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Laboratory Evaluation of the Effect of the Entomopathogenic Fungi, *Hirsutella thompsonii* and *Paecilomyces fumosoroseus,* against the Citrus Brown Mite, *Eutetranychus orientalis* (Acari: Tetranychidae)

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Abstract

El-Sharabasy H.M. (2015): **Laboratory evaluation of the effect of the entomopathogenic fungi,** *Hirsutella thompsonii* **and** *Paecilomyces fumosoroseus,* **against the citrus brown mite**, *Eutetranychus orientalis* (Acari: Tetranychidae). Plant Protect. Sci., 51: 39–45.

Formulations of the entomopathogenic fungi *Hirsutella thompsonii* (Fisher) and *Paecilomyces fumosoroseus* (Wize) Brown and Smith were tested against all stages of this pest under laboratory conditions. Three concentrations: 0.5×10^9, 1.0×10^9, and 2.0×10^9 conidia/ml were sprayed on leaf discs containing larvae, nymphs, and adults, while a single dosage of 1.0×10^9 conidia/ml was sprayed on eggs as ovicide. All the life stages were susceptible to both fungal pathogens at the tested concentrations. Larval and nymphal stages were generally less susceptible than adults. Based on probit analysis, *H. thompsonii* was the highest virulent with LC_{50} and LT_{50} (358, 290, and 146 conidia/ml and 7.78, 7.11, and 6.92 days) and *P. fumosoroseus* (597, 589, and 339 conidia/ml and 7.49, 7.14, and 4.31 days) for larvae, nymphs, and adults, respectively. However the efficacy of the two pathogens evaluated clearly differs from that of the controls. Compared with controls, egg hatchability was reduced at the different pathogen concentrations used. *H. thompsonii* at LC_{50} was more effective against eggs. Also, females in the control laid more eggs than those treated with the fungi. The entomopathogenic fungi *H. thompsonii* and *P. fumosoroseus* could be considered as an environmentally friendly alternative for biocontrol of *E. orientalis*.

Keywords: pathogenicity; biological control; bioassay; mortality

Spider mites (Acari: Tetranychidae) are the most important phytophagous mite pests of agricultural crops worldwide, whose population outbreaks can cause serious damage and yield losses. Among them, the citrus brown mite, *Eutetranychus orientalis* (Klein), is especially significant as the most polyphagous species in field and greenhouses in the Middle East, Africa, and Asia (Sewify & Mabrouk 1991). It usually attacks citrus and is a persistent pest in Egypt, especially in Upper Egypt, preferring lemon and orange. *E. orientalis* is a serious pest of a wide variety of agricultural, ornamental, and medicinal plants (Rasmy 1978). Mites feed on the upper side of the leaf along the midrib, causing the leaves to become chlorotic. Pale-yellow streaks develop along the midrib and veins. In higher infestation, the mites feed and oviposit over the whole upper surface of the leaf (Kandeel *et al*. 1986). *E. orientalis* is primarily controlled by acaricides in Egypt. As long-term reliance of spider mite control on chemical acaricides has caused resistance and public concerns of residues in agroproducts (Iskander *et al*. 1993), it was necessary to search for alternatives to conventional pesticides such as biopesticides to control spider mites. The advantages of biopesticides are their low mammalian toxicity, short environmental persistence, safety to beneficial and non-target organisms, as well as minimum risk of resistance development. Microorganisms and/or their products are the most important sources for developing biopesticides. One of the suggested strategies is microbial control of spider mites by fungal agents (Maniania *et al*. 2008; Afifi *et al.* 2010). Biological control, including the use of entomopathogenic fungi as part of an inte-

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grated pest management (IPM) strategy, is expected to reduce the dependence on synthetic acaricides. Most reports on the subject deal with insects and only few reports are available on Acari. However, research reports on the use of *H. thompsonii* and *P. fumosoroseus* against *E. orientalis* are limited. For instance, the mite specific pathogens, *H. thompsonii* and *P. fumosoroseus,* have been proved to be pathogenic to various mite pests in diverse ecosystems. *H. thompsonii* was tested against *Phyllocoptruta Oleivora* (Latge *et al*. 1988; McCoy 1996) and against *Tetranychus urticae* Koch (Hanna & Heikal 1995). *P. fumosoroseus* was highly virulent against the active stages of *T. urticae* (Alves *et al*. 2002; Kim *et al*. 2008), and on the eggs of the carmine spider mite, *T. cinnabarinus* (Boisduval) (Shi & Feng 2004a,b), *T. evansi* Baker & Pritchard (Wekesa *et al*. 2006), and against broad mite, *Polyphagotarsonemus latus* (Bank) (Acari: Tarsonemidae) (Pena *et al*. 1996).

