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Efficiency of three plant extracts for controlling *Tetranychus urticae* Koch (Tetranychidae) in laboratory and semi-field conditions

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ABSTRACT

Tetranychus urticae Koch (Acari: Tetranychidae), is a major phytophagous mites worldwide. Three essential oils of sweet orange, *Citrus sinensis* (L.) Osbeck (Rutaceae), Eucalyptus, *Eucalyptus globulus* Labill. (Myrtaceae), and Moringa, *Moringa oleifera* Lam. (Morngaceae), were subjected to both laboratory and semi-field studies for toxicity against *T. urticae* adult female. The tested essential oils were purchased as pure oil from National Research Center (NRC), Dokii, Giza, Egypt. Results indicated that mortality increased as both time and concentration increase. Eucalyptus oil was more efficient than Moringa and Sweet orange. After 72 hours of exposure to *T. urticae* adult females, LC_{50} values were 0.106, 0.139, and 0.197% for Eucalyptus, Moringa and Sweet orange, respectively. For the LC_{50} concentrations examined, a significant decrease in the total number of eggs deposited during seven days was observed. Under semi-field conditions, the LC_{50} values for the tested essential oils against *T. urticae* were assessed for effectiveness on cucumber plants. The reduction percentage of *T. urticae* was 73.06, 69.07, and 67.57% for Eucalyptus, Moringa and Sweet orange, respectively after seven days of application without significant differences.

Keywords: eucalyptus; moringa; sweet orange; essential oil; toxicity.

INTRODUCTION

Tetranychus urticae Koch (Acari: Tetranychidae), is a major phytophagous mites worldwide. Vegetables, fruits, crops, weeds, and ornamentals belong to its host plants, which number close to 1513 different plants (Migeon and Dorkeld 2023). The capacity of this mite to rapidly build a resistance to insecticides is its biggest problem (Cranham and Helle 1985). Acaricides has been the primary method of controlling spider mites; however, this strategy has resulted in the accumulation of pesticide residues on harvested goods and pesticide resistance (Attia et al. 2013).

Essential oils have shown promise as agricultural pest control agents because they affect the target insect's nervous system, resulting in physiological and behavioral reactions (Mossi et al. 2013). Aromatic plant-derived essential oils have been the focus of a lot of research due to their perceived promise as a more convenient alternative to synthetic pesticides. Fishes, birds, and mammals are generally not poisoned by essential oils (Isman 2006). Recently, numerous scientists have become interested in the application of essential oils in pest management programs (Khedr and El-Kawas 2013; Waked 2016; Allam et al. 2017). Plant extracts can directly affect pest behavior, physiology, and cuticle disruption through contact, ingestion, and fumigation, influence growth, fecundity decrease, respiratory inhibition, moulting, and cuticle disruption (Gokce et al. 2011). Citrus essential oils have both acaricidal and insecticidal properties. The predominant scion cultivar in orchards in Egypt is the sweet orange, *Citrus sinensis* (L.) Osbeck. Particularly in Neotropical agricultural environments, the essential oils of several citrus cultivars may provide terpenes for integrated pest management (Carvalho et al. 2018). Mahmoudvand et al. (2011) found that *C. sinensis* has a good fumigant effect against stored-grain pests. Dutra et al. (2016) found essential oil concentration inversely proportional to egg and adult growth, with limonene causing moderate toxicity against flies and mosquitoes. Aissaoui et al. (2020) studied the effect of two essential oils extracted from Citrus for *T. urticae* Koch. The majority compound was Limonene. Toxicity tests showed good efficacy against various stages of *T. urticae* development. At 8% concentration, the oils were not persistent on leaves, but they had a repelling effect on adult. These oils have potential for eco-friendly management. Allam et al. (2023) studied the impact of a blend of four essential oils clove, eucalyptus, lavender, and sweet orange on *T. urticae* and its predator, *Phytoseiulus persimilis* Athias-Henriot, using a bioassay to determine its lethal concentration on eggplant and cucumber crops.

Moringa oleifera Lam. (Morngaceae), is a perennial tree, spread in Africa, and in tropical areas. Leaves, seeds, fruit pods, fruits, flowers, and roots of the Moringa have a lot of essential components (Padayachee and Baijnath 2012). A phytochemical examination of the extract from moringa seeds revealed significant levels of phenolic chemicals, specifically flavonoids and tannins (Mohammed and Manan, 2015). Many researchers have reported the toxicity action of *M. oleifera* in arthropods. (Holtz et al. 2016, 2020; Pavela et al. 2018).

