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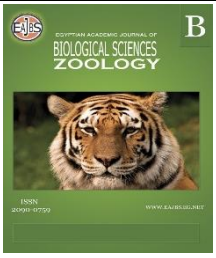

In vitro and *in vivo* Efficacy of Some Acaricides and Two Fungi of *Trichoderma* spp. on Some Biological Aspects of *Tetranychus urticae* Koch and the Predaceous Mite, *Phytoseiulus persimilis* Athias- Henriot on Cucumber Plants

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ABSTRACT

The two-spotted spider mite (TSSM), *Tetranychus urticae* Koch (Acari: Tetranychidae) is a major pest mite infesting vegetables and horticultural ornamental and agronomic crops in Egypt. In biological control strategies, natural enemies were applied to maintain TSSM population below the level where damage occurs for an extended period of time besides utilizing acaricides in a critical situation as a quick practice to avoid deleterious TSSM damage. This study evaluated the potential of acaricides (Abamectin, Chlorfenapyr, Fenpyroximate and Hexythiazox) and the pathogenicity of native species of *Trichoderma* spp.(*Trichoderma harzianum* and *T.album*) on different biological developmental stages of females and males of *T. urticae* and *Phytoseiulus persimilis* Athias- Henriot and their population under greenhouse conditions. Results revealed that the utilization of recommended application rate (RC) of acaricides showed a deleterious effect on the different biological developmental stages including eggs, larvae, protonymh, deutonymph and adults of female and male of TSSM, *T. urticae* and predaceous mite, *P. persimilis* under laboratory conditions. Moreover, the effects varied greatly on immature stages, life cycle, adult stage, longevity and life span of TSSM and *P. persimilis* according to utilized acaricide. Concerning the pathogenicity of the two bio-agents (*T.harzianum* and *T.album* and a mixture of them), *T.harzianum* was more pathogenicity against *T. urticae* and *P. persimilis* than *T.album*. Moreover, *Trichoderma* species showed a negative effect on the population density of *T. urticae* than its predaceous mite *in vitro* treatments and under greenhouse conditions. The results are valuable for producing information on the potential integrated management of two-spotted spider mite (TSSM), *T. urticae* by using acaricides and bio-agents on cucumber plants and their effect on the associated predaceous mite.

INTRODUCTION

The two-spotted spider mite (TSSM), *Tetranychus urticae* Koch (Acari: Tetranychidae), is a main pest of vegetables, many horticultural, ornamental, agronomic crops and other greenhouse-grown plants. (Van de Vrie ,1985 and Stumpf and Nauen ,2002). A formidable problem resulted from TSSM because of its broad host range, good dispersal ability, high reproductive rate, and short life cycle. Moreover, small size and tendency to inhabit the underside of leaves help in the difficulty of noticeable until

populations are already causing deleterious damage. Therefore, growers traditionally rely on applications of chemical miticides to avoid or overcome *T. urticae* problems (Rehab *et al*. 2020). However, chemical control is becoming increasingly difficult as the number of effective and permitted active ingredients are reduced by both pesticide resistance (Bielza, 2008 and Ferna´ndez *et al*.,2009) and market and due to environmental and worker safety. Moreover, authors always exhibited the evaluation of pesticide effects based only on TSSM females and negligence of the overall impacts of pesticides particularly on predatory mites. Therefore, in order to evaluate the overall effects of pesticides on predators, specifying sublethal effects on both sexes is necessary. Therefore, in this study, we estimated demographic parameters with respect to both sexes based on the age-stage, two-sex life table theory (Chi and Liu,1985). The predator mite, *Phytoseiulus persimilis* Athias- Henriot is widely spread throughout the Mediterranean area including Egypt and is considered the most effective biological control agent for control of TSSM. The *P. persimilis* was applied against TSSM in numerous greenhouse vegetable crops (Lee, *et al*., 2002). *In vitro* and greenhouse studies were conducted to evaluate the deleterious effect of *Trichoderma* spp. fungi on TSSM and *P. persimilis* including *Trichoderma harzianum* and *Cladosporium herbarium* (Afifi *et al*.,2007). Recently, entomopathogenic fungi play an important role in the regulation of phytophagous mite populations and sometimes decimate them and consequently reduce the application of acaricides (Van der Geest *et al.*, 2000).