In recent years, the use of microbial control agents has become increasingly attractive and important as one of the biological control components in IPM programmes. Unfortunately, many of these commercial preparations have not been tested on the citrus brown mite, *E. orientalis*. In this respect, this study focused on examining the effectiveness of the commercial entomopathogenic fungi *H. thompsonii* and *P. fumosoroseus* against *E. orientalis* populations under laboratory conditions. The objective was to evaluate their virulence against this important mite pest and facilitate progress in microbial control with fungal pathogens.

MATERIAL AND METHODS

Mite culture and fungal pathogens. The citrus brown mite, *E. orientalis,* was obtained from citrus orchards (*Citrus* spp.) in Ismailia governorate and reared continuously on lima bean plants (*Phaseolus vulgaris* L.) under laboratory condition at 25 ± 2°C and 65 ± 5% relative humidity (RH) with 16 h light : 8 h darkness photoperiodic regime. To obtain fix-aged eggs, larvae, nymphs, and adults for bioassays, adult females were collected from the mite culture and put on leaf discs placed on wet cotton wool in Petri dishes for oviposition. These eggs were used to rear the required life stages. Newly emerged mites were transferred to new leaf discs and thereafter leaf discs were changed every 4 days. Two commercial entomopathogenic fungi were tested on *E. orientalis* in the laboratory (Table 1).

Pathogenicity to larvae, nymphs, and adults. Bioassay was performed in rearing units consisting of four castor oil plant leaf discs (*Ricinus communis* L.) (25 mm in diameter) placed on a moist cotton pad in a Petri dish (90 mm in diameter). Each leaf disc represented one replication. Both sides of leaf discs were sprayed with 10 ml of prepared conidial suspensions for 30 s using manual microsprayer (Cairo, Egypt). Effects of the entomopathogenic fungus were tested separately against larvae, nymphs, and adult mites. For each category of mites, a cohort of 20 mites was used in one replication and was replicated five times. Three concentrations: 0.5×10^9, 1.0×10^9, and 2.0×10^9 conidia/ml were used. Controls were sprayed with distilled water. Mortality was recorded 4, 7, and 10 days after the treatment.

Effects on female fecundity and egg fertility. Twenty deutonymphs per treatment (deutonymphs were exposed to treated leaf discs as described before) were used and replicated five times. Females that survived fungal treatments were maintained individually on fresh leaf discs up to maturity and were allowed to oviposit. The number of eggs laid was recorded daily until the females stopped laying eggs. The number of eggs that hatched was recorded daily for 9 days. A single concentration of 1.0×10^9 conidia/ml (recommended dosage) of *H. thompsonii* and *P. fumosoroseus* was used. Controls were treated with distilled water.

Ovicide bioassay. The effect of different products was evaluated on 100 1-day-old eggs (20/5 replicates) of *E. orientalis*. Freshly laid eggs on castor oil plant leaf discs (25 mm in diameter) were sprayed with 10 ml of conidial suspensions of the same concentrations as mentioned above, for 30 s, using a manual microsprayer. Controls were sprayed with distilled water. Egg hatching rate and offspring development

Table 1. Commercial compounds used against *E. orientalis*

Entomopathogenic fungus	Commercial product	Concentration (recommended)	Manufacturer
H. thompsonii (Fisher)	Bio-Mit	1.0×10^9 (2.5 l/500 l water)	T. Stanes & Co. Ltd., Tamil Nadu, India
P. fumosoroseus (Wize) Brown and Smith	Priority	1.0×10^9 (1 l/500 l water)	

