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Contact, fumigant and repellency effects of some essential oils against the two spotted spider mite *Tetranychus urticae* koch, and the cotton aphid *Aphis gossypii* glover

Heba M Nasr, Noura F Abou-elenien, Hala E Moafi
Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt

Abstract
The contact, fumigant toxicities and repellency effect of three plant essential oils were evaluated against the two spotted spider mite, *Tetranychus urticae* Koch and cotton aphid *Aphis gossypii* Glover. Five concentrations were used for each essential oil and mortality present was measured after 6, 24 and 48 hours.LC50 and LC90 values were calculated. The LC50 effect on the hydrolase- enzymes (ACP & ALP) was detected. The obtained data declared that Marjoram oil was more effective against *T. urticae* than other essential oils causing 100% mortality at 15000 ppm, while, Chamomile oil was more effective against *Aphis gossypii* causing 96.7% mortality at the same concentration. The vapor phase effect of Marjoram oil revealed a remarkable activity (96.67% & 83.3%) adult mortality for both *T. urticae* and *Aphis gossypii* respectively. The repellency effect of the three essential oils was observed. Thyme oil was proven to be a strong repellent for *T. urticae*, wear as, Chamomile oil for *Aphis gossypii*. The optained data for the activity of (ACP&ALP) levels showing increasing in ALP levels for treated *T. urticae*, while that level decreased for treated *Aphis gossypii*. Finally, we can conclude that Marjoram oil and Chamomile oil both had high acaricidal and insecticidal activity.

Keywords: *T. urticae*, *Aphis gossypii*, essential oils, toxicity, repellency, ACP and ALP levels

Introduction

In Agro- food production chain, the limitation of use of chemical control for pests considers a major aim for the new (EU) regulation that dealing with both consumer and environmental health.

Essential oils are one of the methods that developed to propose as alternative to synthetic pesticides. (Regnault-Roger 1997; Isman 2000, Ntallietal, 2009). [9, 14]

Until recently most of studies examine the pesticide activity of essential oil (Tripathi *etal*.2008, Song *etal.* 2011) [15, 16] without knowing what happen inside the pest causing that effect.

Some of these studies examin the volatile or fumigation activity of plant essential oils (Rajendran and Sriranjini 2008;Yang *et al.* 2010). [12, 18]

Effective fumigants are biologically active, sufficiently volatile not absorbed by the agricultural product, and don't affect the nutritional quality or flavor of the agricultural product.

The two spotted spider mite, *Tetranychus urticae* Koch and cotton aphid, *Aphis gossypii* Glover were described as polyphagus pests, usually controlled by pesticides in Egypt and other countries. Two spotted spider mite has more than 1200 host plant (Zhang 2003). [20] Family Tetranychidae has found to develop resistances against 80 different acaricides (Drap, 2004; Dermauw *et al.* 2013). [7]

The cotton aphid, infest more than 200important crops, including cotton, pepper, okra, eggplant and cucumbers. The importance of this pest coming from not only the direct losses by sucking plant sap but also, that its transmite more than 75 plant viruses(Blackman and Eastop,2000) [4]., it also developed a resistance against Pyrethroide and Pirimicarb (Charaabi, *et al*., 2016). [5]

Plant essential oils have toxic effects on pests through contact, ingestion and fumigation moreover; plant essential oils have a significant behavioral effect on pests especially as repellents.

The present study examines essential oils that have shown to be having acaricidal or insecticidal activity and repellency effect against the two spotted spider mite *Tetranychus urticae* Koch and cotton aphid *Aphis gossypii* Glover.

Material and methods

Spider mite rearing

In this study spider mite *Tetranychus urticae* Koch was obtained from castor leaves and kept at 25±2 ℃ on cotton leaves with the lower surface up wards that located on moist- cotton pods in fiber dishes and the cotton pods were moistened every day to prevent mite from escaping.

Cotton aphid rearing

Giza, 86 cotton seed were planted in plastic bags (25× 40× 15 cm) filled with soil and peatmus. Cotton leaves were infested with *Aphis gosypii* Glover and then aphids were cultured under laboratory conditions (30 ±2℃ and 65 ±5 R.H.).

Tested compounds and application methods

Chamomile oil (*Matricaria chamomilla*), Thyme oil (*Thymus vulgarius*) and Marjoram oil (*Origonum majorana*), were tested for their contact, repellency and fumigation toxicity.

five different concentrations (1000. 2500, 5000, 10000 and 15000 PPm) from the three plant essential oil, were tested. Essential oils were dissolved in water containing 0.3% tween20 and then diluted to the target concentrations.

Phytotoxicity test

Three plant essential oils were tested on the two spotted spider mite *Tetranychus urticae* Koch and cotton aphid *Aphis gossypii* Glover, for their toxic effect.

Ten adult females from either the mite or aphid were fixed on leave disk treated with each essential oil with different concentrations. Each concentration has three replicates (10 adults per replicate). Mortality percentages were recorded after 6, 24 and 48 hours.

