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A laboratory study of the acaricidal, repellent and oviposition deterrent effects of three essential and mineral oils on *Tetranychus urticae* (Acari: Tetranychidae)

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Abstract

Essential oils are utilised to manage pests because they are environmentally friendly substances in place of chemical pesticides. Consequently, three essential oils of mint oil (*Mentha spicata*), black cumin oil (*Nigella sativa*) and garlic oil (*Allium sativum*), and a mineral oil, KZ oil 95%EC were tested for their toxicity, ovicidal, egg deposition, repellency, and oviposition deterrence effect against *Tetranychus urticae* Koch (Acari: Tetranychidae) on *Acalypha wilkesiana*, acalypha leaf discs under laboratory conditions. KZ oil was more potent against *T. urticae* females with LC_{50} value of 0.61 mg/l., followed by mint oil, black seed oil and garlic oil recorded LC_{50} values 0.99, 1.01 and1.75 mg/l respectively. KZ oil was the most effective compounds on egg hatching followed by black cumin oil. Whereas garlic oil caused the highest reduction in egg deposition followed by black cumin oil. Mint oil exhibited moderate repellency effects (53.33 %), followed by black cumin oil and garlic oil which recorded 38.78 and 15.29 % respectively. Whereas KZ oil showed no repellency effects (-12%). The oviposition deterrent index (ODI) of garlic oil, mint oil, and black cumin oil recorded at 34.23, 30.08 and 29.30 % respectively. As a result, essential oils can be used on fields and greenhouse crops in a similar way to how synthetic acaricides are now applied.

Introduction

The most polyphagous species is *Tetranychus urticae* Koch (Acari: Tetranychidae), which has been observed in protected and open field settings in tropical, subtropical, and temperate climates. Moving stages suck the sap from a lower surface of leaves which causes yellowing and discoloration (Reddy and Kumar, 2006). However, using chemically created acaricides has a variety of drawbacks, including hazards to human health, pest resistance, incompatibility with natural predators, phytotoxicity, and environmental contamination. In that regard, a supplementary alternative to chemical pesticides in the management of *T. urticae* is the ongoing quest for botanical pesticides. Because the essential oils contained monoterpenes, diterpenes, and sesquiterpenes, they exhibited pesticidal properties. Many years ago, the insecticidal

and acaricidal effects of many essential oils were studied. Additionally, reports of its antinutritional, insect repellant, and infectious properties in a variety of pests (Govindarajan *et al.,* 2016) including *T. urticae* (Roh *et al.,* 2013 and Pavela *et al.,* 2016).

Plant-based pesticides appear to be advised because they often have a very brief persistence in plants (Raina *et al*., 2009). Essential oils' quick action against some insects and mites is evidence that they have neurotoxic effects (Isman, 2006). There is evidence that plant essential oils have an impact on GABA-Gated Chloride Ion Channels and octopamine pathways (Sertkaya *et al.,* 2010). Spider mites and eriophyid mites can be effectively controlled in a variety of fields and greenhouse crops using highly refined petroleum-derived spray oils "PDSO"(Chueca *et al.,* 2010). The most popular theory regarding their mechani sm of action holds that PDSO primarily act physically of obstructing the spiracles in insects (Or the stigmata in mites) and causing suffocation, but it cannot be assumed as the only mode of action (Taverner, 2002). Modern oils, at least, have a variety of cellular disruptions that quickly kill insects (Najar-Rodriguez *et al*., 2008).The purpose of this study is to evaluate the toxicity, ovicidal, egg deposition, repellency and oviposition deterrence effects of one mineral oil KZ oil (95% EC) and three essential oils from different botanical families; mint oil (Lamiaceae) and garlic oil (Amaryllidaceae) and black cumin oil (Ranunculaceae) against *T. urticae*.