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Table 2. Pathogenicity of *H. thompsonii* and *P. fumosoroseus* against movable stages of *E. orientalis*

Fungus	Concentrations (conidia/ml)	Mortality ± SE (%)									
		Day 4			Day 7			Day 10			
		L	N	A	L	N	A	L	N	A	
H. thompsonii	0.5×10^9	33.0 ± 0.1^{a}	44.0 ± 0.9^{a}	53.0 ± 2.6^{a}	38.0 ± 1.8^{a}	49.0 ± 0.9^{a}	59.0 ± 0.6^{a}	39.0 ± 0.9^{a}	49.0 ± 1.6^{a}	63.0 ± 2.8^{a}	
	1.0×10^9	44.0 ± 1.9^{b}	51.0 ± 1.7^{b}	61.0 ± 2.7^{b}	42.0 ± 1.4^{b}	53.0 ± 0.7^{a}	64.0 ± 0.5^{ab}	46.0 ± 0.4^{b}	59.0 ± 2.1^{b}	71.0 ± 3.9^{b}	
	2.0×10^9	49.0 ± 2.8^{b}	58.0 ± 3.6^{b}	69.0 ± 1.7^{b}	51.0 ± 3.0^{c}	61.0 ± 2.6^{c}	72.0 ± 2.8^{c}	53.0 ± 1.3^{c}	62.0 ± 2.8^{bc}	80.0 ± 4.3^{c}	
P. fumosoroseus	0.5×10^9	19.0 ± 1.2^{a}	28.0 ± 1.9^{a}	44.0 ± 2.3^{a}	26.0 ± 2.5^{a}	36.0 ± 1.3^{a}	53.0 ± 0.8^{a}	28.0 ± 1.3^{a}	38.0 ± 1.2^{a}	59.0 ± 1.5^{a}	
	1.0×10^9	26.0 ± 2.8^{b}	34.0 ± 0.8^{b}	49.0 ± 0.5^{ab}	29.0 ± 0.1^{a}	41.0 ± 0.5^{b}	58.0 ± 0.3^{a}	32.0 ± 0.3^{ab}	44.0 ± 2.3^{ab}	66.0 ± 2.9^{ab}	
	2.0×10^9	32.0 ± 1.1^{c}	41.0 ± 3.1^{c}	56.0 ± 2.6^{b}	43.0 ± 0.4^{b}	44.0 ± 0.8^{b}	61.0 ± 0.8^{ab}	36.0 ± 1.5^{b}	46.0 ± 0.8^{b}	72.0 ± 2.2^{b}	
Control	0	0.0	0.0	0.0	0.0	4.0 ± 0.1^{d}	2.0 ± .05^{d}	5.0 ± 0.7^{d}	6.0 ± 1.6^{d}	3.0 ± 2.1^{d}	

L – larvae; N – nymphs; A – adults; $^{a–d}$means followed by the same letters in the same column are not significantly different ($P < 0.05$)

were determined daily by counting the numbers of hatched eggs and larvae on the leaflets.

Statistical analyses. Lethal effects of the fungus were evaluated as percentages of cumulative daily mortality, corrected for mortality in the control variant according to Abbott's (1925) formula. For each concentration-mortality experiment, data from all replicates were pooled and subjected to probit analysis. Lethal concentration (LC_{50}) with 95% confidence limits (CL) and median lethal time (LT_{50}) (Finney 1971). Confidence intervals of varying LT_{50} values were calculated at *P*-level < 0.05. These data were analysed by ANOVA (SAS Institute 1990) and the means were compared with Tukey's test.

