Dispersal of entomopathogenic fungi, *Metarhizium anisopliae* and its synergistic with predatory mite, *Phytoseiulus macropilis* for controlling *Tetranychus urticae*

Dalia A. Waked (✉ dr.dalia188@yahoo.com)

Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt

<https://orcid.org/0000-0002-1404-9214>

Mohamed Elewea

Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt

Abdel Aziz ElNasharty Basha

Plant Protection Department, Agriculture Faculty, Zagazig University

Mohamed Hendawy

Plant Protection Department, Agriculture Faculty, Zagazig University

Galal Shabaan Saleh

Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt

Research Article

Keywords: Dispersal, Loading, *Metarhizium anisopliae*, *Phytoseiulus macropilis*, *Tetranychus urticae*

Posted Date: February 5th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-193652/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

The entomopathogenic fungus "*Metarhizium anisopliae*" and predatory mite "*Phytoseiulus macropilis*" are effective biological controlling agents of *Tetranychus urticae*. Recent studies have shown that predatory mites, used as biocontrol agents can be loaded with entomopathogenic fungal conidia to increase infection rates in pest populations. It was necessary to study the effect of *M. anisopliae* against *P. macropilis* before executing the experiment. The results showed that the predator mite was more effected by 1×10^9 conidia/ml of *M. anisopliae*, while the predator had low effect with 1×10^6 conidia/ml of *M. anisopliae*. It was found that the predator had poor effect at indirect spray. Results showed that dispersal of *M. anisopliae* loaded on *P. macropilis* delivered high numbers of conidia to *T. urticae* infested leaves, thereby increased the proportion of *T. urticae* that came into contact with the fungus.

Our study suggests that loading certain predatory mite species with fungal conidia can increase their capacity to suppress thrips populations by combining predation and dispersing pathogens when releasing the loading predatory mite with *M. anisopliae* for controlling *T. urticae* on cucumber crop under greenhouse conditions

Introduction

Two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a cosmopolitan and polyphagous species feeding on more than 900 plants including field crops, horticultural crops and ornamental plants, with great economic importance for crops in greenhouses and in the field (Janssen *et al.* 1997; Zhang 2003; Kumar *et al.* 2010; Clotuche *et al.* 2011). Phytophagous mite, *Tetranychus urticae* Koch as a global agricultural pest, is economically important because it causes high and very quick damaging population levels for host plant due to its rapid developmental rate and high reproductive potential when its growth conditions are good (Van de Vrie, 1972; Meyer, 1996, Navajas, 1998; Nauen *et al.*, 2001; Farouk and Osman, 2011 and Kanika, 2014). Fungal pathogens used as biocontrol agents, now are accepted and have become important as one of the biological controlling components in the IPM programs. (Nugroho and Bin Ibrahim 2004) indicated potential importance of the two entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* against mite adults. *M. anisopliae* has shown higher activity and rapid mortality against mite adults. (Hughes *et al.* 2004). The entomopathogens play an important role in the regulation of phytophagous mite populations and sometimes to decimate it (Van der Geest *et al.*, 2000). Many predaceous Phytoseiid mites are now used as biological controlling agents in various agricultural ecosystems, and are important predators of phytophagous mite populations in IPM programs on outdoor and greenhouse crops. *Phytoseiulus macropilis* is one of the most important generalist indigenous predators of tetranychid mites and is widely found on various crops and it is considered one of the main predatory mite used in IPM in Egypt. Pathogens have evolved several ways to disperse and increase the probability of encountering their host. A pathogen can be transferred directly from an infected individual to an uninfected one, indirectly when the host encounters the free-living infectious stage of the pathogen in the environment. A number of studies has shown that arthropods can act as dispersal agents and transmit pathogens passively to potential hosts without becoming

themselves infected. Arthropod vectors therefore have the potential to shape direct and indirect interactions between a microorganism and its host and consequently, influence their population dynamics, as well as the structure and stability of communities. Hence, biological control may offer a reliable controlling method compatible with other components of an integrated pest management (IPM) programme (Jacobson et al. 2001). Predatory mites should be closely associated to the target pest and have the ability to search for, locate and engage in interactions with the pest on the plant, so they can disperse spores on the plant. In this study, the dispersal and the compatibility of *Metarhizium anisopliae* with *Phytoseiulus macropilis* for the management of *Tetranychus urticae* has been evaluated.