Materials and methods

1.*Tetranychus urticae* stock cultures:

T. urticae colonies were obtained from the Acarology laboratory of the Plant Protection Research Institute (PPRI). In the Virus Laboratory of the Agriculture Directorate in Tanta, El-Gharbia Governorate, Egypt, *T. urticae* colonies were raised under laboratory conditions at 25± 5 °C and 65 ± 5% RH. with a 12 L: 12 D h photoperiod on *Acalypha wilkesiana* ornamental plant arena. By placing heavily infested leaves on fresh ones that were then placed on wet cotton on arenas, mites were transferred from old to fresh plant leaves. Before being used in research, the mites were reared for several generations.

2. Tested compounds:

Using four compound formulations and dosages calculated according to the basis of mg/l of an active ingredient.

2.1. Mineral oil:

KZ oil (95% EC)**,** Lubricant fraction of petroleum oil. It was supplied by Amerya Petrol Ref. Co. (Local: Kafer El Zayat for Pesticides and Chemicals).

2.2. Essential oils:

Cold pressed oils supplied by El-Masrayia for natural oils extraction company. The oils were pure, free of preservatives, 100% natural. Mint oil (Spearmint *Mentha spicata* extract), black cumin oil (*Nigella sativa* extract) and garlic oil (Extracted from *Allium sativum*).

3. Experimental techniques:

3.1. Preparation of leaf discs:

A. wilkesiana used in the study were grown in the garden of the Agriculture Directorate, Tanta, El-Gharbia Governorate, Egypt. *A. wilkesiana* leaf discs were cut from fresh healthy leaves using a cork borer and placed upper side down on a moist sponge covered with moist paper tissue in Petri dishes. The size of the disc varied depending on the kind of experiment.

3.2. Preparation of the emulsions:

Emulsions of mineral oil and essential oils were prepared by mixing tween 80 as an emulsifier (0.1 tween/1 L water). A series of aqueous concentrations (1, 2,4,8,16 mg/l) were prepared from the stock solution.

4. Toxicity of mineral oil (KZ oil) and three essential oils of (Black cumin, mint and

garlic oils) against *Tetranychus urticae* females:

A. wilkesiana leaf discs measuring 2.5 cm in diameter were dipped in each concentration for 5 seconds before being allowed to air dry at room temperature. Water + tween, were used to submerge control leaf discs. Each leaf disc received ten female mites of the same age; four replicates of each treatment were performed. The Petri dishes were housed in a growth chamber with a 12 L: 12 D h photoperiod at 25 ± 5 °C and 65 ± 5 % RH. After treatment, mortality was checked 24, 48, and 72 hours later. Mortality percentages were corrected by Abbott's formula (1925) and LC_{90}, LC_{50} with 95% confidence limit and slope values were calculated according to Finney (1971) using "LDP Line" software (Baker, 2000).

5. Ovicidal effects of mineral oil (KZ oil) and three essential oils of (Black cumin, mint and garlic oils) against *Tetranychus urticae*:

Treatments' concentrations or control were applied to four *A. wilkesiana* leaf discs, each measuring 2.5 cm in diameter. Ten females were added to each leaf disc in the Petri dishes overnight in preparation for oviposition. Leaving only 25 eggs per leaf disc, the remaining eggs were removed. In each treatment, treated leaf discs were submerged in LC_{50} for 5 seconds before being allowed to dry. Control leaf discs received treatment with (Water + tween). Six days were spent determining the viability of the eggs (Yanar *et al.*, 2011). Egg mortality: The percentage of mortality was calculated as follows: **Egg mortality = (a/b) X 100** Where a= unhatched eggs, b= number of total eggs which counted before treatment with toxicant.

6. Effects of mineral oil (KZ oil) and three essential oils of (Black cumin, Mint and Garlic oils) on fecundity of *Tetranychus urticae* females:

The LC_{50} concentrations of the oils' emulsions or the control were applied to each leaf disc in 7 replicates, which held a set of seven preovipositing females and three males per replicate. Leaf discs used as a control were dipped in water and tween. Cohorts with 30 females were chosen from the survivors after a 24-hour exposure period and were individually placed on untreated leaf discs. The number of eggs laid over the next five days were simultaneously counted, the technique advised by Marčić and Ogurlić (2007) was used with slight changes.