RESULTS

Pathogenicity to larvae, nymphs, and adults. Pathogenicity of the tested entomopathogenic fungi against motile stages of *E. orientalis* is shown in Table 2. Data revealed that both tested fungi caused mortality against all stages at all three conidial concentrations. Control mortality for larvae, nymphs, and adults was 5.0 ± 0.7, 6.0 ± 1.6, and 3.0 ± 2.1%, respectively, at 10 days post-treatments (Table 1). Percentage mortality of mites due to entomopathogenic fungi with 2.0×10^9 conidia/ml at 4 days of spraying with *H. thompsonii* and *P. fumosoroseus* were: 49.0 ± 2.8, 58.0 ± 3.6, and 69.0 ± 2.7% and 32.0 ± 1.1, 41.0 ± 3.1, and 56.0 ± 2.6% for larvae, nymphs, and adults, respectively. On the 7th day, it increased to 51.0 ± 3.0, 61.0 ± 2.6, and 72.0 ± 2.8% and 43.0 ± 0.4, 44.0 ± 0.8, and 61.0 ± 0.8%, respectively. On the 9th day, it was up to 53.0 ± 1.3, 62.0 ± 2.8, and 80.0 ± 4.3% and 36.0 ± 1.5, 46.0 ± 0.8, and 72.0 ± 2.2%, respectively. There was a significant increase in mortality after 10 days between the different stages compared to the control: larvae (F = 63.4; df = 3.0; $P < 0.05$); nymphs (F = 89.7; df = 3.0; $P < 0.05$); adults (F = 99.2; df = 3.0; $P < 0.05$). The lowest median lethal concentration (LC_{50}) 10 days after treatments was recorded for *H. thompsonii* (358, 290, and 146 conidia/ml) against larvae, nymphs, and adults of *E. orientalis*, respectively, showing the highest virulence. Increased LC_{50} was registered for *P. fumosoroseus* (597, 589, and 339 conidia/ml), against larvae, nymphs, and adults, respectively (Table 3). On the other hand, median lethal time (LT_{50}) for *H. hompsonii* was 7.78, 7.11, and 6.92 days for larvae, nymphs, and adults, respectively, while it was 7.49, 7.14, and 4.31 days for *P. fumosoroseus,* respectively.

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Table 3. Probit analysis of different concentration bioassays with *H. thompsonii* and *P. fumosoroseus* against movable stages of *E. orientalis* 10 days post-treatments

Fungus	Mite stage	LT_{50} (95% CL) (days)	LC_{50} (95% CL) (conidia/ml)	Slope ± SE	χ^2 (*df*)
H. thompsonii	larvae	7.49 (5.14–5.91)	358 (324–421)	2.21 ± 0.34	3.92 (3)
	nymphs	7.14 (5.27–5.75)	290 (298–311)	0.63 ± 0.12	4.71 (3)
	adults	4.31 (3.66–4.97)	146 (126–195)	0.53 ± 0.31	3.65 (3)
P. fumosoroseus	larvae	7.78 (5.61–5.93)	597 (477–611)	1.32 ± 0.21	0.84 (3)
	nymphs	7.11 (5.03–5.22)	589 (497–599)	0.83 ± 0.11	1.33 (3)
	adults	6.92 (4.56–4.86)	339 (237–397)	0.54 ± 0.15	1.98 (3)

Effects on female fecundity. The eggs laid by treated females and the daily fecundity of *E. orientalis* are presented in Table 4. Females in the control laid more eggs than those treated with the fungi. Minimum fecundity was recorded on the 1st and 2nd day of oviposition in controls. On the 4th and 5th oviposition day, fecundity reached its maximum but showed a gradual decline from the 8th day, and females stopped oviposition on the 9th day. There was no significant difference between the number of eggs laid in both fungal treatments, and the highest numbers of eggs were laid on the 1st and 2nd day. Females treated stopped ovipostion on the 4th day with total number of eggs: 7.0 ± 0.34 and 7.8 ± 0.84 compared with control (32.9 ± 2.7 eggs).