Materials And Methods

Rearing technique of mite *Tetranychus urticae*

Samples of eggplant leaves infested by, *Tetranychus urticae*. All samples placed in paper bags and transferred immediately to the laboratory. The mass culture was initiated by transferred individuals of females and males using a camel's hair brush placed in petri-dishes 10 cm. diameter, which provided with untreated fresh leaves discs of mulberry (*Morus alba* L.), about 2.4 cm. diameter placed on a pad of cotton wool fully saturated with water as a source of moisture and to prevent mite escaping. Newly laid eggs and hatched larvae were placed on fresh leaf discs in prepared petri-dishes as mentioned above. The old leaf discs were removed wherever, it was necessary. All colonies were kept in an incubator at $27\pm 2^{\circ}\text{C}$ and $65\pm 5\text{R.H.}$

Rearing technique of predaceous mite, *Phytoseiulus macropilis*:

Predaceous mite was reared on the kidney bean leaves highly infested with *T. urticae* as prey that putted in large metal box $35 \times 25 \times 5$ cm, pieces of sponge, 2cm thick were put in the middle of each box, putted filter paper on sponge and leaving a space provided with water as a barrier to prevent predatory mites from escaping. *T. urticae* individuals provided every day as food source. The boxes were kept in an incubator at $27\pm 2^{\circ}\text{C}$ and $65\pm 5\%$ R.H. water was added daily to maintain suitable moisture for the predator rearing.

Fungus culture:

The fungus *M. anisopliae* was isolated from the soil. Virulence of fungus against *T. urticae* was studied and maintained on sabouraud dextrose agar (SDA). SDA media (Glucose =4.0%, Mycological peptone = 1% and Agar = 1.5 %) was autoclaved for 20 minutes at 121°C and then poured on to sterilized petridishes separately (Alves *et al.*, 1998). Left for 24 hr.in air flow to cool down, and then incubated at $25\pm 2^{\circ}\text{C}$ and $80\pm 5\%$ R.H. spore production was observed after 2 weeks of fungal culturing.

Efficiency evaluation of the entomopathogenic fungus, *M. anisopliae* on some biological aspects of *P. macropilis*.

Thirty-five adult females from the predator were reared individually, placed on clean mulberry leaf discs upper side down on moist cell cotton in petri- dishes and sprayed with two concentrations 1×10^6 and 1×10^9 conidia/ml of *M. anisopliae* (Direct spray). The treated predator females with *M. anisopliae* were fed on all stages of *T. urticae*, that added to each arena daily to ensure an abundance of food. Treatment (Indirect spray) contained adult females from *T. urticae* were sprayed with 1×10^6 and 1×10^9 conidia/ml of *M. anisopliae* by a glass manual atomizer. After 24 hr, one starved female of *P. macropilis* was added with known number of treated *T. urticae* females. The petri-dishes were incubated under constant temperature $27 \pm 2^\circ\text{C}$ and R.H approximately 65%. The control check discs were treated with sterile water mixed with a wetting agent (tween 80). Longevity, fecundity, egg fertility and consumption for survived predator individuals for the treated and untreated females (control) were calculated.

