis* when treated directly.

Table 3: Effect of LC_{50} of mint oil, mint extract and menthol on the predator,*Neoseiulus*sp

Treatments	One day	Three days	Total mortality %
Mint oil	0	30	30
Mint extract	10	10	20
Menthol	3.33	10	13.33

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