Pathogenicity of Two Fungi; *Trichoderma harzianum* and *Cladosporium herbarium* on The Two-spotted Spider Mite; *Tetranychus urticae* Koch

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ABSTRACT

Two fungi; *Trichoderma harzianum* and *Cladosporium herbarium* were isolated from dead bodies of the two-spotted spider mite *Tetranychus urticae* Koch. Pathogenicity of the two fungi against *T. urticae* were studied and evaluated under laboratory and semi-field conditions. Three fungal spore suspensions were prepared; *T. harzianum* ($2x10^6$ spores/ml), *C. herbarium* ($1x10^6$ spores/ml) and a mixture of both fungi (50%: 50%). The effect of fungal spore suspensions on the deferent stages of *T. urticae* was studied at 30 & 35°C and 90 % R.H. The mortality percentages of all mite stages increased with prolonging the period after spraying. The mixture of the two fungi gave higher mortality than a single one after 3 and 7 days for all mite stages reaching 46.8% and 59.8% after 3 and 7 days respectively at 30°C, while at 35°C, it increased to 65.1% and 83.0% after the two periods respectively. Under semi-field conditions, the mean reduction in mite population resulted from using suspensions of *T. harzianum* and *C. herbarium* recorded 42.4 and 43.9% respectively after 10 days of spraying, while it increased to 58.2% when using the mixture of the two fungi. Both fungi *T. harzianum* and C. *herbarium* exhibited a positive effect in the pathogenicity of the two-spotted spider mite, *T. urticae*, but it is recommended to use a mixture of both fungi to obtain more efficient control.

KEY WORDS: Pathogenicity, fungi, *Trichoderma harzianum*, *Cladosporium herbarium* spider mite, *Tetranychus urticae*.

INTRODUCTION

The two-spotted spider mite *Tetranychus urticae* Koch is considered an economically important pest of commercial strawberry production. Its decreases plant vigor which eventually results in decrease fruit size and yield (Lola-luzd 2003). In 2004 and during the application of integrated pest management (IPM) program in strawberry farm, Badr district, Behira Governorate, Egypt, it was noted that, the population of *T. urticae* deeply declined in the greenhouses used for mass culturing the predatory mites *Phytoseiulus persimilis* (Athias-Henroit) and *Amblyseius californicus* (McGregor). This phenomenon encouraged us to collect dead bodies of *T. urticae* and examined in the laboratory, where the two fungi; *Trichoderma harzianum* and *Cladosporium herbarium* were isolated. Knowledge of mite diseases is still fragmentary, but in recent years, more attention has been paid to acaropathogens, because of the economic importance of many phytophagous mites. The entomo-pathogenic fungi play an important role in the regulation of phytophagous mite populations and sometimes to decimate it (Van der Geest *et al*, 2000) and concequently reduce the application of acaricides.In Egypt, climatic conditions are considered more suitable for fungal pathogens (Sewify, 1989). Several species of pathogenic fungi were applied against mite pests; *Hirsutella thompsonii* Fisher against the citrus rust mite, *Phyllocoptruta oleivora* (Ashmed) (McCoy, 1975 and Latge *et al*, 1988) against *T. urticae* (Hanna and Heikal, 1995) and against *Aceria gurreronis* (Kumar, 2006); *Verticillium lecanii* against *Eutetranychus orientalis* (Klein) (Sewify and Mabrouk, 1991) and *Beauveria bassiana* against *T. urticae* (Yousri, 1994; Hassan, 2003 and Afifi *et al*. 2004 & 2006); The objective of this work was to study the pathogenicity of two fungi, *T. harzianum* and *C. herbarium* on *T. urticae* in the laboratory and semi-field conditions.

MATERIALS AND METHODS

Fungi Isolation, Identification and Preparation:

Dead bodies of *T. urticae* were collected from three greenhouses used for mass rearing and production of the predatory mites, *Phytoseiulus persimilis* (Athias-Henroit) and *Amblyseius californicus* (McGregor). For fungal isolation, the dead mites were surface sterilized by immersing it in 3.0% Sodium hypochloride solution and immediately left up, then rinsed in sterilized water. Sterilized mites were dried between two sterilized filter papers, and then transferred to Potato dextrose agar (PDA) medium. Inoculated (PDA) plates were incubated at 25±2°C and examined daily for occurrence of the fungal growth. The isolated fungi were purified using the single spore technique (Hildbrand, 1948). Detected fungi were transferred to slants of PDA medium and kept at 5°C. Fungal isolates were identified at Mycology Research and Disease Survey, Dept., Plant Pathology, Research Institute, Agricultural Research Center, according to

Bisset (1991) and Ellis (1993). A mycelial agar block from a stock culture of *T. harzianum* and *C. herbarium* was transferred each to a 9 cm.-diameter Petri-dish of PDA. Cultures were grown at room temperature (24±2° C) for ten days, then spores were liberated by gentle agitation with a sterile wire loop in the presence of 10 ml of sterile water, followed by filtration through two layers of sterilized cheese cloth to reduce mycelium clumping. The concentration of fungal spores was determined using a haemocytometer, and the suspension was diluted with sterile water to inoculum levels of $2x10^6$ and $1x10^6$ spores/ml for *T. harzianum* and *C. herbarium*, respectively.

Three fungal spore suspensions were prepared; *T. harzianum* ($2x10^6$ spores/ml), *C. herbarium* ($1x10^6$ spores/ml) and a mixture of both fungi (50%/50%).

