Pathogenicity of two entomopathogenic fungi and toxicity, oviposition deterrent, and repellency of two essential oils on *Eutetranychus orientalis*

Ashraf S. Elhalawany[1*], Naglaa F.R. Ahmad[1] & Sahar S. Ali[2]

[1*]*Fruit Trees Mites Department, Plant Protection Research Institute, Agricultural Research Centre, Dokii, Giza, Egypt, E-mail: dr_ashraf_said@yahoo.com, ashrafelhalawany@arc.sci.eg, ORCID https://orcid.org/0000–0001–5195–3942; naglaaahmad@gmail.com*
[2]*Bio-Insecticide Production Unit, Plant Protection Research Institute, Agricultural Research Centre, Dokii, Giza, Egypt, E-mail: sahar_s_ali@yahoo.com*
**Corresponding author: dr_ashraf_said@yahoo.com*

ABSTRACT

Eutetranychus orientalis (Klein) (Tetranychidae) is a worldwide polyphagous mite causing economic damage to several crops. A laboratory experiments was carried out to evaluate the pathogenicity of two entomopathogenic fungi, *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin, on *E. orientalis* at three spore concentrations (10^6, 10^7, and 10^8 conidia ml^{-1}). Probit analysis indicated that *B. bassiana* was significantly effective and more virulent (LC_{90} = 1.34×10^8 conidia ml^{-1}) than *M. anisopliae* (LC_{90} = 2.46×10^{11} conidia ml^{-1}), with relative toxicity of 100: 0.2. Toxicity of two essential oils: neem (*Azadirachta indica* A.Juss. (Meliaceae) and caraway, *Carum carvi* L. (Apiaceae) against *E. orientals* was also evaluated at concentrations of 0.125, 0.25, 0.5, and 0.75%. Based on LC_{90}, caraway oil caused higher mortality percentage of *E. orientalis* females than neem oil after 72 h (i.e., LC_{90} = 0.198 and 0.753%, respectively) with relative toxicity of 100: 26.30. Oviposition deterrent index increased as concentration increase. It was highest for caraway as 59.91 and 39.2% for neem at concentration of 0.75%. Repellency effect increased as oil concentration increase, and decreased as time increase. Environmentally suitable options for bio-control of *E. orientalis* might consider the obtained results.

Keywords: Citrus brown mite, *Beauveria bassiana*, *Metarhizium anisopliae*, neem, caraway, control, fecundity, repellency

INTRODUCTION

Citrus brown mite, *E. orientalis* is a polyphagous pest species reported on about 228 host plants in 58 families of economic importance worldwide including ornamental, flowering, and forest plants, fruit orchards, vegetables, legumes, cereals and others. It prefers citrus plants (Migeon and Dorkeld 2022). Feeding on upper leaf surface and fruits causes stippling. At high population densities, the trees become silver-grey, the leaves stop growth, may fall and the shoots die back (Jeppson et al. 1975).

Biological control is a key component of environmentally sustainable integrated crop protection systems. However, many potential biological control agents remain to be discovered. Numerous studies have been conducted using *B. bassiana* and *M. anisopliae* as potential biological control agents for agricultural pests. They frequently have a significant influence in reducing native populations of phytophagous mites (Chandler et al. 2000). Few reports have been reported on the usage of *M. anisopliae* or *B. bassiana* against *E. orientalis* (El-Hady 2004). Most were on *Tetranychus urticae* Koch (Negash et al. 2014) and *Bryobia cristata* (Dugès) (Nada et al. 2012).

Neem, *A. indica* is a tropical and subtropical evergreen tree. Their seeds are used to produce oil. Azadirachtin, nimbin, picrin, and sialin are some of its constituent active components. A triterpenoid and azadirachtin found in neem seeds are one of the most efficient natural insecticides nowadays. Laboratory bioassays on *T. urticae* have revealed toxic and sub lethal effects (Abdel-Aziz and Kelany 2001).

Caraway, *C. carvi* is a biennial herbaceous plant. It is among Egypt's most significant medicinal and aromatic herbs. It is the first plants to be domesticated in Asia, Africa, and Europe. About 95% of the total essential oil production is made up of two compounds: carvone and limonene (Raal et al. 2012).