Moreover, the pathogenicity of *Trichoderma* spp. extended to use against other pests like root-knot nematodes (RKN), *Meloidogyne* spp. under greenhouse conditions due to their effectiveness as bio-agents against mites (acaropathogens) as well as their economic importance of many phytophagous mites (Ali *et al*., 2022). Under Egyptian climates, Afifi *et al*., (2007) mentioned that the various species of *Trichoderma* fungi used against other mite species like *Hirsutella thompsonii* Fisher against the citrus rust mite, *Phyllocoptruta oleivora* (Ashmed) (McCoy, 1975 and Latge *et al.*, 1988) and also against *T. urticae* (Hanna and Heikal, 1995) but the fungi, *Beauveria bassiana*, was used against *T. urticae* (Hassan, 2003). van de Vrie *et al*.,(1972) mentioned to the effect of pesticides and their residues often have direct effects on TSSM and *P. persimilis*, including mortality, decreased longevity and reduced or increased fecundity. The objective of this study was to evaluate the efficacy of some synthetic acaricides and biological control agents *Trichoderma* spp. on some biological aspects of male and female of the two-spotted spider mite (TSSM) *Tetranychus urticae* Koch to achieve acceptable control in integrated pest management programmers, by its predator *Phytoseiulus persimilis* Athias-Henriot under laboratory and greenhouse conditions.

MATERIALS AND METHODS

Test Organism:

The laboratory strains of spider mites Two-spotted Spider Mites (TSSM), *Tetranychus urticae* Koch (Acari: Tetranychidae) were collected from infected plants from citrus orchards in Bassaten Barakat (Belles District, Sharqia Governorate, Egypt) from September to October 2021.

Various host plants such as cucumber (*Cucumis sativus* L. cv. Chief (SC 4145), bean (*Phaseolus vulgaris* L. cv. Local) were grown in plastic pots (15 cm in diameter × 21 cm in height) filled with sterilized media (sand: clay, 95 1:1), and normal agriculture practices, including irrigation, and fertilization, were applied. After 17 days. In the current study, cucumber leaves only were detached and used for leaf disc preparation to conduct the following trials. All plants were irrigated simultaneously during the experiments, and

no pesticides were used. Cucumber and bean plants were maintained in the greenhouse at 27±3°C, 65±5% RH and a photoperiod of 16:8 (L: D) h. for several generations. All plants were irrigated simultaneously during the experiments, and no pesticides were used.

Tested Acaricides:

Four registered commercial formulations of pesticides available in the market and used for controlling insect and nematode pests in Egypt were obtained from the Central Laboratory of Pesticides, Dokki, Giza. The tested pesticides were used at recommended application rate (RC) as follows:

Abamectin (Abazeen 1.8 % EC), 50 cm^3 /100 L water; Chlorfenapyr (Acinapyr 24% SC) 60 cm3/100 L water; Fenpyroximate **(**Abroch 5 % SC), 50 cm^3/100 L water and Hexythiazox (Prince 10% EC), 20 cm^3/100 L water.

Preparation of Tested *Trichoderma* spp. Used:

Trichoderma album and *T.harizianum* were obtained from the Plant Pathology Laboratory, Faculty of Agricultural, Zagazig University. The following technique was used to culture *Trichoderma* species. Petri dishes filled with potato dextrose agar (PDA) were used in culturing for 7 days at 25°C. Ten Petri dishes for each fungus were used to collect sufficient conidia for the experiment. The conidia suspension of the tested *Trichoderma* species was prepared by flooding each dish with 10 ml of sterilized distilled water containing 0.01% (*V/V*) Tween 80, and the agar surface was scraped gently with sterile glass rods and collected in 250 ml beakers. The suspension was filtered through a sterile double-layered muslin cloth to remove mycelium fragments. The density of conidia was assessed using Neubauer hemocytometer to use these concentrations as stock to prepare a suspension of different densities: $2x10^6$ spores/ml and stored at 4 °C.

Planting for the Rearing of Predacious Mite, *Phytoseiulus presimilis*:

Bean plant *Phaseoulus vulgaris* L. cv. Local was used as the host plant and planted in mentioned plastic pots. These trays were used in rearing the predatory mite *Phytoseiulus persimilis* Athias Henriot, (Acarina: Phytoseiidae) which was used as a bio-agent predacious mite under laboratory and greenhouse conditions. As well as, two-spotted Spider Mites (TSSM), *Tetranychus urticae* Koch-infested leaves of cucumber plants under laboratory conditions were collected and produced for predatory mites, *P. persemilis* as a food source during the study.

Mass Rearing of Predator:

The following producer was maintained to obtain the appropriate population density of predaceous mites.

Ten females of predatory mite *P. persimilis were* transferred to each bean plant, there were followed by the relation between the predator and the prey *T. urticae* when it needs prey; it was supported with more prey. About one month later, the population of predatory mite *P. persimilis* reached 40-50 individuals/ leaflet and then picked for the following tests in a small paper bag with a few preys on bean leaves and transferred to an inside ice box.

Experimental Design:

The leaf disc method was used according to Salonaz E. Awad *et al*., 2022. Each leaf disc had a $5cm^2$ area cut from the center of the leaves. A plastic Petri dish (9 cm in diameter ×1.5 cm high with a hole in its center) filled with one leaf disc of cucumber plants prepared by collecting the third leaf below the apical meristem of 3-weeks-old plants to avoid the effect of plant age on biological parameters of tested mites such as mite growth and reproduction. The leaves of cucumber plants were selected from all replications, cut into a leaf disc (2.5 × 2.5 cm), and then placed on water-saturated cotton in the Petri dish with the underside facing upward. Moreover, cucumber and bean seeds weekly were planted to deliver the mites with new leaf discs.