Ovicidal activity. Hatch rate observed on days 3, 6, and 9 post-treatment are recorded in Table 5. There was a highly significant difference in egg hatchability between fungus-treated eggs and the control after spraying at all concentrations ($F = 79.3$; $df = 3.0$; $P < 0.05$). All the eggs in controls hatched on day 10 (100% for both fungus-treated). Hatchability after Day 4 was 36 ± 0.83, 27 ± 0.17, 22 ± 0.39%, and after Day 10 it reached 46 ± 2.34, 48 ± 3.36, 52 ± 3.11% with *H. thompsonii* at 0.5×10^9, 1.0×10^9, and 2.0×10^9 conidia/ml respectively, while it was 48 ± 0.13, 46 ± 0.84, and 46 ± 1.56% with *P. fumosoroseus*, at the same concentrations, respectively (Table 5). However, hatched larvae from treated eggs became infected, and their larval mortality reached 100% 10 days after spraying with *H. thompsonii* at all three concentrations, compared with control (5.02% mortality). Larval mortality by *P. fumosoroseus* ranged from 95.78 to 98.22%, compared to control (5.35%). Based on the LC_{50} estimates determined by the concentration–mortality relationships from the probit analysis, *H. thompsonii* was highly infectious to *E. orientalis* eggs at LC_{50} with 173 conidia/ml, followed by *P. fumosoroseus* (226 conidia/ml). It seems as if *P. fumosoroseus* was more effective than *H. thompsonii* (based on LC_{50} *H. thompsonii* seems more effective than *P. fumosoroseus*).

DISCUSSION

The different motile stages of *E. orientalis* varied in their susceptibility to both fungi treatments. Larval and nymphal stages were generally less susceptible to fungal infection than adults. Data showed that the highest mortality was recorded at the highest concentration and mortality increased with the age of mites, and adults were the most susceptible to fungal infection (Table 2). The results obtained are close to those reported by Sewify and Mabrouk (1991) who found that all stages of the citrus brown mite, *E. orientalis,* were susceptible to the entomopathogenic fungus, *Verticillium lecanii* (Zimn.). Also, El-Hady (2004) found that treatment with *V. lecanii* was effective at all stages of *E. orientalis*, and the adult

Table 4. Number of eggs laid and daily oviposition (mean ± SD) by females of *E. orientalis* treated with *H. thompsonii* and *P. fumosoroseus* ($n = 20$)

Fungus	Days									Total number of eggs laid
	1	2	3	4	5	6	7	8	9	
H. thompsonii	2.0 ± 1.00	2.8 ± 0.57	2.2 ± 1.22	0.8 ± 0.00	0	0	0	0	0	7.0 ± 0.34
P. fumosoroseus	1.8 ± 0.58	2.6 ± 1.52	2.0 ± 1.00	0.6 ± 0.00	0	0	0	0	0	7.8 ± 0.84
Control	2.4 ± 0.35	3.6 ± 0.84	5.8 ± 0.84	8.2 ± 0.53	5.4 ± 0.84	3.8 ± 0.84	2.4 ± 0.35	1.3 ± 0.58	0	32.9 ± 2.7

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Table 5. Hatch rate (% ± SD) of *E. orientalis* eggs after treatments with different concentrations of *P. fumosoroseus* and *H. thompsonii*

Fungus	Concentration (conidia/ml)	Hatchability (%)			Larval mortality (%)	LC_{50} (95% CL) (conidia/ml)
		Day 4	Day 7	Day 10		
H. thompsonii	0.5×10^9	36 ± 0.83[a]	32 ± 2.36[a]	46 ± 2.34[a]	95.98	173 (113–346)
	1.0×10^9	27 ± 0.17[a]	30 ± 2.58[a]	48 ± 3.36[a]	95.73	
	2.0×10^9	22 ± 0.39[a]	29 ± 1.69[a]	52 ± 3.11[a]	98.22	
	Control	59 ± 0.81[b]	76 ± 1.52[b]	100 ± 3.23[b]	5.35	
P. fumosoroseus	0.5×10^9	33 ± 1.67[a]	37 ± 0.84[a]	48 ± 0.13[a]	100	226 (134–850)
	1.0×10^9	29 ± 1.31[a]	33 ± 1.95[a]	46 ± 0.84[a]	100	
	2.0×10^9	19 ± 0.84[a]	31 ± 0.71[a]	46 ± 1.56[a]	100	
	Control	66 ± 1.65[c]	72 ± 3.21[c]	100 ± 2.13[b]	5.02	