Several studies globally reported the effect of various vegetable oils and entomopathogenic fungi on specific phytophagous mites (Dimetry et al. 1993; Abdel-Aziz and Kelany 2001; Tsolakis et al. 2002; Wekesa et al. 2006; Amjad et al. 2012; Silva et al. 2013; Habashy et al. 2016; Hassan et al. 2017; Elhalawany et al. 2019). Therefore, the objective of the present study is to evaluate the effect of two entomopathogenic fungi (*B. bassiana* and *M. anisopliae*) and two essential oils (caraway and neem) on *E. orientalis* control under laboratory conditions that can be used afterwards as biocontrol products.

MATERIALS AND METHODS

Colony of *E. orientalis*

Colony of *E. orientalis* was collected from infested neem plants. Castor bean plant seeds were sown in plastic trays with soil and leaf compost. The castor bean plants were infested with *E. orientalis* after four weeks. The stock culture was maintained for several generations for the experiments.

Preparation of conidial suspension of *B. bassiana* and *M. anisopliae*

Two entomopathogenic fungi were prepared by the Bio-insecticides Production Unit, Plant Protection Research Institute, Agricultural Research Centre, Dokii, Giza, Egypt. They were isolated according to Ali et al. (2020). Three concentrations (10^6, 10^7, and 10^8 conidia ml^{-1}) in 0.1% Triton X-100 (added as surfactant) were prepared and used. The control treatment was sprayed with 0.1% Triton X-100 solution in distilled water. *Eutetranychus orientalis* adults were sprayed with both fungi preparations and mortality rates were calculated after 3, 5, 7, and 10 days.

Oils source and preparation of the emulsions

Commercial essential oil of caraway and neem were obtained from "EL-HAWAG Company for Extracted Oils", Nasr City, Cairo, Egypt. The two oils were mixed with Triton X-100 to create emulsions for various concentrations, which were then completed with distilled water.

Experimental design

An experimental foam dishes (15 x 20 cm in diameter) with a citrus leaf disc kept upside down on moistened cotton pads resting on sponge were used. Water was added when needed to avoid mites from fleeing and to keep the culture healthy. A total of 36 experimental foam dishes were divided into two treatments and a check (untreated), with four replicates in each treatment. In each treatment, four concentrations (0.75, 0.5, 0.25, and 0.125%) were used for each oil.

Treatment of *E. orientalis* females with caraway and neem oils

Ten *E. orientalis* adult females were placed on each citrus leaf disc using a fine camel hairbrush. Sixty individuals were used per each replication. Leaf discs were sprayed with different concentrations of each oil. Untreated treatment used in each test was sprayed with only distilled water and two drops of Triton X-100. Mortality was record after 24, 48, and 72h for each treatment under a stereo-microscope (BS-3030B, China). Mites were considered to be dead if their bodies or appendages did not move when poked with a fine camel hairbrush (Kim et al. 2004). The mean mortality was calculated and corrected for control mortality counts according to Abbott's formula (1925).

Effect of plant essential oils on *E. orientalis* females' fecundity and mortality

Different concentrations: 0.75, 0.5, 0.25, and 0.125% of the two oils were sprayed over the leaf discs of neem plants. Newly emerged females were placed individually on leaf discs. For each concentration, 20 replicate leaf discs were used. A clean leaf discs with the same number of females were used as control. Females' mortality and fecundity were recorded for seven days. The oviposition deterrent index (ODI) was calculated according to Lundgren (1975) as follows:

ODI = (A-B/A+B) X 100%, where: A: Number of eggs in untreated treatment and B: Number of eggs in treated treatment.