In vitro Bioassay for *Trichoderma* spp.:

To study the effect of fungal spore suspensions of TSSM, *Tetranychus urticae* Koch and predaceous mite, *P. persimilis in vitro* treatments on the various stages, sterilized cucumber leaf discs (2.5 × 2.5 cm) were put on moist cotton wool pads placed in plastic Petri-dishes, where few drops of water were added daily to maintain suitable moisture content. Treatments consist of the two prepared fungal spore suspensions; *T. harzianum* and *T. album* at a concentration of individually and mixture of both fungi (50%/50%) on eggs, immatures, or adult females of *T. urticae* and the same manner was used with predaceous mite, *P. persimilis*. Ten individuals from eggs, immature stages and adult females of *T. urticae* and *P. persimilis* and replicated five times. A hand atomizer was used to spray *Trichoderma* spp. by direct spray (spraying in the presence of the mite individuals) then kept at incubators adjusted at 30 ±3°C and 85± 5% R.H and water only was used in the control treatment.

Mortality was calculated daily up to 10 days post-treatment. The equation of Henderson and Tilton (1955) was used to assess the percentage of mite population reduction

Screening of Acaricides:

To assess the efficacy of tested acaricides against various stages of *T. urticae* and *P. persimilis in vitro* treatments, the current trials were conducted. The leaf dips method of Castagnoli *et al.*, 2005 was utilized on each mite egg. Females of TSSM, *T.urticae* Koch and *P. persimilis* were on cucumber and bean leaves to oviposit the eggs for 24 h and then removed.

Leaves with each mite egg were carefully collected directly five discs with 10 eggs of TSSM and five discs with 5 eggs of *P. persimilis* were dipped in each recommended application rate (RC) of the tested acaricides for 30 seconds and immediately placed upside down on a wet cotton pad. Hatched eggs were evaluated for five successive days of acaricides application. Thereafter, twenty of each *T. urticae* and *P. persimilis* hatched larvae were reared singly on cucumber leaf discs up to adulthood.

Developmental periods of the different stages of two mites were verified and the predaceous mite; *P. persimilis* was supplied by 20 *T. urticae* larvae and the consumed larvae were recorded and replaced daily by new ones. The developmental time (life cycle) and developmental rate (1/developmental time) of immature stages, survival from eggs to adult stage was recorded. Also, the growth index was asses by the following equation:

$$\text{Growth index} = \frac{\text{Mean of adult emergence \%}}{\text{Average of immature stages (life cycle}}$$

Developmental time for egg, protonymph and deutonymph as well as total immature stages was used to calculate developmental rates regressed against pesticide treatments (Omkar and James, 2004). All tests were conducted under laboratory conditions of 27 ± 3 °C and 65 ± 5% R.H.

Data Analysis:

Data for the developmental time of immature stages, adults, pre-oviposition, oviposition, and postoviposition periods, and longevity of females and males were subjected to one-way analysis of variance (ANOVA), and the means were separated using Duncan's Multiple Range Test (COSTAT (2005).

RESULTS AND DISCUSSION

The current study aimed to determine the biological aspects of using the acaricides Abamectin, Chlorfenapyr, Fenpyroximate and Hexythiazox recommended application rates (RC) to evaluate the different biological developmental stages of female and male of

T. urticae and *P. persimilis* after treating eggs with mentioned tested under laboratory conditions.

Incubation Period:

Results obtained the effectiveness of various tested acaricides on incubation periods of *T. urticae* and its predator *P. persimilis* female and male (Tables 1-4). Also, showed clearly a significant effect of egg incubation periods of female and male of *T. urticae* and the predator, *P. persimilis* after being treated with different egg acaricides except with Abamectin, Chlorfenapyr, Fenpyroximate and Hexythiazox when compared with control treatment (Tables 1&2).

Table 1: Developmental duration (Mean ± S.E. by days) of two-spotted Spider Mites (TSSM), *Tetranychus urticae* Koch female after eggs treatment with tested acaricides at 28 ±2 °C.