[a–c]values indicated by the same letters in the same column are not significantly different ($P < 0.05$)

stage appeared highly susceptible compared to other motile stages, with mortality of 72.61, 73.66, and 89.38% for larvae, nymphs, and adults, respectively. The susceptibility can be attributed to integument being penetrated by the fungus and ecdysis. Moulting has been reported to be an important factor in arthropod resistance to fungal infection, especially in arthropods with short ecdysis intervals (Sewify & Mabrouk 1991). The lowest median lethal concentration (LC_{50}) and median lethal time (LT_{50}) at 10 days after treatments against larvae, nymphs, and adults of *E. orientalis* show *H. thompsonii* to be more effective than *P. fumosoroseus.* Amjad *et al*. (2012) found that *P. fumosoroseus* (n = 32) caused mortality (79.16%) at a concentration of 1×10^8 conidia/ml against adult females of *T. urticae*, at LC_{50} (9.1×10^4 conidia/ml on the 8th day post-treatment), and at LT_{50} (4.58 days at 1×10^8 conidia/ml) after 8 days of inoculation. According to Ghosh *et al*. (2007) the LC_{50} and LT_{50} values of *H. thompsonii* were 19.91 conidia/ml and 124.3 h and 29.92 conidia/ml and 119.5 h for *P. fumosoroseus* showing to be more virulent to *T. urticae* adults. The main characteristic that plays an important role in the virulence of entomopathogenic fungi is the production of enzymes necessary for the penetration of the arthropod cuticle. The extracellular proteases enzymes are considered the most important to penetrate the cuticle allowing the toxic compounds to invade the host's haemolymph (Gupta *et al*. 1992).

Egg hatchability was reduced at the different concentrations, and the different fungal species varied in ability to infect *E. orientalis* eggs and their impact on the egg mortalities largely depended on the conidial concentrations sprayed. Shaw *et al*. (2002) mentioned that the bioassays of fungal biocontrol agents on *Varroa destructor* eggs are different from those dealing with immature or adults, where the eggs are immobile. Similar results were reported for *E. orientalis* (Sewify & Mabrouk 1991; El-Hady 2004). However, all larvae hatched from treated eggs became infected with the fungus and their mortality reached 100% with *H. thompsonii*. Based on our observations, the two fungal species were capable of killing *E. orientalis* eggs. This is in accord with Shi and Feng (2004b), who evaluated the action of some entomopathogenic fungi against *T. cinnabarinus* eggs, and their results after 9 days post inoculation showed that un-hatched eggs observed on a given day after spraying could not be classified as dead or alive until fungal outgrowths were visible or the mites hatched. Sewify and Mabrouk (1991) found that the fungi were more susceptible to newly deposited (1-day-old) than to 7-day-old eggs. They also mentioned that newly laid eggs, being highly elastic and resilient, are more susceptible to the fungi than older, less resilient eggs which thus may escape the infection. For contamination of newly hatched larvae, Rodrigues-Reudan and Fargues (1980) suggested that fungal infestation already took place on the chorion surface of the eggs before hatching. Females in the control laid more eggs than those fungus-treated and infection of females affected the reproductive potential. Shi and Feng (2009) found that the infection of *P. fumosoroseus* not only killed *T. urticae* females, but greatly reduced their fecundity. The effect of fungal infection on the fecundity of pests is often revealed by fecundity differences between infected and non-infected mites (Wekesa *et al*. 2006) and ticks (Samish *et al*. 2001). Thus, a

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modelling analysis of the fecundity probability might be an interesting method to evaluate the effect of the fungal infection on pest fecundity (Shi & Feng 2009).

Generally, data in this study clearly indicate the potential of *H. thompsonii* and *P. fumosoroseus* to control *E. orientalis*, as mycoacaricides comparable to standard bioacaricides and synthetic acaricides (Iskander *et al.* 1993), which indicates a potential use of these species for bio-control of this pest in Egypt. It is difficult to compare these candidates with others because their LC_{50} to spider mites are expressed as a number of conidia/ml (Alves *et al.* 2002) rather than number of conidia/mm^2 (Wekesa *et al.* 2005) and several factors may affect the deposits of sprayed conidia. Considering the spider mite resistance to acaricides, mycoacaricides may be a successful alternative to conventional chemical control. However, more research on the control of the citrus brown mite is needed, especially on compatibility of these products with predatory mites and other acaricides.

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