Repellency assay

Castor bean leaf discs (5 cm in diameter) were prepared by placing leaf discs with the surfaces upside-down in Petri-dish. Four concentrations (0.75, 0.5, 0.25, and 0.125%) of each oil were applied to half of each disc for ten sec before being allowed to dry. The other half was left untreated as a check. Using a fine camel hairbrush, ten females of same age were positioned in the centre of the leaf disc. The number of mites on the treated and untreated half was counted after 12, 24, and 48 hr. For each oil concentration, ten replications of leaf discs were used; each treatment was repeated three times. The repellency index was calculated according to Pascual-Villalobos and Robledo (1999) as follows:

RI = (C–T/ C+T) × 100, where: the number of treated mites and C: the number of untreated mites

Statistical analysis

Data from each dose-response bioassay were submitted to Probit analysis (Finney 1971) to determine the LC_{50}, LC_{90}, and slope values using Ldp line software (Bakr 2000). Simple correlations and partial regression were used for the effect of time and concentration of two essential oils repellency effects using Procs Corr, and Reg, in SAS (Anonymous 2003).

RESULTS AND DISCUSSION

Entomopathogenic fungi

Pathogenicity **of *B. bassiana* and *M. anisopliae* on *E. orientalis* adults**

Mites treated with *B. bassiana* conidial suspensions cased mortality values ranged from (10.0, 17.16, and 24.09%) after three days to (72.67, 85.07, and 89.05%) after ten days, for 10^6, 10^7, and 10^8 concentrations, respectively (Table 1). The same trends were observed for *M. anisopliae* as mortality percentage gradually increased as concentrations and exposure time increase. Highest mortality was recorded as 42.79, 54.46, and 64.04% for tested concentrations after ten days. *Beauveria bassiana* was more efficient against *E. orientalis* adults compared with *M. anisopliae*. Probit analysis indicated that *B. bassiana* was significantly effective and more virulent (LC_{90} = $1.34×10^8$ conidia ml^{-1}) with slope (0.33), than *M. anisopliae* (LC_{90} = $2.46×10^{11}$ conidia ml^{-1}), with slope (0.28), with relative toxicity (100: 0.2) (Table 2). Similar results were reported on *Tetranychus evansi* Baker & Pritchard with *B. bassiana* and *M. anisopliae*, the maximum mortality was occurred at 10^8 conidia ml^{-1} (Wekesa et al. 2006). Mortality percentage of *E. orientalis* adults ranged between 75.9 to 77.64% when sprayed with *M. anisopliae* (10^7 spores ml^{-1}) (El-Hady 2004). Mortality in *T. urticae* adult females increased as conidial concentration of *M. anisopliae* increase (10^6, 10^7, and 10^8 conidia ml^{-1}) (Amjad et al. 2012). *Metarhizium anisopliae* was more effective with highest mortality (87.1–98%) on *T. urticae* after seven days (Habashy et al. 2016). The mortality percentage of *T. urticae* to both *B. bassiana* and *M. anisopliae* gradually increased with spores concentration as ranged between (29.89–46%) at lowest concentration (10^6 spores ml^{-1}) to (65.63–88.52%) at highest concentration (10^8 spores ml^{-1}) (Hassan et al. 2017).

Toxicity effect of caraway and neem essential oils on *E. orientalis* adults

Probit analysis for caraway and neem oils efficacy against *E. orientalis* adults after 1 to 3 days are presented in Table (3). Mortality increased as both time and concentration increase. Caraway oil was more efficient than neem. The corresponding LC_{50} values after three days were 0.032 and 0.028% and the LC_{90} values were respectively 0.198 and 0.50%. The slope values of regression line were respectively 1.63 and 0.90 for caraway and neem after three days, with relative toxicity (100: 26.3) (Table 3).

Effect of caraway and neem essential oils on fecundity and oviposition deterrent index (ODI) of *E. orientalis* adult females

Females' percent mortality increased as oils concentration increase (Table 4). Fecundity of *E. orientalis* females was highly affected by tested oils concentration as reflect to occurred mortality. For all concentrations, a decrease in fecundity over a 7-day period was observed. Highest fecundity was in the control as 36.7 eggs/female/7 days. Lowest fecundity was 9.2 and 16.03 eggs/female/7 days in caraway and neem oils with highest tested concentration. The highest oviposition deterrent index (ODI) after seven days was 59.91% for caraway and 39.2% for neem. The lowest values at 0.125% concentration were 18.82 and 16.94% on caraway and neem oils, respectively (Table 4). The present result agrees with Silva et al. (2013) on another tetranychid mite species, as the fecundity of *Mononychellus tanajoa* (Bondar) was affected by the LC_{50} of neem oil resulting in fewer deposited eggs than in the untreated ones, the egg loss (> 80%) has a negative impact on *M. tanajoa* fertility.