Treatment		Control	Abamectin	Chlorfenapyr	Fenpyroximate	Hexythiazo
	Egg	3.52 ±0.16 c	5.28 ±0.16 a	4.24 ±0.25b	4.90 ±0.22 b	5.32 ±0.14 a
Immature stages	Larva	1.62 ±0.15 bc	1.34 ± 0.20ab	1.95 ±0.24a	1.35 ±0.23 ab	1.21 ±0.13 c
	Protonymph	1.90 ±0.19 b	2.44 ±0.42 b	2.56±0.31ab	3.26 ±0.46 a	1.33 ±0.24 c
	Deutonymph	3.25 ±0.28 a	3.29 ±0.37 a	3.22±0.29 a	1.77 ±0.54±b	1.08 ±0.41c
Life cycle		10.29 ±0.19 b	12.35 ±0.29a	11.97±27a	11.28 ±0.28 a	8.94 ±0.27 c
	Pre- Oviposition	1.49 ± 0.29 b	2.21 ±0.23 a	-	-	-
Adult stage	Oviposition	8.20 ±0.37 a	3.41 ±0.56 b	-	-	-
	Post-oviposition	1.42 ±0.28 a	1.64 ±0.24 a	-	-	-
Longevity		10.40± 0.34 a	7.26 ±0.64 b	-	-	-
life span		21.51 ± 0.32 a	19.61±0.29 b	11.97 ±27c	11.28 ±0.28 c	8.94 ±0.27 c

*Means in each column followed by the same letter(s) are not significantly different at the 5% level of probability according to Duncan's multiple range test.

Table 2: Developmental duration (Mean ± S.E. by days) of two-spotted Spider Mites (TSSM), *Tetranychus urticae* Koch male after eggs treatment with tested acaricides at 27 ±3°C.

Treatment		Control	Abamectin	Chlorfenapyr	Fenpyroximat	Hexythiazox
	Egg	2.15±0.21 c	4.78± 0.22 ab	3.66 ±0.22 b	4.35± 0.62 ab	5.29 ±0.56 a
Immature stages	Larva	1.24±0.23 c	1.80±0.34 a	1.59 ±0.38ab	1.95±0.65 a	1.19±0.98 c
	Protonymph	1.62±0.17 b	1.90± 0.12 ab	2.34±0.34 ab	2.75± 0.59 a	1.20± 0.67 c
	Deutonymph	2.78±0.19 a	2.43 ±0.22 b	2.98 ± 0.42 a	2.13 ±0.12 ab	2.28±0.75 b
Life cycle		7.69 ±0.20 d	10.91± 0.19 ab	10.57± 0.34ab	11.18±0.28 a	9.96± 0.74c
Longevity		8.67 ±0.36 a	-	-	-	-
Life span		16.36 ±0.56 a	10.91± 0.19 b	10.57± 0.34b	11.18±0.28 a	9.96± 0.74c

*Means in each column followed by the same letter(s) are not significantly different at the 5% level of probability according to Duncan's multiple range test.

The female and male incubation periods of *T. urticae* duration (5.28 & 2.15) days in control but these were (3.28 & 4.78), (4.24 & 3.66), (4.90 & 4.35) and (5.32 & 5.29) days after egg treatment with Abamectin, Chlorfenapyr, Fenpyroximate and Hexythiazox, respectively.

The parallel values in female and male phytoseiid mite, *P. persimilis* were (3.57 & 3.05) days in the control treatment but were (5.65 &4.54), (5.47 &3.79), (5.65 &4.80) and (4.26 &5.24) days after the same treated egg with the mentioned acaricides (Tables 3 &4).

Life cycle:

Two-spotted Spider Mites (TSSM):

Compared with the control (10.29 days), the life cycle of *T. urticae* female treated with mentioned acaricides was significantly varied and averaged 12.35, 11.97,11.28 and 8.94 days after eggs treated with acaricides. Whereas, the male of TSSM showed a shorter life cycle and recorded 7.69, 10.91, 10.57, 11.18 and 9.96 days compared

with the control treatment, Abamectin, Chlorfenapyr, Fenpyroximate and Hexythiazox, respectively.

Predatory mite, *P. persimilis*:

Results exhibited the same trend with a shorter life cycle in predaceous mite males compared to females. The life cycle of male *P. persimilis* was 7.32, 11.57, 10.13, 11.14, 10.27 days in control, Abamectin, Chlorfenapyr, Fenpyroximate and Hexythiazox, respectively.

Longevity:

Two-spotted Spider Mites (TSSM):

The tested acaricides showed high effects on the longevity of TSSM females after the eggs were treated with RC of all mentioned pesticides except with Abamectin. The longevity was 7.26 days with the treatment of Abamectin compared with 10.40 days of female control.

Unfortunately, the male of *T. urticae* died with egg treatment of Abamectin, Chlorfenapyr, Fenpyroximate and Hexythiazox while the longevity of control treatment was 8.67 days.