Eucalyptus is one of the major genera of the Myrtaceae family, is cultivated worldwide and native to Australia. Its perennial leaves emit odorous essential oils, rich in monoterpenes and sesquiterpenes, used in pharmaceuticals and perfumery (Araujo et al. 2010). Many researchers have reported the toxicity of *Eucalyptus* on mites (Choi et al. 2004; Han et al. 2010; Afify et al. 2012 *Amizadeh* et al. 2013).

This study aims to assess the effectiveness of three essential oil extracts from Eucalyptus Moringa, *and Sweet orange* on *T. urticae* adult under laboratory and semi field conditions.

MATERIALS AND METHODS

Oils source and preparation of the emulsions

Commercial essential oil of Eucalyptus, Moringa *and Sweet orange* were purchased as pure oil from National Research Center, Dokii, Giza, Egypt. The three oils were mixed with Triton X-100 to create emulsions for various concentrations, which were then completed with distilled water.

Culture of *T. urticae*:

The stock colony of *T. urticae* was collected from infested cucumber plants (*Cucumis sativus* L.). They were reared on a plastic tray (23 x 20 x 70 cm) on *Phaseolus vulgaris* L., adult mites were moved to a clean castor bean leaf, lower side up, and set on cotton pads that had been moistened and were sitting on sponges in the foam dish (15 x 20 cm). The colonies were kept in a laboratory setting at room temperature. Water was added when needed. Mites were housed in a laboratory at 25°C with appropriate moisture levels at 70% RH. The same environmental conditions used for the culture were used for all bioassays.

Experimental design

A castor bean leaf disc (3.0 cm in diameter) was flipped over and placed on cotton pads that had been wet that were sitting on sponge in an experimental foam dish (15 x 20 cm). In order to keep the culture healthy and stop the mites from escaping, water was introduced when needed. Three treatments and a control were applied, totally 40 experimental foam dishes, with 10 duplicates for each treatment.

Laboratory experiment:

Twenty adult female of *T. urticae* individuals were placed on the lower surface of a newly castor bean leaf disc. Four concentrations (0.25, 0.5, 1.0, and 2.0%) in each treatment and 10 replicates of each concentration were given a prior treatment with a fine camel hairbrush. One of the earlier treatments was applied to the leaf discs. Two drops of Triton X-100 and pure water were used as a control in each test. Four duplicates of each treatment were conducted. Mortality was calculated using a binocular microscope at 24, 48, and 72 h post treatments and corrected using Abbott's method (1925), and Finney's (1971) estimates of the LC_{50}, LC_{90}, and slope values were used. The castor bean discs were maintained at room temperature (25±2°C, 70±5% RH). A mite was considered dead when its body or appendages did not move when it was probed with a fine camel brush (Elhalawany and Dewidar 2017; Elhalawany et al. 2019).

Effect of LC_{50} concentration of tested essential oils on *T. urticae* fecundity:

Tested oils were sprayed at LC_{50} concentrations over the leaf disks of castor bean plants. Females that had just emerged were transferred individually onto sprayed leaf discs. For every concentration, ten leaf disks were used, and a control group consisting of an equal number of females on clean leaf disks was also included. For seven days, the fecundity of the females was noted. Lundgren (1975) revealed the following methodology for calculating the oviposition deterrent indices (ODI): ODI = (C–T/C+T) x 100 where, C = total number of eggs in control, T = total number of eggs in treatment.

Semi field experiment:

Cucumber plants were grown in pots under open field at Qaha Station, Plant Protection Research Institute, Qalubiya governorate. Each pot contains three plants, after two weeks of planting, the plants was infested with 40 adults of *T. urticae* per each pot. After one week the LC_{50} of the three essential oils were sprayed and control (untreated) was sprayed with distilled water. Twenty leaves were chosen randomly from each treatment and the number of motile mite stages was counted before spraying and after three, five and seven days of application using the aid of a stereomicroscope. Henderson and Tilton's (1955) equation was used to estimate the reduction percentage of the spider mite.

Statistical analysis

Probit analysis (Finney, 1971) was used to the data from each dose-response bioassay in order to determine the LC_{50} and LC_{90} values using Ldp line software (Bakr 2005). Reduction percentage of *T. urticae* moving stages was analyzed by one-way ANOVA and means were compared by using Tukey's HSD test at α=0.05 in SAS Program version 9.1.3 (SAS Institute, 2003).

RESULTS AND DISCUSSION

Plant essential oil extracts offer an effective natural product alternative for *T. urticae* control. These extracts simplify handling and application easier, and they may also be a less expensive option when it comes to chemical control.