Fumigation effect

The toxicity by fumigation with essential oil vapor phase was assessed in a small glass jar (230ml.), both mites and aphids were placed on filter paper at the bottom of the jar, the top of the jar was sealed with a sealing lid with a hole in its center, with rubber septum. The pure essential oil was injected by a syringe through the rubber septum into a cotton plug. Distilled water was used for the untreated control. Three replicates were done for each treatment. The mortality of mites and aphids were observed after 24 hours.

Repellency effect

The repellence test was adapted from the method described by (Pascual- Villalobos and Robleda, 1998). [11] 4.5 cm cotton leaf disks were used. Half of the disks (2 cm) were immersed in each essential oil solution for 5 seconds, after drying in room temperature for 10 seconds, the other half (2cm) immersed in distilled water containing 0.3% tween20 as control.

Each leave was immersed in such way as to leave a (0.5cm) area intact between the two halves, where the mite and aphid were initially placed.

The leaf was placed on filter paper on poly ethylene foam disk and then all was placed on Petri dish containing water.

10 female of mite or aphid were transferred to each disk. Each treatment was repeated three times. The numbers of mites or aphids were counted in each half after 24 hours.

Repellency index was calculated by the equation RI= (C – T/ C+ T) x 100, were RI = repellency index, T= the number of mites or aphids in the treatment and C= the number of mites or aphids in the control.

Preparation of samples

Treated mites and aphids were prepared after three days from treatment to describe according to Amin, 1998. [3] They were homogenized in 1 ml. distilled water and then centrifuged in refrigerated- centrifuge with 5000/r.p.p. twice for 15 minute at 2℃**.**

Biochemical studies

The concentration (5000ppm) of the three plant oils was determined on hydrolase- enzymes to determine physiological affects against the two spotted spider mite *Tetranychus urticae* Koch and cotton aphid *Aphis gosypii* Glover. Acid phosphatase and alkaline phosphatase were calculated after treatment on three days comparison with control.

Statistical analysis

The mortality percentage was estimated and corrected according to the Abbotts formula, 1925. [1] Activity determination of acid phosphatase and alkaline phosphatase were estimated according to Powel and Smith method, 1954).

Result and discussion

A. Phytotoxicity effect

1. Efficacy of three different plant essential oils against the two spotted spider mite *Tetranychus urticae* adult females

Results illustrated in table (1) showed that Marjoram oil caused high mortality rate (93.3%) of adult females at 10000ppm while, Thyme oil caused the lowest mortality rate (36.7%) at the same concentration. Mortality rate increased as the concentration increased.

The toxicity index for Marjoram oil was 100.0 while for Chamomile oil and Thyme oil were (25.5 & 11.6) respectively.

Results also indicated that Marjoram oil also was more effective than Thyme oil or Chamomile oil, with LC50 (1336.80 ppm, 11558.98ppm and 5238.46ppm) respectively. These results agreed with (Rania and Enas, 2022) and also with (Isman, 2000). [9, 13]

2. Efficacy of three different plant essential oils against the cotton aphid *Aphis gossypii*

Data in table (2) demonstrated that Chamomile oil had the highest mortality rate (96.7%) followed by Thyme oil and Marjoram oil with moderate mortality rate, (76.7% & 70.0%) respectively at the same concentration (15000ppm). The toxicity index for Chamomile oil was (100.0), while it was so close for Thyme oil and Marjoram oil (67.11 & 55.37) respectively. That effect also appear in the result of LC50 which was (4440.95ppm) for Chamomile oil, and (6617.37ppm &8020.06ppm) for Thyme oil and Marjoram oil, respectively. These results agreed with (Regnault-Roger, 1997) and also with (Isman, 2006). [10, 14]

Table 1: Efficacy of three different plant essential oils against the two spotted spider mite *Tetranychus urticae* Koch.

Treatments	Conc. (ppm)	Mortality after treatments %			Total Mortality %	LC_{50}	LC_{90}	Toxicity index
		6 hours	24 hours	48 hours				
Marjoram oil	1000	20.0	16.7	3.3	40.0	1336.80	9436.70	100.0
	2500	50.0	6.7	10	66.7			
	5000	53.3	20.0	13.3	86.7			
	10000	60.0	23.3	10.0	93.3			
	15000	66.7	26.7	6.7	100.0			
Chamomile oil	1000	6.7	3.3	0.0	10.0	5238.46	23722.53	25.5
	2500	13.3	10.0	0.0	23.3			
	5000	30.0	16.7	0.0	46.7			
	10000	56.7	13.3	3.3	73.3			
	15000	60.0	20.0	0.0	80.0			
Thyme oil	1000	6.7	0.0	0.0	6. 7	11558.98	91278.27	11.6
	2500	10.0	6.7	0.0	16.7			
	5000	16.7	10.0	3.3	30.0			
	10000	23.3	13.3	0.0	36.7			
	15000	33.3	13.3	0.0	46.7			

Table 2: Efficacy of three different plant essential oils against the cotton aphid *Aphis gossypii* Glover.