Table 3: Developmental duration (Mean ± S.E. in days) of *Phytoseiulus persimilis* female after egg treatment with tested acaricides at 27 ±3°C

Treatment		Control	Abamectin	Chlorfenapyr	Fenpyroxim	Hexythiazox
Immature stages	Egg	3.57 ±0.14 c	5. 65 ±0.28 a	5.47 ±0.29 a	5.65 ±0.14 a	4.26 ±0.78 b
	Larva	1.45 ±0.11 b	2.50 ±0.33 a	2.68 ±0.77 a	2.69 ±0.12 a	2.55 ±0.33 a
	Protonymph	1.55 ±0.13 c	2.78 ±0.24a	2.10 ±0.53 b	2.57 ±0.34ab	2.65 ±0.67 a
	Deutonymph	2.45 ±0.12 ab	2.50 ±0.23 a	2.35 ±0.46 ab	2.29 ±0.28 ab	2.44 ±0.75 a
Life cycle		9.02 ±0.12 c	13.43 ±0.27a	12.60 ±0.51 ab	13.20 ±0.22 ab	11.90 ± 0.63 b
Adult stage	Pre-oviposition	2.56 ±0.21ab	1.50 ±0.49 b	-	-	2.00 ±0.53 b
	Oviposition	8.18 ±0.37 a	2.00 ±1.13 b	-	-	-
	Post-oviposition	1.74 ±0.25 a	-	-	-	-
Longevity		12.48 ±0.27 a	3.50 ±0.81 b	-	-	-
life span		21.50 ±0.19 a	16.93 ±0.54b	12.60 ±0.51c	13.20 ±0.22 c	13.90 ± 0.58 c

*Means in each column followed by the same letter(s) are not significantly different at the 5% level of probability according to Duncan's multiple range test.

Table 4: Developmental duration (Mean ± S.E. in days) of *Phytoseiulus persimilis* male after egg treatment with tested acaricides at 27 ±3°C.

Treatment		Control	Abamectin	Chlorfenapyr	Fenpyroxim	Hexythiazox
Phytoseiulus	Egg	3.05 ±0.11 b	4.54 ± 0.12 ab	3.79 ±0.14 b	4.8 ± 0.15 ab	5.24 ±0.12 a
Immature stages	Larva	1.05 ±0.12 c	2.20 ±0.19 b	2.25 ± 0.12 ab	2.41 ±0.18 a	2.33 ±0.39 a
	Protonymph	1.05 ±0.14 c	2.34 ± 0.16 a	2.37 ±0.14 a	1.79 ± 0.17 b	2.14 ± 0.37 a
	Deutonymph	2.14 ±0.16 ab	1.59± 0.18 c	2.15 ± 0.29 a	2.10 ± 0.19 b	1.94 ± 0.38 c
Life cycle		7.32 ± 0.28 c	10.27 ± 0.36 b	10.13± 0.32b	11.14 ±0.35ab	11.57 ± 0.63ab
Longevity		5.85 ±0.28 a	2.24 ±0.41 b	2.33 ±0.35 b	2.42 ± 0.45 b	1.98 ±0.69 c
Life span		13.12 ±0.48 a	12.49 ±0.59 b	12.52 ±0.68 b	13.49 ±0.59 a	13.52 ±1.18 a

*Means in each column followed by the same letter(s) are not significantly different at the 5% level of probability according to Duncan's multiple range test.

Predatory Mite, *P. persimilis*:

When referring to males and females of predaceous *P. persimilis*, unfortunately, results showed the same trend and females of *P. persimilis* completely died except with control and Abamectin treatments and the longevity was 3.50 days with the treatment of Abamectin compared with 12.48 days of female's control. Contrarily, males of *P. persimilis* survived after reaching the adult stage with the four egg acaricides treatment but the longevities were less compared with the control. The longevity of *P. persimilis*

male was 5.85, 2.24, 2.33, 2.42 and 1.98 days with control, Abamectin, Chlorfenapyr, Fenpyroximate and Hexythiazox respectively.

Life Span:

Two-spotted Spider Mites (TSSM)*:*

The life span of females and males of TSSM, *T. urticae* varied greatly according to the type of tested acaricides compared with the control.

For instance, the life span of females recorded 19.61, 11.97, 11.28 and 8.94 days with Abamectin, Chlorfenapyr, Fenpyroximate and Hexythiazox compared to 21.51 days in control.

Whereas, the parallel values with the male of TSSM, *T. urticae* were 10.91, 10.57, 11.18, 9.96 and 16.36 days with Abamectin, Chlorfenapyr, Fenpyroximate, Hexythiazox and control respectively. Moreover, significant differences (P> 0.05) were observed with all applied RC of acaricides and the least Life span recorded with Hexythiazox.

Predatory Mite, *P. persimilis:*

The same trend was observed with the life span of females and males of *P. parsimilis* treated with mentioned acaricides and Hexythiazox recorded with the least Life span (13.90 and 13.52 days) in females and males, respectively.

Growth Index (GI) and Developmental Rate (DR):

Two-spotted Spider Mites (TSSM)*:*

Tested acaricides, Abamectin, Chlorfenapyr, Fenpyroximate, and Hexythiazox showed varied growth index (GI) and developmental rate (DR) in *T. urticae* females as shown in Table (5) when compared with untreated females.GI of *T. urticae* female were 5.51, 7.04, 7.40, and 7.98 when eggs were treated with Abamectin, Chlorfenapyr, Fenpyroximate and Hexythiazox compared with 10.05 in control. Whereas, DR of *T. urticae* female were 0.101, 0.085, 0.029, 0.098 and 0.080 with treatments of control, Abamectin, Chlorfenapyr, Fenpyroximate and Hexythiazox, respectively.