Repellency effect of caraway and neem essential oils on *E. orientalis* adult females

Repellency of all concentrations of caraway and neem oils on *E. orientalis* females are presented in Table (5). Simple correlation and multiple regression values for the effect of time and concentration of caraway and neem oils repellency on *E. orientalis* showed significant correlations between both oils repellency and time or concentration (negative with time and positive with concentration) (Table 6). Repellency increased as concentration increase and decreased with time increase. Similar results with other oils, i.e., coriander essential oil was more potent and repellant for *E. orientalis* than rosemary (Elhalawany et al. 2019). Repellency percentages of rosemary oil for *E. orientalis* adults were 32.35, 30.56, and 52.77% after 72 hr at 10% concentration (El-Safty 1993).

CONCLUSION

The results of the present study indicated the pathogenicity of *B. bassiana* is highly effective and virulent to *E. orientalis* than *M. anisopliae.* The caraway oil cause a highest mortality percentage against *E. orientalis* adult females than neem oil based on LC_{90}. The repellency effect is increased with oils concentration increase. The oviposition deterrent index of the vegetable oils after seven days is highest on caraway at 0.75% concentration. This finding should be considered in *E. orientalis* control program as an alternative bio-control method to chemical control in the sustainable ago-ecosystem.

ACKNOWLEDGMENTS

Deepest gratitude to Prof. Mohammed Abou-Setta (Plant Protection Research Institute, Agricultural Research Centre) for his help in the data statistical analysis and revising the earlier draft of the manuscript. Many thanks to Dr Reham Abo-Shnaf (Plant Protection Research Institute, Agricultural Research Centre) for valuable comments and suggestions.

Table 1. Mortality rate of *Eutetranychus orientalis* adults exposure to various concentrations of *Beauveria bassiana* and *Metarhizium anisopliae*

Fungi	Concentration	Mortality % after			
		3 days	5 days	7 days	10 days
B. bassiana	10^6	10.00	21.33	52.00	72.67
	10^7	17.16	44.78	76.12	85.07
	10^8	24.09	57.66	83.21	89.05
M. anisopliae	10^6	2.49	9.95	29.85	42.79
	10^7	4.95	17.82	38.61	54.46
	10^8	9.85	25.12	44.33	64.04

Table 2. Toxicity effect of different conidial concentrations of *Beauveria bassiana* and *Metarhizium anisopliae* on *Eutetranychus orientalis* adults after ten days

Fungi	LC_{50}	LC_{90}	Lower limit	Upper limit	Slope	Toxicity index
B. bassiana	1.8×10^4	1.34×10^8	24.51	1.6×10^5	0.33	100
M. anisopliae	9.1×10^6	2.46×10^{11}	3.6×10^6	2.1×10^7	0.29	0.2

Table 3. Toxicity effect of two essential oils on *Eutetranychus orientalis* adults after 1–3 days

Essential oil	Time (days)	LC_{50}	LC_{90}	Confidence limits LC_{90}		Slope	Toxicity index
				Lower	Upper		
Caraway	1	0.163	1.014	0.81	1.38	1.62	19.53
	2	0.057	0.556	0.45	0.76	1.3	35.61
	3	0.032	0.198	0.16	0.24	1.63	100
Neem	1	0.193	2.828	1.78	6.06	1.1	7
	2	0.035	1.393	0.87	3.69	0.8	14.21
	3	0.028	0.753	0.54	1.38	0.9	26.3

Table 4. Effect of various concentrations of two essential oils on females' mortality, oviposition and deterrent index (ODI) over seven days

Concentration (%)	Caraway			Neem		
	Females mortality %	Eggs/♀	ODI	Females mortality %	Eggs/♀	ODI
Control	25	36.7	0	25	36.7	0
0.125	40	25.08	18.82	35	26.07	16.94
0.25	55	18.38	33.26	55	18.73	32.42
0.50	65	16.93	36.86	60	19.07	31.61
0.75	70	9.2	59.91	65	16.03	39.2