Dispersal of fungus, *M. anisopliae*:

Plastic pots were planted with kidney bean and when it reached 20 days old, known number from *T. urticae* has been put on leaf of plant at position (2) after that put Eppendorf with loaded predator (which treated with 1×10^6 conidia /ml of *M. anisoplias* on the stem of plant, position (A). Recording *M. anisopliae* on plant and prey, *T. urticae*. To detect the presence or absence of *M. anisopliae* on living *T. urticae* that remained on plants until the end of the experiment, *T. urticae* were individually picked with a sterilized toothpick or clean fine brush (sterilized with 90% ethanol and rinsed with 0.1% Tween-80 between samples) and placed in a small Petri dish ($\emptyset$ 3.5 cm) containing 2.5 ml of selective media for *M. anisopliae*. Petri dishes were examined 10 days later when colony-forming units (CFUs) can be visualized. The proportion of *T. urticae* bearing conidia was calculated. To assess the number of conidia on each plant part following arthropod removal, leaves and stems were cut into small pieces (<2 cm in width or length) and put back into the solo cup. Conidia were washed off by adding 5 ml of 0.1% Tween-80 into each solo cup and the cups were put on a rotary shaker for 2 hours at a speed of 125 rpm. Next, one aliquot of a 0.5 ml suspension was transferred onto the selective media for *M. anisopliae* and CFUs were counted 9 days later.

Release of loaded *P. macropilis* with *M. anisopliae* for synergistic management of *T. urticae* on cucumber crop in greenhouse:

Prey culture

The two spotted spider mite, *Tetranychus urticae* was reared on kidney bean plants, *Phaseolus vulgaris* L. planted in greenhouse at Sharkia Governorate. When kidney bean plants had 20 days old age, were infested with *T. urticae* collected from leaves of eggplant and then individuals of the *T. urticae* mite moved off the infested leaves to the new foliage.

Predators rearing

After one week from infestation kidney plant the phytoseiid predator mite, *P. macropilis* was reared on kidney bean plants, *Phaseolus vulgaris* L. planted in

greenhouse at Sharkia Governorate. Excised bean leaves highly infested with *T. urticae* were provided every other day as food source.

Sowing practices

The experimental area (greenhouse) was (9×45m²) planted with cucumber seeds that were divided into 3 plots representing the different treatments, (releasing loaded predator with *M. anisopliae*, unloaded predator and control) each plot (about 9 m²). Seeds were sown in hills beds, on one side of the ridge of 80 cm in width. The different treatments were applied after three weeks from complete germination. Treatments were arranged in plots each design with three replicates (each replicate contains 25 plants).

Releasing of the predatory mite; *P. macropilis*

Releasing of the predatory mite was carried out in greenhouse planted with cucumber plants. The treatments were releasing loaded predator at 1×10⁶ conidia / ml of *M. anisopliae*, unloaded predator and control with ratio, 1:10 predator: prey *T. urticae*, respectively. Each treatment was represented by 3 replicates each about 3 × 3 m². A plastic sheet was fixed between each replicate to avoid the predatory mite escaping to the other one. The required population numbers of predatory mite individuals were calculated according to the following formula:

Released number = Total no. of *T. urticae* in treatment × Predator ratio / Prey ratio. Bean leaves with the loaded and unloaded predatory mites were transferred in an ice-box to the greenhouse and then distributed on infested cucumber plants with *T. urticae* except the treatment which was kept free from any controlling agents. After one week of releasing, 20 cucumber leaves were investigated to count the movable stages of *T. urticae* and the predatory mite for 10 subsequent weeks. The reduction percentages were calculated using equation of Henderson and Tilton (1955) was also applied.

Statistical analysis

Data were subjected to statistical analysis using one-way analysis of variance, ANOVA Duncan (1955).

Results And Discussion

Efficiency evaluation of the entomopathogenic fungus, *M. anisopliae* on some biological aspects of *P. macropilis*.