Predatory Mite, *P. persimilis:*

In predatory mites, *P. parsimilis* GI were 7.42, 7.38, 8.31, and 8.49 after being treated with RC of Abamectin, Chlorfenapyr, Fenpyroximate and Hexythiazox, respectively. Whereas, the DR of *P. parsimilis* female after treating eggs with the aforementioned acaricides were 0.089, 0.082, 0.100, and 0.094, respectively compared to 0.129 in control.

Table 5: Growth index (GI) and developmental rates (DR) of *T. urticae* and *P. persimilis* females after egg treatment with acaricides at 27 ±3°C.

Mite Species	Treatment	Growth index (GI)	Developmental rate (DR)
	Control	10.05	0.101
T. urticae	Abamectin	5.51	0.085
	Chlorfenapyr	7.04	0.029
	Fenpyroximate	7.40	0.098
	Hexythiazox	7.98	0.080
	Control	11.14	0.129
Phytoseiulus persimilis	Abamectin	7.42	0.089
	Chlorfenapyr	7.38	0.082
	Fenpyroximate	8.31	0.100
	Hexythiazox	8.49	0.094

Survival Percentage:

From the current result, Chlorfenapyr was the most effective acaricide on the mean survival percentage in immature stages of *T. urticae* followed by Fenpyroximate and

Hexythiazox whereas Abamectin was the least effective one. The mean survival percentage of *T. urticae* were 74.34, 47.60, 63.47 and 67.47 with Abamectin, Chlorfenapyr, Fenpyroximate and Hexythiazox, respectively. Moreover, Chlorfenapyr was the most effective acaricide on the mean survival percentage of *P. persimilis* immature stages as well as on the mean of adult emergence (Table 6).

Table 6: In vitro survival percentages of *T. urticae* and *P. persimilis* immature and adult females after egg treatment with abamectin, chlorfenapyr, fenpyroximate and hexythiazox at 27 ±3°C.

Mite Species	Treatment	In vitro survival percentages of *T. urticae* and *P. persimilis* immature and their adult females			
		Larva	Protonymp	Deutonymph	Mean of adult mergence
T. urticae	Control	100.0	100.00	100.0	100.0
	Abamectin	68.00	77.00	78.0	74.34
	Chlorfenapyr	52.80	40.00	50.0	47.6
	Fenpyroximate	55.40	64.00	71.0	63.47
	Hexythiazox	64.60	67.90	69.90	67.47
P. persimilis	Control	100.0	100.0	100.0	100.0
	Abamectin	95.0	98.0	80.0	91.0
	Chlorfenapyr	62.40	48.80	55.70	55.63
	Fenpyroximate	59.60	52.40	58.60	56.87
	Hexythiazox	57.20	55.60	64.50	59.10

Table (7): In vitro effect of *Trichoderma harzianum* ($2x10^6$ spores/ml) and *Trichoderma album* ($2x10^6$ spores/ml) and their mixture on the mortality percentages of *Tetranychus urticae* and Phytoseiulus *persimilis* in different stages at 30±3°C and 85±5% R.H.

Days after treatment	Mite species/ stage		(%) Mortality after fungi treatments			(%) Mortality in Control
			T. harzianum	*T. album*	*T. harzianum* +*T. album*	Control
3 days	*T.urticae*	Adult	58.20	47.70	64.30	3.70
		Immature	54.60	42.80	58.90	3.90
		Egg	60.80	69.60	74.90	3.20
		Mean	57.86	53.36	66.03	2.93
7 days		Adult	86.40	80.30	89.20	4.70
		Immature	71.90	62.80	74.80	4.10
		Egg	77.60	78.60	99.90	4.60
		Mean	78.63	73.90	87.96	4.46
3 days	*P. persimilis*	Adult	38.10	21.80	40.60	2.00
		Immature	42.60	40.90	43.80	3.10
		Egg	51.90	49.20	52.70	3.40
		Mean	44.20	37.30	45.70	2.83
7 days		Adult	39.40	36.80	41.20	4.60
		Immature	45.90	42.60	49.80	4.00
		Egg	57.60	48.20	52.90	4.20
		Mean	47.63	42.53	47.96	4.26

Results illustrated in (Table 7) were assessing the effect of two *Trichoderma* spp. (*Trichoderma harzianum* and *Trichoderma album*) and a mixture of them on all mite stages of TSSM, *T. urticae,* and *Phytoseiulus persimilis* under laboratory conditions as well as, on the population of *Tetranychus urticae* and *Phytoseiulus persimilis* at semi-field

conditions was found in (Table 8). Results concluded that percentages of mortality were increased in all tested mite stages as time elapsed from 3 days post-treatment to 7 days after treatment with *T.harzianum* and *T.album* and the mixture of them under laboratory conditions. Moreover, results in (Table 7) clearly exhibited that *T.harzianum* was more efficacy against eggs, immature and adult stages of *T. urticae* and *P. persimilis* than *T.album* at 3 and 7 days of treatment. As well, the mixture of the two fungi showed higher mortality after 3 and 7 days in all mite stages than that of either one alone.