Treatments	Conc. (ppm)	Mortality after treatments %			Total Mortality %	LC_{50}	LC_{90}	Toxicity index
		6 hours	24 hours	48 hours				
Marjoram oil	1000	6.7	6.7	0.0	13.3	8020.06	45799.44	55.37
	2500	10.0	6.7	3.3	20.0			
	5000	23.3	10.0	3.3	36.7			
	10000	36.7	13.3	3.3	53.3			
	15000	60.0	10.0	0.0	70.0			
Chamomile oil	1000	6.7	6.7	3.3	16.7	4440.95	13803.61	100.0
	2500	13.3	10.0	3.3	26.7			
	5000	26.7	20.0	6.7	53.3			
	10000	50.0	26.7	6.7	83.3			
	15000	73.3	23.3	0.0	96.7			
Thyme oil	1000	6.7	3.3	0.0	10.0	6617.37	36113.30	67.11
	2500	13.3	10.0	0.0	23.3			
	5000	20.0	20.0	3.3	43.3			
	10000	33.3	16.7	6.7	56.7			
	15000	63.3	10.0	3.3	76.7			

B. Fumigation effect

The testing for vapour phase effect in air- tight conditions for mite and insect exposure as shown in table (3) was revealed a remarkable acaricidal and insecticidal activity of Marjoram oil against the tested mite, *Tetranychus urticae* causing (96.67% adult mortality), and also for the tested insect, *Aphis gossypii* causing (83.30% adult mortality). These results agrees with the studies did on greenhouses conditions with essential oils that revealed a fumigation effects on *T. cinnabarinus* and *Aphis gossypii* (Tuni and Sahinkaya, 1998), [17] and also with (Abou- El Saad *et al*., 2011). [2]

Table 3: Fumigation activity of three different plant essential oils against the two spotted spider mite *Tetranychus urticae* and cotton aphid, *Aphis gossypii.*

Treatments	Mortality % of adult *Tetranychus urticae*	Mortality % ofadult *Aphis gossypii*
Marjoram oil	96.67	83.30
Chamomile oil	70.00	73.30
Thyme oil	73.30	50.00

C. Repellency effect

The repellent activity for the three plant essential oils were tested at LC50 concentration represented in table (4). The Thyme oil was found to had the highest repellency effect against the two spotted spider mite, *Tetranychus urticae* with repellency index (67%), while Chamomile oil had the highest repellency index (49%) against the cotton aphid, *Aphis gossypii*. *Marjoram oil* had the lowest repellency index for both tested mite and insect with, (0% & 7%) repellency index respectively. These results were agreed with (Mansour *et al*., 1986). Essential oil is used as repellent, Isman, 2000 &2006. [9, 10] Studies also confirm the repellency effect of some essential oils against two spotted spider mite (El- Zemmity *et al.*, 2006),(Yorulmaz- Salman and Erbas,2014). [8, 19]

Table 4: Repellency effect of three different plant essential oils against *Tetranychus urticae* Koch and *Aphis gossypii* Glover according to repellency index by (Pascual- Villalobos and Robleda, 1998). [11]

Treatments	*Tetranychus urticae*		*Aphis gossypii*	
	Concentration LC_{50}	RI %	Concentration LC_{50}	RI %
Marjoram oil	1336.80	0.0%	8020.06	7%
Chamomile oil	5238.46	34%	4440.95	49%
Thyme oil	11558.98	67%	6617.37	27%

D. Biochemical studies

The effect of essential oils on hydrolase- enzymes acid phosphatase (ACP) and alkaline phosphatase (ALP) as shown in table (5), reported that the level of (ACP) decreased compared with control for both tested mite and insect treated with Marjoram oil, but that level increased when tested mite and insect treated with Thyme oil and Chamomile oil. While, (ALP) level increased compared with control for *Tetranychus urticae* when treated with all three tested essential oils. On the other hand, the (ALP) level was decreased compared with control for *Aphis gossypii* treated with all three tested essential oils. These results agree with (Rania and Enas 2022). [13]

Table 5: LC_{50} effects of three different plant essential oils on hydrolase- enzymes against the two spotted spider mite *etranychus urticae* Koch and cotton aphid *Aphis gossypii* Glover.

Treatments	*Tetranychus urticae*		*Aphis gossypii*	
	ACP	ALP	ACP	ALP
Marjoram oil	1.3	83.0	2.1	6.0
Chamomile oil	2.4	46.0	3.4	4.0
Thyme oil	4.7	33.3	4.6	7.0
control	1.8	30.0	3.1	11.0

ACP = Acid phosphatase ALP = Alkaline phosphatase

Conclusion

We concluded that all three plant essential oils had acaricidal and insecticidal activity and can be used to control the two spotted spider mite *Tetranychus urticae* Koch and cotton aphid *Aphis gossypii* Glover with different degrees. Also, the tested essential oils were not detected to have any phyto toxic effect on plant. However, it's important to perform experments under greenhouses conditions with these three plant essential oils and compare results with the laboratory one.

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