Two concentrations, 1×10⁶ and 1×10⁹ conidia/ml of *M. anisopliae* were used against *P. macropilis* with two treatments, direct and indirect spray. It was noticed that two treatments shortened the longevity and reduced the fecundity of adult females of *P. macropilis* with different percent. Results in table (1) reveal that, the longevity of individuals was 20.53 and 15.42 days at concentration 1×10⁶ and 1×10⁹ conidia/ml on the predator with direct spray, while in indirect spray of predator it was 23.09 and 21.11 days at the same previous concentrations compared with 23.03 days for control. There was no any significant

difference in the fecundity, between indirect spray and the control, the total number was 35.12 and 32.43 eggs compared with 38.75 eggs for control. The total consumption of *T. urticae* was near similar in indirect spray with the control 87.26 and 82.19 preys and compared with 95.54 preys. while, the consumption reduced in direct spray. The previous results showed that the predator mite more effected by 1×10^9 conidia/ml of *M. anisopliae* while, the predator had low effect with 1×10^6 conidia/ml of *M. anisopliae*. It was found that the predator had poorly effect at indirect spray. Generally, it would be appeared that conjoint usage of tested *M. anisopliae* with predatory mite; *P. macropilis* in IPM may require applying biological control agents, entomopathogenic fungi and the predator in sequence unseparated by time intervals. Midthassel *et al.* (2016) reveled that the results from the study the susceptibility of *A. swirskii* as a physiological host for *B. bassiana* is confirmed with slight virulence depending on exposure type.

Table (1). Functional response of *P. macropilis* treated by *M. anisopliae* and its feeding on treated prey

Treatments	Conc. (No. conidia/ml)	Longevity	Fecundity	Egg fertility %	Consumption	
					Oviposition period	Total
Direct spray	1×10^6	20.53 ± 0.97^a	28.46 ± 2.26^b	96	60.18 ± 3.45	77.37 ± 4.08^b
	1×10^9	15.42 ± 0.89^b	21.37 ± 1.72^c	95	27.57 ± 1.87	53.28 ± 3.52^c
Indirect spray	1×10^6	23.09 ± 1.34^a	35.12 ± 2.87^a	98	75.62 ± 4.08	87.26 ± 4.76^a
	1×10^9	21.11 ± 1.02^a	32.43 ± 2.64^a	97	70.15 ± 4.11	82.19 ± 4.38^b
Control		23.03 ± 1.52^a	38.75 ± 2.89^a	98	81.65 ± 4.57	95.54 ± 5.22^a

Means in column followed by the same letter are not significantly different at 5 % level (Duncan's 1955 multiple range tests). Indirect spray = Predator feed on treated prey with *M. anisopliae*

Data in table (2) and fig. (2) show dispersal of loaded predator with *M. anisopliae* on kidney bean plant and *T. urticae* individuals that exist on the plant after 3,7 and 10 days of releasing the predator on the plant that found number of *M. anisopliae* conidia distributed that fall down from the predator on kidney bean leaves in addition to the conidia reached to the prey, *T. urticae* resulted from the predator feeds and touched the prey. It was found that population of conidia increased with increasing the time after the releasing till reached maximum 23.50 conidia /prey after 10 days of the predator release while it was 13.27 conidia/leaf. These results are in agreement with Gongyu *et al.* (2019) loaded certain predatory mite, *Neoseiulus cucumeris* with fungal conidia, *Beauveria bassiana* can increase their capacity to suppress thrips, *Frankliniella occidentalis* populations by combining predation and dispersing pathogens.

Table (2). Dispersal of fungus, *M. anisopliae* loaded on *P. macropilis* after 10 days from its release on Kidney bean infested with *T. urticae*

Days after release the predator	Number of <i>M. anisopliae</i> conidia	
	<i>T. urticae</i> prey	Kidney bean leaf
3	04.63 ± 0.02	02.03±0.03
7	16.43±0.15	07.15±0.06
10	23.50±1.03	13.27±0.09