7. Repellency effect of mineral oil (KZ oil) and three essential oils of (Black cumin, Mint and Garlic oils) against females of *Tetranychus urticae*:

A. wilkesiana leaf discs (5 cm in diameter) were divided in half by midrib, one half being used as a control and being dipped in a (water + tween) emulsion, the other half dipped in the LC_{50} concentration of selected essential oil or mineral oil. In Petri dishes, treated leaf discs were allowed to dry. Ten *T. urticae* females were attached to each leaf disc's midrib. There were ten replicates for each LC_{50} value. Females orientations were noted 1, 2, and 24 hours after treatment. After 24 hours, the number of eggs laid on each half was counted (Hussein *et al.*, 2006). Repellency effect (%) was calculated using Obeng-Ofori *et al.* (1997).

Repellency effect (%) = [(Nc-Nt) / (Nc+Nt)] × 100: Where Nt is the number of individuals who received treatment, and Nc is the number of individuals who were in the control area. The repellency index **(RI**) was calculated according to Kogan and Goeden (1970).

The repellency index RI=2G/(G+P): Where P is the number of control mites and G is the number of mites in the treatment. The average of the calculated RI and its standard deviation (SD) was used to determine the safety interval time that was utilised to determine whether or not the

treatment is repellant. Oviposition deterrent index (ODI) was estimated according to Dimetry *et al.* (1993).

Oviposition deterrent index (ODI): = {(C-T)/(C+T)} × 100: Where T was the number of eggs laid on the treated side**,** C was the number of eggs laid on the control side.

8. Statistical analysis:

Data were analyzed using SPSS version 20 for Windows, and the statistical analysis was done by using one-way ANOVA analysis followed by using post-hoc multiple comparison, least significant difference tests, Duncan. The differences were statistically significant at $p < 0.05$.

Results and discussion

1. Toxicity of three essential oils and mineral oil (KZ) on *Tetranychus urticae*:

Mineral oil (KZ) 95% EC showed the highest toxicity with the lowest LC_{50} value of 0.61 mg/l., followed by mint oil and black cumin oil recorded LC_{50} values of 0.99 and 1.01 mg/l respectively. Whereas garlic oil gave the lowest toxicity and recorded the highest LC_{50} value 1.75 mg/l. Toxicity Index compared with KZ oil, recorded 100,62.01,60.61 and 35.06 for KZ oil, mint oil, black cumin oil and garlic oil respectively, Table (1) and Figure (1). Similar results were obtained by El-Shiekh and El-Shereif (2011) who reported that KZ oil 95% EC was the most effective compound against *T. urticae*, while W 95% EC was the least active one. However, Amer *et al.* (2001) found that KZ oil was more harmful to *T. urticae* eggs than mature females. When spearmint essential oil was examined by Elhalawany and Dewidar (2017) for its toxicity against female *T. urticae* Koch, the LC_{50} value after 72 hours was 0.85%.

Choi *et al.* (2004) stated that lemon eucalyptus, pennyroyal, and spearmint oils achieved more than 90% mortality against adult *T. urticae*. At the same dose, peppermint oils caused 89% death. In a similar vein, *Allium sativum* extract was identified by Keratum *et al.* (2010) as the *T. urticae* adult female's least poisonous substance. According to Dabrowsky and Seredynska (2007) report, 48–57% of *T. urticae* died after receiving garlic extract. In 2011, Ismail *et al.* investigated how garlic oil affected *T. urticae* on sweet potato leaves, garlic oil's LC_{50} was 2.1%.