Efficacy of Eucalyptus, Moringa, and *Sweet orange* on *T. urticae* adult female under laboratory conditions:

Data illustrated in Figure (1) showed that Eucalyptus essential oil extract was the most potent effective plant extracts against *T. urticae* adult females, which greater the highest adult female mortality. Adult female mortality percentages after 24 h were 40.4, 65.0, 79.2, and 84.2% for Eucalyptus by spraying the different concentrations of 0.25, 0.5, 1.0, and 2.0%, respectively. The percentages of corresponding mortalities for Moringa were 41.25, 56.6, 65.8, and 75.4%, while 12.4, 20.7, 36.5, and 58.5% for Sweet orange, respectively.

The same trends were observed when we used the previous concentrations of Eucalyptus, Moringa, and *Sweet orange after 48h the mortality percentage increased as time increased.* Adult mortality percentages after 72 h were 69.8, 74.0, 87.5, and 95.7% for Eucalyptus; 62.4, 73.2, 85.0, and 91.3%, for Moringa and 50.9, 68.58, 74.0, and 80.6% for *Sweet orange*, by spraying the different concentrations of 0.25, 0.5, 1.0, and 2.0%, respectively. For control treatment no-mortality of *T. urticae* adults was recorded when treated with Triton X-100 at a rate of 0.1 Figure (1).

The present result agrees with Choi *et al*. (2004) found *Eucalyptus citriodora* Hook's essential oil can cause over 90% mortality on *T. urticae* adults. Han et al. (2010) tested the same oil on the same mite species using a vapor-phase mortality bioassay, finding similar fumigant activity results. Aissaoui et al. (2020) found that essential oils *Citrus sinensis* and *C. aurantium* effectively reduced mortality rates in *T. urticae*, with adult mortality rates at 63.33% and 68.74%, larvae at 100% and 75%, and eggs at 85.41% and 95%. Pavela et al. (2018) report that 100% of the *Aedes aegypti* (L.) (Diptera) insects died after 24 h of exposure to the aqueous extract of Moringa seeds. Holtz et al. (2020) investigated Moringa's acaricidal effect on *T. urticae*, finding toxic extracts with higher mortality rates, with green seed extract showing lower LC_{50} potential.

Toxicity effect of Eucalyptus, Moringa and *Sweet orange* on *T. urticae* adult females

Probit analysis for Eucalyptus, Moringa and *Sweet orange* oils efficacy against *T. urticae* adults after 24, 48, and 72h are presented in Table (1). Mortality increased as both time and concentration increased. Eucalyptus oil was more efficient than Moringa and *Sweet orange*. The LC_{50} values after 24h were 0.317, 0.384, and 1.555% and the LC_{90} values were respectively 2.541, 7.624, and 10.485% for Eucalyptus, Moringa and *Sweet orange, respectively*. These values as time increase, the corresponding LC_{90} values were respectively

1.332, 1.171, and 5.082%. Whereas, The LC_{50} values after 72h was 0.106, 0.139, and 0.197% for Eucalyptus, Moringa and *Sweet orange*, respectively. The slope values of regression line were 1.166, 1.175, and 0.907 for Eucalyptus, Moringa and *Sweet orange*, respectively after 72h. The curve showed a high slope for Sweet orange, resulting in a lower LC_{90} of 27.82 %. Relative toxicity was 100.0, 77.94, and 26.21. The results obtained coincide with what was found by Afify et al. (2012) showed that the LC_{50} values of Eucalyptus for eggs and adults were 7.33 and 2.18, respectively. Amizadeh et al. (2013) tested the fumigant effect of essential oils from Eucalyptus on *T. urticae* eggs and adult females. The results showed LD_{50}s of 1.52 and 5.7 µL/L for leaves and fruits, respectively. Holtz et al. (2016) evaluated the acaricide effect of M. oleifera extracts on *T. urticae*. They showed the seeds extract was the most toxic, with an estimated LC_{50} value of 12.39%, indicating its potential for controlling the mite. Elhalwany et al. (2019) evaluated the toxicity and repellency of Rosemary and Coriander essential oils against Eutetranychus orientalis. Results showed significant differences in their efficacy on different *E. orientalis* developmental stages. Compared to rosemary oils, coriander essential oil was shown to be more toxic, with a higher LC_{50} and LC_{90} for eggs and adult stages. Mortality increased with concentration and decreased with time.