To study the effect of fungal spore suspensions on the deferent stages of *T. urticae*, sterilized potato leaf discs (1 inch diameter) were put on moist cotton wool pads placed in glass Petri-dishes, where few drops of water were added daily to maintain suitable moisture content. Ten leaf discs, each contained ten individuals of homogenous eggs or immatures or adult females of *T. urticae*. Ten replicates were used for each suspension, in addition to the check, which were treated with water only. Plant discs were treated by direct spray (spraying in the presence of the mite individuals) using a hand atomizer, then kept at incubators adjusted at 30° C, 35°C and 90% R.H. Mortality was determined after 3 and 7 days.

To study fungi effects at semi-field condition, 20 plastic pots (10cm diam. & 15cm high) cultivated with mite infected bean plants, were sprayed, five pots for each treatment, in addition to the check. The percentage of mite population reduction was determined after 3, 7 and 10 days according to the equation of Henderson and Tilton (1955).

RESULTS AND DISCUSSION

Data presented in Table 1 show that the mortality percentages of all mite stages increased after 7 days more than those after 3 days of fungal suspensions spraying. *T. harzianum* exhibited higher efficiency on eggs and immature stages of *T. urticae*, while *C. herbarium* was more effective to the adults. The mixture of the two fungi showed higher mortality after 3 and 7 days in all mite stages than that of either one alone. When this experiment was conducted at 35C° and 90% R.H. (Table 2), the obtained results show that *T. harzianum* gave higher mortality on adults and immatures after 3 days than *C. herbarium*, while the latter gave higher mortality on eggs than the former. After 7 days, *C. herbarium* gave high mortality on mite adults and eggs. Mortality percentage was higher when the mixture of the two fungi was applied. However total mean mortality percentage of *T. urticae* was nearly greater when using *C. herbarium* especially at 35°C.

Under semi-field conditions, the four submentioned treatments were applied on infested bean plants planted in plastic pots. Table (3) show that, the mixture of two fungi gave the highest population reduction percentage (58.2%) followed by that of *C. herbarium* (42.4%) then *T. harzianum* (43.9%) after 10 days of spraying.

Therefore, it could be concluded that *C. herbarium* and *T. harzianum* have a positive effect in the pathogenicity of *T. urticae*, but using the mixture of both fungi is recommended to obtain more efficient results in controlling this noxious pest.

Lenteren (2000) stated that green-houses support favorable conditions for fast reproduction of pests and diseases, thus it demanded successive chemical treatments. About 100 species of beneficial organisms are commercially available for control all important mite and insect pests. Chandler and Van

Table (1): Effect of *Trichoderma harzianum* ($2x10^6$ spores/ml) and *Cladosporium herbarium* ($1x10^6$ spores/ml) and their mixture on the mortality percentages of Tetranychus urticae different stages at 30°C and 90% R.H.

Days after treatment	Mite stage	(%) Mortality after fungi treatments			
		T .harzianum	*C. herbarium*	*T. harzianum* + *C. herbarium*	Check
3 days	Adult	42.0	45.3	62.0	0.0
	Immature	39.6	36.9	58.4	2.0
	Egg	21.4	16.6	20.0	3.3
	Mean	34.3	32.9	46.8	1.8
7 days	Adult	62.4	63.3	72.0	4.5
	Immature	45.0	47.1	65.7	0.6
	Egg	25.4	23.1	41.5	13.3
	Mean	44.3	44.5	59.8	6.2

Table (2): Effect of *Trichoderma harzianum*($2x10^6$ spores/ml) and *Cladosporium herbarium* ($1x10^6$ spores/ml) and their mixture on the mortality percentages of *Tetranychus urticae* different stages at 35°C and 90% R.H.

Days after treatment	Mite stage	(%) Mortality after fungi treatments			
		T .harzianum	*C. herbarium*	*T. harzianum + C. herbarium*	Check
3 days	Adult	57.1	47.7	64.3	6.7
	Immature	46.5	42.8	57.1	6.7
	Egg	53.1	69.6	73.9	3.3
	Mean	52.2	53.4	65.1	5.6
7 days	Adult	75.5	81.1	81.2	11.7
	Immature	60.7	60.7	67.8	6.1
	Egg	68.3	78.6	99.9	8.6
	Mean	68.1	73.5	83.0	8.8

Table (3): Effect of *Trichoderma harzianum* ($2x10^6$ spores/ml), *Cladosporium herbarium* ($1x10^6$ spores/ml) and their mixture on the population of *Tetranychus urticae* at semi- field conditions

Fungus	(%) Reduction after			Mean (%) reduction
	3days	7 days	10 days	
T. harzianum	34.1	46.8	46.3	42.4
C. herbarium	34.8	48.5	48.5	43.9
Fungi mixture	48.3	63.2	63.1	58.2

der Geest (2006) indicated that the best documented pathogens of Acari are fungi; close to 60 species of fungi have been reported infesting at least 70 species of acarines across nearly all orders.

In Brazil, Van der Geest *et al*, (2002) isolated pathogenic fungus *Cladosporium sp.* from the eriophyid mite *Retracus johnstoni Keifer* (Phytoptidae) and cultured it in Potato-dextrose agar. Moreover, Kovach (1996) and Lola-Luz (2003) reported that the fungus *T. harzianum* could control some plant diseases and not harmful to mammals and other animals. It attacked the fungus *Botrytis cinerea* which causes gray mould disease in strawberries fruits and prevents it from developing .Now, manufacturing of various formulations of these biocontrol agents has been going on.

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