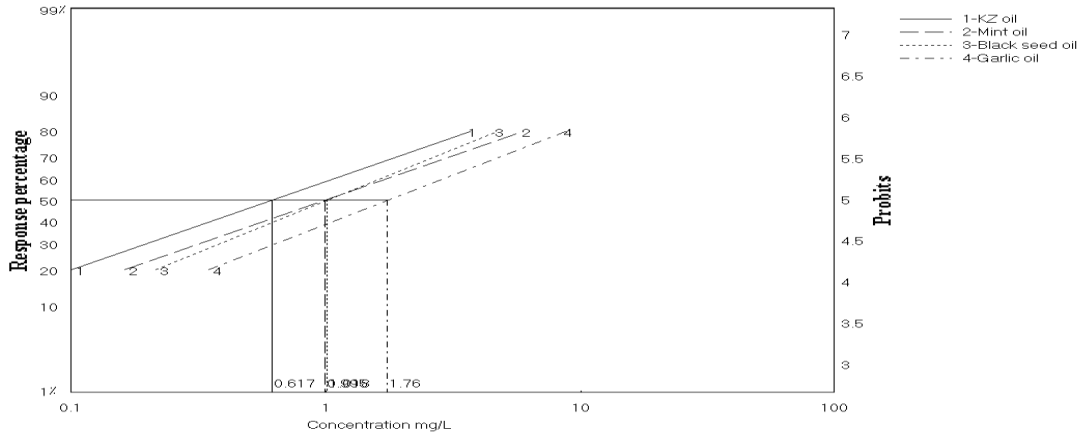

Figure (1): Toxicity lines of mineral oil (KZ) and three essential oils (Black cumin, mint and garlic oils) against *Tetranychus urticae* females.

Table (1): Efficacy of three essential oils of (Black cumin, mint and garlic oils) and mineral oil (KZ) against *Tetranychus urticae* females.

Treatment	Conc. mg/l	Corrected mortality%	LC_{50}	LC_{90}	Slope ±/SE	X2 (Tabulated =7.8)	Toxicity index
KZ oil	1	65	0.61	9.62	1.07 ± 0.25	6.48	100
	2	67.5					
	4	75					
	8	87.5					
	16	97.5					
Mint oil	1	52.63	0.99	15.57	1.07 ± 0.24	2.44	62.01
	2	63.15					
	4	68.42					
	8	84.21					
	16	92.1					
Black cumin oil	1	55.26	1.01	10.71	1.25 ± 0.25	5.92	60.61
	2	60.52					
	4	73.68					
	8	84.21					
	16	97.36					
Garlic oil	1	36.84	1.75	20.61	1.19 ± 0.23	2.26	35.06
	2	55.26					
	4	68.42					
	8	73.68					
	16	89.47					

Toxicity Index compare with KZ oil.

2. Ovicidal effect of three essential oils and KZ oil on *Tetranychus urticae* eggs:

The effect of LC_{50} values of the tested oils on the hatchability of *T. urticae* eggs is indicated in Table (2). KZ oil and black cumin oil were effective compounds on egg hatching, with 45 % and 22% egg mortality, while mint oil and garlic oil gave 6% and 5% egg mortality respectively. The obtained results showed significant differences between tested LC_{50} (F*:* 8.84, LSD: 4.61, P*:*0.001). Our results agreed with that obtained by Keratum *et al.* (2010) who reported that the mineral oil Nat-1 was more harmful to the *T. urticae* egg stage than the extract of *A. sativum*. According to Derbalah *et al*. (2013), black cumin extract and mineral $CAPL_2$ were found to record 52.96 and 70.4% of egg hatchability, respectively. EL-Kasser *et al.* (2015) stated that the greatest reduction in *T. urticae* egg hatchability on several host plants was induced by suprmasrona oil, while, *Nigella sativa* extract was the least hazardous substance to *T. urticae* eggs and adult females on several host plants. Similar studies have shown that mineral oils significantly reduce the number of *T. urticae* eggs (Sertkaya *et al.,* 2010; Ismail *et al.,* 2011; Mead, 2012 and Elsadany *et al*., 2020).