Table 5. Repellency effects of two essential oils on *Eutetranychus orientalis* females

Concentration %	Caraway			Neem		
	12 h	24 h	48 h	12 h	24 h	48 h
0.125	54.0±19.0	46.0±13.5	36.0±20.7	42.0±14.8	40.0±13.3	24.0±12.6
0.25	64.0±15.8	62.0±14.8	46.0±9.7	48.0±14.0	42.0±14.8	30.0±19.4
0.50	88.0±14.0	80.0±16.3	58.0±22.0	62.0±14.8	56.4±17.9	46.0±21.2
0.75	96.0±8.40	88.0±14.0	74.3±24.8	82.0±19.9	72.0±19.3	50.0±19.4

Table 6. Simple correlation and multiple regression analysis of the effect of time and concentration of two essential oils repellency on *Eutetranychus orientalis*

Oil	Factor	Simple correlation		Multiple regression				
		r	P	b	P	F	P	E.V. %
Caraway	Time	-0.37	0.0001	-0.61	0.0001	72.61	0.0001	55.38
	Conc.	0.64	0.0001	64.9	0.0001			
Neem	Time	-0.39	0.0001	-0.59	0.0001	52.72	0.0001	47.4
	Conc.	0.56	0.0001	53.49	0.0001			

REFERENCES

Abbott WS. 1925. A method of computing the effectiveness of an insecticide. *Journal Economic Entomology*, 18, 265–267. DOI:10.1093/jee/18.2.265a

Abdel-Aziz EB, Kelany IM. 2001. Laboratory studies on the effects of NeemAzal-T/S and NeemAzal-T on some biological aspects of the two-spotted spider mite *Tetranychus urticae* Koch. *In*: Kleeberg H, Kelany IM (Eds.) *Practice oriented results on use of plant extracts and pheromones in integrated and biological pest control* (Proceedings of the 10th workshop, Dokki, Giza, Egypt, February 10–11, 2001). Lahnau, Trifolio-M GmbH, p. 291–299.

Ali SS, Haron EN, Ahmed MA, Abas AA, Elshaier ME. 2020. Isolation of entomopathogenic fungi and efficacy as a biological control agent on red spider mite *Tetranychus urticae* (Acari: Tetranychidae), *Egyptian Journal of Plant Protection Research Institute*, 3 (2), 761–770.

Amjad M, Bashir MH, Afzal M, Sabri MA, Javed N. 2012. Synergistic effect of some entomopathogenic fungi and synthetic pesticides, against two spotted spider mite, *Tetranychus urticae* Koch. (Acari: Tetranychidae). *Pakistan Journal of Zoology*, 44 (4), 977–984.

Anonymous. 2003. *SAS Statistics and Graphics Guide, Release* 9.1. SAS Institute, Cary, North Carolina 27513, USA.

Bakr EM. 2000. Ldp line 3. Available from: http//www.ehab soft.com.

Chandler D, Davidson G, Pell JK, Ball BV, Shaw K, Sunderland KD. 2000. Fungal control of Acari. *Biocontrol Science and Technology*, 10 (4), 357–384.

Dimetry NI, Amer SAA, Reda AS. 1993. Biological activity of two neem seed kernel extracts against the two-spotted spider mite *Tetranychus urticae* Koch. *Journal of Applied Entomology*, 116: 308–312.

El-Hady MM. 2004. Susceptibility of the citrus brown mite, *Eutetranychus orientalis* (Klein) to the entomopathogenic fungi *Verticillium lecanii* and *Metahrrizium anisopliae*. *Egyptian Journal of Biological Pest Control*, 14, 409–410.

Elhalawany AS, Abou-Zaid AM, Amer AI. 2019. Laboratory bioassay for the efficacy of coriander and rosemary extracted volatile oils on the citrus brown mite, *Eutetranychus orientalis* (Actinidida: Tetranychidae). *ACARINES: Journal of the Egyptian Society of Acarology*, 13, 15–20. DOI:10.21608/AJESA.2019.164149

El-Safty AF. 1993. *Toxicological and biological studies on citrus brown mite Eutetranychus orientalis (Kelin)*. M.Sc. Thesis, Faculty of Agriculture, Cairo University, Egypt, 120 pp.