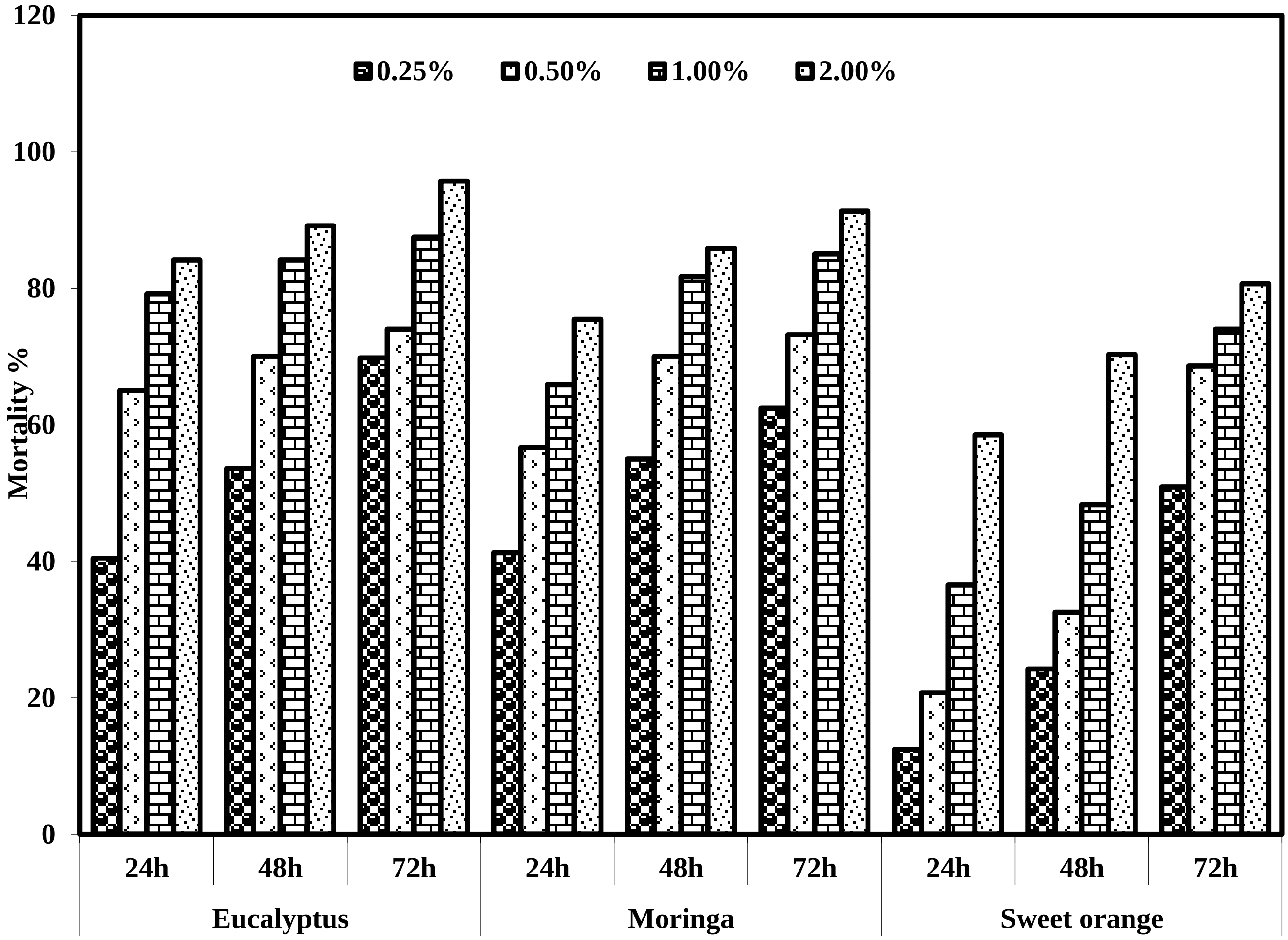

Figure 1. Mortality percentage of *T. urticae* adult females treated with different concentrations of essential oils after seven days in laboratory.

Table 1. Toxicity effect of three essential oils against *T. urticae* adult females after 24, 48, and 72 h post treatments in laboratory.

Essential oils	Time (hours)	LC_{50}	LC_{90}	Confidence limit LC_{90}		Slope	Toxicity index	X^2
				upper	lower			
Eucalyptus	24	0.317	2.541	1.755	4.758	1.418	52.42	1.91
	48	0.204	1.892	1.327	3.538	1.326	70.402	1.42
	72	0.106	1.332	0.936	2.605	1.166	100	1.00
Moringa	24	0.384	7.624	3.702	36.89	0.988	17.471	5.72
	48	0.176	2.642	1.652	6.883	1.09	50.416	1.98
	72	0.139	1.709	1.171	3.52	1.175	77.94	1.28
Sweet orange	24	1.555	10.485	5.919	27.82	1.547	12.704	7.87
	48	0.941	8.099	4.603	21.8	1.371	16.446	6.08
	72	0.197	5.082	2.567	26.2	0.907	26.21	3.82

The most effective compounds, LC_{90} (Eucalyptus 72h), was used to calculate the toxicity index.