Data in table (3) indicate that, the examined cucumber leaves before releasing *P. macropilis* and infestations with the two-spotted spider mite, *T. urticae* were generally high as its populations at the pre-count 135, 122 and 152 individual / 20 leaves for loaded predator, unloaded predator and control, respectively. The number of the target pest in treatment as *T. urticae* movable stages was reduced after one week till 9th week after loaded predator release while, the number was reduced after one week till 6th week after unloaded predator release. The reduction percentage in mite count after one week from release was 24.93 and 27.51% for loaded predator and unloaded predator. Concerning, the reduction percentage of the pest that increased gradually to reach its maximum in the 9th week after loaded predator release as the calculates reduction percentages was 90.72% and 79.33% for unloaded predator release at 5th week. It was found significant differences at the mean reduction percentage of loaded predator that was 70.59% and 59.27% for unloaded predator. These results are in agreement with El- Nagggar *et al.* (2008) who reported that, the reduction percentage after 10 weeks of release the predatory mites *P. persimilis* at level 1: 10 of treatment was 72.84% under greenhouse and Heikal and Fawzy (2003) released the predatory mite, *P. macropilis* for controlling the two spotted spider mite, *T. urticae* in greenhouse that revealed the release of this predator was successful in reducing the population of *T. urticae*.

Table (3). Release of *P. macropilis* on cucumber plants in greenhouse for controlling *T. urticae*

Sampling date		Loaded predator		Unloaded predator		Control /20 leaves
		NO. <i>T. urticae</i> /20 leaves	R%	NO. <i>T. urticae</i> /20 leaves	R%	
Pre-count /20 leaves		135	-	122	-	152
Weeks after releasing	1 st	110	24.93	96	27.51	165
	2 nd	90	39.58	65	45.28	148
	3 rd	53	64.89	47	65.55	170
	4 th	40	76.90	42	73.16	195
	5 th	50	73.31	35	79.33	211
	6 th	39	80.48	48	73.42	225
	7 th	34	82.51	57	67.57	219
	8 th	22	87.91	66	59.88	205
	9 th	14	90.72	63	53.82	170
	10 th	17	84.68	53	47.17	125
Mean R%		-	70.59 ^a	-	59.27 ^b	-

Means in row followed by the same letter are not significantly different at 5 % level (Duncan's 1955 multiple range tests).

Conclusion

The fungus *M. anisopliae* and the predatory mite, *P. macropilis* are regarded among of the most important biological control agents of the spider mite, *T. urticae*. It was possible to use both agents together in the control process, where a weak concentration of *M. anisopliae* was used and the predator loaded with it for the possibility of its spread in the infected fields. The results were satisfactory as it was possible to load the predator with *M. anisopliae* and release it on the cucumber crop in the fight against the spider mite present on the cucumber leaves. Conidia of *M. anisopliae* present on the leaves and *T. urticae* individuals were recorded and counted as a result of the fall of these conidia from the back of the predator during walking and feeding on *T. urticae* individuals. And the reason for a reduction in the population exceeded 90 % and by a significant increase over the release of the unloaded predator with *M. anisopliae*.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data material: Not applicable

Competing interests: The authors declare that they have no competing interests

Funding: No funding was obtained for this study All authors read and approved the final manuscript.

Acknowledgements: We would like to acknowledge all members of acarology department in Plant Protection Research Institute, ARC.