3. Effect of three essential and KZ oils on egg deposition of *Tetranychus urticae*:

The data shown in Table (2) indicated that garlic oil caused the highest reduction in egg deposition followed by black cumin oil and KZ oil 2.53 ± 1.05; 4.33 ± 1.11 and 9.7 ± 1.7 (Mean no. of egg /females after 5 days) respectively, whereas mint oil gave the lowest reduction in egg deposition 21.6 ± 2.4 (Mean no. of egg /females after 5 days). The obtained results showed significant differences between tested LC_{50} (F: 49.44, LSD: 4.24, P:0.0001). According to our findings, garlic oil caused the highest reduction in egg deposition (90.5%), followed by black cumin oil (83.74%) and KZ oil (63.57%), even though garlic oil had the lowest toxicity to adult females of *T. urticae*. While the least impact on fecundity was caused by mint oil (18.89%).

Additionally, a study by Erdogan *et al.* (2012) in Turkey showed that five plant extracts, including *Allium sativum* L. (Amaryllidaceae), were effective in reducing adult mites' capacity to lay eggs in all treatments, even at the lowest concentrations as compared to the control. According to Abd El-Rahman and Farag (2021) *A. sativum* and *M. azedarach* oils decreased mite fecundity by 20.83 and 6.63%, respectively. Ismail *et al.* (2011) reported that in comparison to the other tested oils, garlic oil (0.5%) caused the most pronounced significant reduction in the number of deposited eggs. According to Habashy (2018), garlic aqueous extract dramatically decreased egg deposition and egg hatchability in *T. urticae*. According to Attia *et al.* (2011), the concentration of garlic extracts increased female mortality and decreased fecundity.

According to Derbalah *et al.* (2013), who stated that the percentages of black cumin and mineral oil $CAPL_2$ that inhibited the ability of *T. urticae*/5 females to lay eggs were 31.7 and 14.9%, respectively. On the other hand, Hosny *et al.* (2010) indicated that LC_{25} of Nat-1 and *A.sativum* extract caused about the same effect on egg deposition of adult female mites (29.2 and 21.7% reduction.

Table (2): Effect of three essential oils of (Black cumin, mint and garlic oils) and mineral oil (KZ oil) on egg hatchability and fecundity of *Tetranychus urticae* females.

Treatment	Ovicidal effect	Egg deposition effect
	Mean no. of unhatched egg /replicate 25 eggs (Mortality %)	Mean no. of egg/female after 5 days (% reduction)
KZ oil	11.25 ± 2.28 a (45)	9.7 ± 1.7 c (63.57)
Black cumin	5.5 ± 2.50 b (22)	4.33 ± 1.11 d (83.74)
Mint oil	1.5 ± 0.28 bc (6)	21.6 ± 2.41 b (18.89)
Garlic oil	1.25 ± 0.25 bc (5)	2.53 ± 1.05 d (90.5)
Control (Water +tween)	0.25 ± 0.2 c	26.63 ± 0.69 a
F	8.84	49.44
P	0.001	0.0001
L.S.D	4.61	4.24

4. Repellency and oviposition deterrence effects of three essential oils and KZ oil on *Tetranychus urticae*:

Results showed that Mint oil exhibited moderat repellency effects (53.33 %), followed by Black cumin oil and Garlic oil which recorded 38.78 and 15.29 respectively. Whereas KZ oil showed no repellency effects (-12%). Mint oil has the lowest RI value (0.48), followed by black cumin oil (0.62), and garlic oil (0.9) While, KZ oil showed the highest RI value (1.12). Thence, Mint oil, was classified as repellent to *T. urticae.* Meanwhile, black cumin oil, garlic oil and KZ oil, were classified as neutral to *T. urticae.*