Summary of *Trichoderma* species the effectiveness for tested concentration ($2x10^6$ spores/ml) under laboratory conditions (at 30±3°C and 85±5% R.H) compared to the control of distilled water against stages of two mentioned mites showed the highest effect against TSSM, *T. urticae* than *P. persimilis.* For example, mean mortality percentages (%) after 7 days post-treatment were 78.63, 73.90 and 87.96% for the application of *T.harzianum, T.album* and *T.harzianum+T. album* against TSSM and *T. urticae, respectively* compared with *P. persimilis (* 47.63, 42.53and 47.96 %).

Even though *T.harzianum* and *T.album* and a mixture of them were more pathogenicities against TSSM than *P. persimilis*, it could be concluded that *Trichoderma* species have a negative effect on the survival of predaceous mite *P. persimilis* than *T. urticae*, under optimum laboratory conditions.

Table 8: Effect of *Trichoderma harzianum* ($2x10^6$ spores/ml), *Trichoderma album* ($2x10^6$ spores/ml) and their mixture on the population of *Tetranychus urticae* and *Phytoseiulus persimilis* under greenhouse conditions.

Mite Species	Fungus species	(%) Reduction after			Mean (%)
		3days	7 days	10 days	Reduction
T. urticae	*T. harzianum*	34.10	46.80	57.30	46.06
	T. album	34.80	48.50	52.50	45.26
	Fungi mixture	48.30	63.20	69.10	60.20
P. persimilis	*T. harzianum*	25.80	34.90	49.30	36.66
	T. album	22.40	29.70	40.60	30.90
	Fungi mixture	36.40	39.40	47.30	41.03

On the other hand, under greenhouse conditions, results support mentioned in vitro results that were obtained from *harzianum* and *T.album* and a mixture of them. After 3-, 7- and 10-days post-treatment, the percentages of population densities of two mites decreased (Table 8).

Maximum population reduction percentages were obtained from a mixture of *Tichoderma* species. For instance, after 10 days post-treatment, the mean population reduction percentages were 46.06, 45.26 and 60.20% resulting from *harzianum* and *T.album* and a mixture of them respectively in *T. urticae* compared with its predacous mite (36.66, 30.90 and 41.03%, respectively).

It could be concluded that *Trichoderma* species have a negative effect on the population density of predaceous mites, *P. persimilis* than *T. urticae*, under greenhouse conditions.

Results obtained from in vitro assessment of acaricides and *Trichoderma* species under *in vitro* and *in vivo*, many authors were in harmony with those findings. (Opit *et al.*, 2005) mentioned that fewer pesticides are used in integrated pest management (IPM) and could contribute to mitigating the development of pesticide resistance. Also, the same author, 2009 illustrated that biological control of *T. urticae by* using *P. persimilis* can be recommended as a safe and reliable pest management alternative to miticides on herbaceous ornamental plants. Moreover, based on comparative efficacy and economic

data under commercial greenhouse conditions, the density-based release of *P. persimilis* is as effective as scheduled pesticide applications for the control of *T. urticae.*

Seyed-Talebi *et al*., (2012) studied the effects of the entomopathogenic fungus *Beauveria bassiana* on life table parameters of TSSM, *T. urticae* feeding on bean and cucumber under laboratory conditions and mentioned that female and male longevity, oviposition period and fecundity were significantly lower on fungus treated mites and only the mean generation time was influenced considering the effect of the host plant, which was shorter on cucumber.

As well as, the adult stage of *T. urticae* was probably the most damaging stage because of its longevity and feeding rate which also affect the build-up of the mite population resulting in less progeny and consequently less number of generations produced per season (Seyed-Talebi *et al*., 2012).

Abd El-Mageed *et al*., (2012) reported that chlorfenapyr and etoxazole were much less toxic to *P. persimilis* adult females than those of *T. urticae*. Kim and Yoo (2002) reported that the survival after treatment with etoxazole was 86% and 66 % for *P. persimilis* and *T. urticae* adult females, respectively.