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Effect of Eucalyptus, Moringa and *Sweet orange* essential oils on *T. urticae* fecundity and oviposition deterrent index (ODI)

The fecundity of *T. urticae* females was highly affected by treated with LC_{50} of essential oil concentrations after 72 h, (Table 2). For all concentrations, a decrease in fecundity over a 7-day period was observed. The highest fecundity in the control was 32.1 eggs/female/7 days. Whereas, the lowest fecundity was 7.3, 14.0, and 17.6 eggs/female/7 days for Eucalyptus, Moringa, and Sweet Orange, respectively. The highest oviposition deterrent index (ODI) after seven days was 62.94% for Eucalyptus, 39.26% for Moringa, and 29.18% for Sweet Orange. These results agree with finding by Elhalwany et al. (2022) evaluated the toxicity of neem and caraway essential oils against *E. orientalis.* Caraway oil caused higher mortality percentages and a higher oviposition deterrent index than neem oil. Habashy (2018) found that garlic aqueous extract significantly decreased egg deposition in *T. urticae*.

Table 2. Impact of LC_{50} concentrations of tested three essential oils *on* oviposition deterrent index (ODI) *of T.urticae* adult females *over seven days*

Concentration (%)	Eucalyptus		Moringa		*Sweet orange*	
	Eggs/♀	ODI	Eggs/♀	ODI	Eggs/♀	ODI
Control	32.1	-	32.1	-	32.1	-
LC_{50}	7.3	62.94	14.0	39.26	17.6	29.18

Semi-field experiment

The medium lethal concentration (LC_{50}) of the three essential oils was sprayed on cucumber plants under semi-field, and the control was sprayed with distilled water. The data in Table (3) demonstrated that there was no significant difference between the three essential oils in number before application. Results indicated that the motile stages of *T. urticae* on cucumber leaves after spray decreased gradually till the end of the test. The reduction percentage of *T. urticae* individuals was 80.80, 78.47, and 76.84% after three days post-treatment for Eucalyptus, Moringa, and Sweet orange, respectively. The reduction decreased as the time increased; after seven days, it was 73.06, 69.07, and 67.57%, respectively, without a significant difference between the tested three essential oils ($F = 3.3$, $P = 0.0611$).

The current findings are consistent with those reported by Habashy *et al.* (2016), who investigated the effectiveness of garlic aqueous extract against two tetranychid mites, *T. urticae* and *Tetranychus cinnabarinus* in semi-field conditions, observing the greatest reductions in the mite populations one day after treatment, at 89.75 and 80.14%, respectively. Allam et al. (2023) used a 12,000 ppm mixture of essential oils on eggplant and cucumber in plastic-houses to control *T. urticae*. They found the oil plays a role in controlling *T. urticae*, despite the presence of predators. Habashy et al. (2023) found those three oils, eucalyptus, thyme, and garlic, effectively reduced *T. urticae* under semi-field conditions. Eucalyptus oil showed the highest reduction (65.741%), while thyme and garlic oils were less toxic (55.386%) and (55.002%), respectively. So, these oils effective in controlling *T. urticae*.

Table 3. Mean number and reduction percentage of *Tetranychus urticae* treated with LC_{50} concentration of Eucalyptus, Moringa, *and sweet orange* under semi- field conditions.

Treatments	Mean no. of moving stages before spray	Mean number and reduction percentage in moving stages of *T. urticae* after spray at indicated periods:					
		3 days		5 days		7days	
		No.	% R.	No.	% R.	No.	% R.
Eucalyptus	5.20	1.20	80.80 a	1.80	74.10 a	2.00	73.06 a
Moringa	5.40	1.40	78.47 a	2.20	69.51 a	2.40	69.07 a
Sweet orange	5.60	1.60	76.94 a	2.40	67.93 a	2.80	67.57 a
Control	5.50	6.70		7.35		8.25	
F		1.46		1.22		3.30	
P-value		0.3019		0.3857		0.0611	
$HSD_{0.05}$		12.2		11.64		10.77	

% R.= Reduction percentage, Tukey's HSD test at α=0.05 indicates a significant difference when different letters appear in the same column.

CONCLUSION

Eucalyptus, Moringa, and sweet orange essential oils show potential as eco-friendly and effective acaricides for controlling *T. urticae* in IPM programs, but further research is needed to evaluate their mode of action and cost-effectiveness.

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