Finney M. 1971. Probit Analysis. Cambridge Univ. Press, 3rd ed., London, 333 pp.

Habashy MG, Al-Akhdar HH, Elsherbiny EA, Nofal AM. 2016. Efficacy of entomopathogenic fungi *Metarhizium anisopliae* and *Cladosporium cladosporioides* as biocontrol agents against two Tetranychid mites (Acari: Tetranychidae). *Egyptian Journal of Biological Pest Control*, 2 6(2), 197–201.

Hassan DM, Rizk MA, Sobhy HM, Mikhail WZ, Nada MS. 2017. Virulent entomopathogenic fungi against the two-spotted spider mite *Tetranychus urticae* and some associated predator mites as non-target organisms. *Egyptian Academic Journal of Biological Sciences A. Entomology*, 10 (6), 37–56.

Jeppson LR, Keifer HH, Baker EW. 1975. *Mites Injurious to Economic Plants*. University of California Press, Berkeley and Los Angeles, California, 614 pp.

Kim Y-J, Lee S-H, Lee S-W, Ahn Y-J. 2004. Fenproximate resistance in *Tetranychus urticae* (Acari: Tetranychidae): cross-resistance and biochemical resistance mechanisms. *Pest Management Science*, 60, 1001–1006. DOI:10.1002/ps.909

Lundgren L. 1975. Natural plant chemicals acting as oviposition deterrent on cabbage butterflies *Pieris brassica* (L.) and *Pieris rapi* (L.). *Zoological Science*, 4, 253–258.

Migeon A, Dorkeld F. 2022. Spider mites web: a comprehensive database for the Tetranychidae. Available from: http://www1.montpellier.inra.fr/CBGP/spmweb (Accessed 1/7/2022)

Nada MS, Mahgoub MHA, Abo-Shnaf R. 2012. Susceptibility of *Bryobia cristata* (Acarina: Tetranychidae) adults to infection by *Metarhizium anisopliae* and *Beauveria bassiana*. ACARINES: *Journal of the Egyptian Society of Acarology*, 6, 31–33. DOI:10.21608/ajesa.2012.163622

Negash R, Dawd M, Azerefegne F. 2014. Pathogenecity of *Beauveria bassiana* and *Metarhizium anisopliae,* to the two spotted spider mites, *Tetranychus urticae*, (Acari: Tetranychidae) at different temperatures and in greenhouse condition. *Ethiopian Journal of Agricultural Sciences*, 24, 51–58.

Pascual-Villalobos MJ, Robledo A. 1999. Anti-insect activity of plant extracts from the wild flora in Southern Spain. *Biochemical Systematics and Ecology*, 27, 1–10.

Raal A, Arak E, Orav A. 2012. The content and composition of the essential oil found in *Carum carvi* L. commercial fruits obtained from different countries, *Journal of Essential Oil Research*, 24 (1), 53–59.

Silva ACB, Teodoro AV, Oliveira EE, Rego AS, Silva RR. 2013. Toxicity of neem oil to the cassava green mite *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae). *Chilean Journal of Agricultural Research*, 73, 315–319. DOI:10.4067/S0718-58392013000300016

Tsolakis H, Ragusa E, Ragusa S. 2002. Effects of Neem oil (*Azadirachta indica* A. Juss) on *Tetranychus urticae* Koch (Acariformes, Tetranychidae) in laboratory tests *In*: Bernini F, Nannetti R, Nuzzaci C, de Lillo E. (Eds.), *Acarid Phylogeny and Evolution. Adaptations in miles and ticks*. Proceedings of the IV Symposium of the European Association of Acarologists 2002, Kluwer Academic Publishers, p. 351–362.

Wekesa VW, Knapp M, Maniania NK, Boga HI. 2006. Effects of *Beauveria bassiana* and *Metarhizium anisopliae* on mortality, fecundity and egg fertility of *Tetranychus evansi*. *Journal of Applied Entomology*, 130 (3), 155–159. DOI:10.1111/j.1439-0418.2006.01043.x