References

- Alves, R. T.; R. P. Bateman; C. Prior and S. R. Leather (1998). Effect of simulated solar radiation on conidial germination of *Metarhizium anisopliae* in different formulations. Crop Prot., 17: 675-679.
- Clotuche, G.; A.C. Mailleux; A.A. Ferná'ndez; J.L. Deneubourg and C. Detrain, (2011). The formation of collective silk balls in the spider mite *Tetranychus urticae* Koch. PLoS ONE, 6(4): 1804-1817
- El-Naggar, M.E.; A.M. Metwally; Zakia, E. A. Darwish and Azzia, M. M. Abou Zaid (2008). Biological control of *Tetranychus urticae* Koch on cucumber plants by using of two predaceous mites, *Phytoseiulus persimilis* and *Neoseiulus californicus* under greenhouse condition. Egypt. J. Agric. Res., 86 (1): 269- 279.
- Farouk, S. and M.A. Osman, (2011). The effect of plant defence elicitors on common bean (*Phaseolus vulgaris* L.) growth and yield in absence or presence of spider mite (*Tetranychus urticae* Koch) infestation. J. Stress Physio. and Biochem.; 7(3): 6-22.
- Gongyu L.; C. Guertin; D.P. Sean-Anthony; T. Silvia and B. Jacques (2019). Phytoseiid predatory mites can disperse entomopathogenic fungi to prey patches. Scientific Reports 9:19435 doi.org/10.1038/s41598-019-55499-8
- Heikal, I.H. and M.M. Fawzy (2003). Preliminary study of biological control of *Tetranychus urticae* Koch on cucumber (Acari: Tetranychidae). Egypt. J. Agric. Res., 81 (1): 93 - 100
- Henderson, C.F. and E.W. Tilton (1955). Tests with acaricides against the brown white mite. J. Econ. Entomol., 48:157-161.
- Hughes, W.O.H.; K.S. Petersen; L.V. Ugelvig; D. Pedersen; L. Tomsen; M. Poulsen and J.J. Boomsma (2004). Density-dependence and within- host competition in a semelparous parasite of leaf-cutting ants. Bio. Med. Central Evolutionary Biology: 4: 45.
- Jacobson R.J.; D. Chandler; J. Fenlon and K.M. Russell (2001). Compatibility of *Beauveria bassiana* (Balsamo) Vuillemin with *Amblyseius cucumeris* Oudemans (Acarina: Phytoseiidae) to control *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) on cucumber plants. Biocontrol Science and Technology, 11, 391-400
- Janssen, A.; J. Bruin; G. Jacobs; R. Schraag and M.W. Sabelis (1997). Predators use volatiles to avoid prey patches with conspecifics. Journal of Animal Ecology, 66: 223-232

- Kanika, T. (2014). A review on reproductive strategies in two spotted spider mite, *Tetranychus urticae* Koch 1836 (Acari: Tetranychidae) Indian J. of Entom. and Zoo. Studies; 2 (5): 48-52.
- Kumar, S.V.; C. Chinniah; C. Muthiah and A. Sadasakthi (2010). Management of two spotted spider mite *Tetranychus urticae* Koch a serious pest of brinjal, by integrating biorational methods of pest control. Journal of Biopesticides, 3(1): 361-368
- Meyer, M. K. P. S. (1996). Mite pests and their predators on cultivated plants in southern Africa. Vegetables and berries. ARC, South Africa.
- Midthassel A.; Simon R. Leather; Denis J. Wright and Ian H. Baxter (2016). Compatibility of *Amblyseius swirskii* with *Beauveria bassiana*: two potentially complimentary biocontrol agents. BioControl. 61:437-447
- Nauen, R.; N. Stumph; A. Elbert; C.P. Zebitz and W. Kraus (2001). Acaricide toxicity and resistance in larvae of different strains of *Tetranychus urticae* and *Panonychus ulmi* (Acari: Tetranychidae). Pest Manag. Sci.; 57: 253-261.
- Navajas M. (1998). Host plant associations in the spider mite *Tetranychus urticae* (Acari: Tetranychidae): insights from molecular phylogeography. Exp. Appl. Acarol.; 22: 201-214.
- Van de Vrie, M.; J.A. McMurty and C.B. Huffaker (1972). Ecology of tetranychid mites and their natural enemies: a review. III. Biology, ecology and pest status, and host plant relations of tetranychids. Hilgardia; 41: 343-432.
- Van der Geest, L.P.S.; S.L. Elliot; J.A.J. Breeuwer and E.A.M. Beerling (2000). Diseases of mites. Experimental and Applied Acarology, 24: 497-560.
- Zhang, Z.Q. (2003). Mites of Greenhouses: Identification, Biology and control. CABI Publishing, Wallingford, UK, xii pp. 244.

Figures

Figure 1

Dispersal of fungus, *M. anisopliae* loaded on *P. macropilis* An Eppendorf tube containing predatory mites and fungal conidia to be released was attached on stem in position (A) Distribution of *T. urticae* on leaflet of kidney bean in position (2)

Figure 2

M. anisopliae conidia on *T. urticae* and kidney bean leaf