Acaricides treatments reduced the longevity and the total life span of males and female *T.urticae* and this reduction increased with increasing concentration. Also, the application of acaricide at lower rates with the release of the appropriate predatory mites could lead to efficient control of *T. urticae*. Conversely, when choosing the RC of acaricides, it should affect different life stages of the target pest (Alinejad *et al*.,2015). Wang *et al.*, (2021) showed that sublethal and low-lethal concentrations of bifenazate could significantly affect the development duration and fecundity of *P. citri.* Concerning Acari pathogens, Chandler and Van der Geest (2006) mentioned that close to 60 species of fungi including *Trichoderma* species have been reported infesting at least 70 species of acarines across nearly all orders. Kovach (1996) and Lola-Luz (2003) reported that the fungus *T. harzianum* could control some plant diseases and is not harmful to mammals and other animals. It attacked the fungus *Botrytis cinerea* which causes gray mould disease in strawberry fruits and prevents it from developing. Nowadays, commercial products of fungi are available to use as biocontrol agents against various pests including plant parasitic nematodes (Ali *et al.,* 2022) to keep Root-Knot Nematode, *Meloidogyne incognita* population below the economic threshold and mites.

Recent results were in harmony with Afifi *et al.,* (2007). that mentioned the percentages of mortality of all mite stages increased with prolonging the period after spraying and the mixture of the two fungi, *T.harzianum* and *Cladosporium herbarium* gave higher mortality than a single one after 3 and 7 days for all mite stages and recommended to use a mixture of both fungi to obtain more efficient control.

Conclusion:

From the present study, it is concluded utilization of RC of acaricides against the two-spotted spider mite, *Tetranychus urticae* and predaceous mite, *P. persimilis* showed a great effect on the different biological developmental stages of female and male of *T. urticae* and *P. persimilis* under greenhouse conditions. Moreover, the two bio-agents (*T.harzianum* and *T.album* and a mixture of them) were more pathogenicity against TSSM than *P. persimilis* and have a negative effect on the population of predaceous mite, *P. persimilis* than *T. urticae* in vitro treatments and under greenhouse conditions.

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ARABIC SUMMARY

فاعلية بعض المبيدات الأكاروسية ونوعين من فطر الترايكودرما على بعض النواحى البيولوجية لكلا من حلم العنكبوت الأحمر ذو البقعتين *Tetranychus urticae* Koch والحلم المفترس *Phytoseiulus persimilis* Athias- Henriot على نباتات الخيار

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يعتبر حلم العنكبوت الأحمر ذو البقعتين *Tetranychus urticae* من أهم الافات الرئيسية والتى تصيب محاصيل الخضر، نباتات الزينة والمحاصيل الحقلية. وفى استراتيجات المكافحة البيولوجية، تستخدم الأعداء الحيوية لتصل أعداد حلم العكنبوت الأحمر تحت الحد الاقتصادى والذي قد يمتد لفترات طويلة بجانب استخدام المبيدات الأكاروسية فى الأوقات الحرجة كوسيلة سريعة لتجنب الأضرار الناتجة من هذا الحلم. وتقيم هذه الدراسة فاعلية المبيدات الأكاروسية مثل Abamectin و Chlorfenapyr و Fenpyroximate و Hexythiazox والقدرة المرضية لنوعين من فطر التريكودرما وهم *Trichoderma harzianum* و *T.album* على الاطوارالبيولوجية لكلا من إناث وذكور حلم العكبوت الأحمر ذو البقعتين *T. urticae* والحلم المفترس *Phytoseiulus persimilis* وتعدادهما تحت ظروف الصوبة.اوضحت النتائج أن استخدام التركيز الموصى به من المبيدات الأكاروسية أظهرت تأثيرات ضارة على مختلف مراحل الأطوار البيولوجيه مثل طور البيضة واليرقة و العمرالحورى الأول والثاني والأفراد البالغة لكلا من الذكور والإناث لحلم العنكبوت الأحمر *T. urticae* والحلم المفترس *P. persimilis* تحت ظروف المعمل. كما اختلفت هذه التأثيرات كثيرا على الأطوار غير البالغة ودورة الحياة وطول فترةعمرالأفراد الكامله تبعا لنوع المبيد الأكاروسى المستخدم. وفيما يتعلق بالقدرة المرضية لعاملى المكافحة البيولوجية وهما فطر *T.harzianum* وفطر *T.album* وخلطهما معا ، فكانت الفطر *Trichoderma harzianum* أكثر قدرة مرضية ضد حلم العنكبوت الأحمر ذو البقتين *T. urticae* و الحلم المفترس *P.persimilis* من تريكودرما ألبم *T.album* . أضف إلى ذلك ، أن نوعى التريكودرما أظهرا تأثيرا سلبيا على الكثافة العددية لحلم العنكبوت الأحمر ذو البقعتين *T. urticae* أكثر من الحلم المفترس *P. persimilis* معمليا وتحت ظروف الصوبة. وتعد هذه النتائج ذات قيمة حيث تزيد من فاعلية المكافحة المتكاملة للحلم العنكبوت الأحمر ذو البقعتين بإستخدام المبيدات الأكاروسية والفطريات التريكودارما على نباتات الخيار وتأثيرهما على الأكاروس المفترس *P. persimilis* المصاحب له.