Similarly, Sathyaseelan *et al*. (2020) found that repellency was noted in the case of Mentha oil (65.00%). Motazedian *et al.* (2012) reported that *Mentha longifolia* essential oil has been shown to have a repellent effect on *T. urticae*. Moreover, Momen *et al.* (2001) demonstrated that *T. urticae* females were substantially repelled by mint oil. They concluded that the harmful effect was caused by *M. virdis's* higher concentration of hydrocarbons. Kheradmand *et al.* (2015) mentioned that oils from spearmint *Mentha spicata*, and cumin *Cuminum cyminum* had the strongest repellent effects on *T. urticae*. RI value of spearmint oil (0.40), and cumin oil (0.56). Sararit and Auamcharoen (2020) found that *Allium sativum* essential oils had a 15–20% repellency effect on mature female *T. urticae.* Garlic oil had the highest oviposition deterrent index (ODI) 34.23 % followed by mint oil and black cumin oil recorded 30.08 and 29.30 % respectively. While ODI of KZ oil recorded (-13.01), (Table 3). Similar investigations were recorded by Elhalawany and Dewidar (2017) who showed that spearmint oil had a 29.6% oviposition deterrence index (ODI) at 1% concentration. *A. sativum* shown a repellant effect against *T. urticae* (Carlos *et al.* 2008).

Our findings are different from some earlier studies of essential oils repelling *T. urticae*. Peppermint essential oil's miticidal and repulsive action failed to demonstrate any noticeable toxicity (6.7± 1.7 (Mortality%± SE) and no repulsive impact (-33.3 26.7) Repellency% ±SE) (Yoon and Tak 2018). The different doses applied in this research may be the source of these variations. Additionally, it's possible that the chemical compositions of the essential oils used in our study were different from those used in other studies because essential oils from the same plant species can have different chemical compositions depending on the extraction techniques, plant parts extracted, and the stage of plant development (Dvaranauskaite *et al.*, 2009).

Finally, this investigation demonstrated the toxicity, ovicidal, oviposition deterrent, and repellency effects of all test materials against *T. urticae*. The existence of certain alkaloids, terpenoids, flavonoids, and other oxygenated hydrocarbon chemicals, which are in charge of many of plants' insecticidal and/or acaricidal abilities, may be the cause of these effects (Pavela *et al.*, 2016 and Sharopov *et al.*, 2016). The use of these acaricides for plant protection is encouraged by the possibility for quick degradation of botanical pesticides. Future field research should be carried out to look into their possible acaricidal effects in unrestricted environments.

Table (3): Repellency and oviposition deterrence effect of mineral oil and three essential oils at LC_{50} concentration against *Tetranychus urticae.*

Treatment	Mean No. ± SE after 24 hr.		Repellen - cy %	Mean No. of eggs ± SE after 24 hr.		ODI%	RI ± SD	Classific- ation
	LC_{50}	Control		LC_{50}	Control			
Mint oil	2.1 ± 0.41	6.9 ± 0.75	53.33	9.3 ±2.35	17.3 ±2.69	30.08	0.48 ± 0.38	R
Black cumin oil	3.0 ± 0.89	6.8 ± 0.86	38.78	11.1± 3.63	20.3± 3.97	29.30	0.62 ± 0.54	N
Garlic oil	3.6 ± 0.43	4.9 ± 0.55	15.29	4.9 ±0.77	10 ± 2.12	34.23	0.9 ± 0.54	N
KZ oil	5.6 ± 0.16	4.4 ± 0.28	-12	13.9±2.4	10.7 ±2.35	-13.01	1.12 ± 0.39	N

% Repelency = (Nc-Nt)/ (Nc+Nt) *100 Nc = no. of individuals in control (after 24 hr), Nt no of individuals in treatment (after 24 hr). ODI = {(C-T)/(C+T)} × 100: Where C was the number of eggs laid on the control side and T was the number of eggs laid on the treated side. The average of RI was lower than 1 - SD,(R: repellent). RI was higher than 1 + SD, (A: attractive); and RI was between 1- SD and 1 + SD (N: neutral).

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