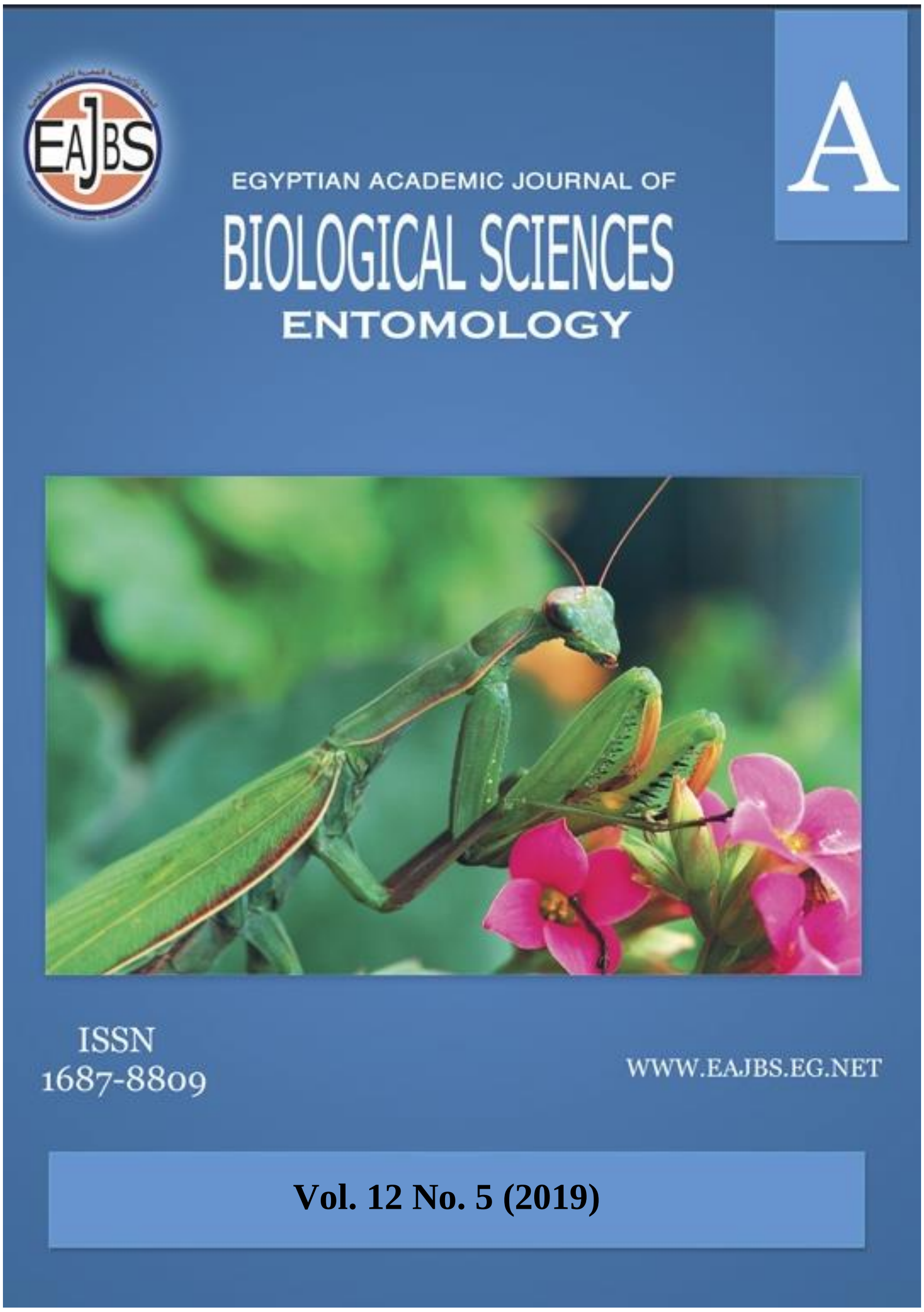

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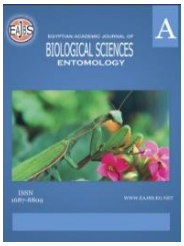

Compatibility of *Phytoseiulus persimilis* with *Isaria fumosorosea* against Two-Spotted Spider Mites (*Tetranychus urticae*) on Soybean

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ABSTRACT

In this investigation, the impact of the predatory mite, *Phytoseiulus persimilis* (PM) and entomopathogenic fungus, *Isaria fumosorosea* (F) at three levels (F1=10^4, F2=10^6 and F3=10^8 conidia/ml) alone or in grouping state as compared with Ortus (acaricide) was evaluated on eggs and motile stages of two-spotted spider mites (TSSM). Nine treatments (CK=control, F1, F2, F3, PM, F1+PM, F2+PM, F3+PM and Ortus) were performed. On the other hand, the effect of the used entomopathogenic fungus on numbers of eggs and motile stages of the predatory mite was detected only at four treatments (PM, PM+F1, PM+F2, and PM+F3). Applications of *I. fumosorosea* at all chosen doses caused significant decreases in the mean population density of TSSM eggs (50.15-58.81% in 2016 and 59.63-68.37% in 2017) and motile stages (62.61-74-66% in 2016 and 68.47-75.32%) in comparison to the control. The effect of PM addition on TSSM eggs was higher than that of *I. fumosorosea* using but on TSSM motile stages, the pattern differed. The combination of both *I. fumosorosea* and *P. persimilis* showed greater influence on TSSM eggs and motile stages than the use of *I. fumosorosea* or *P. persimilis* individually. Spraying *I. fumosorosea with releasing P. persimilis* led to small reductions in eggs (11.88-22.42% in 2016 and 7.86-21.73% in 2017), and motile stages (9.84-21.33% in 2016 and 6.75-17%) of *P. persimilis*. Results of this study concluded that the combination of entomopathogenic fungus, *I. fumosorosea* and predatory mite, *P. persimilis* was strongly succeeded in the biological control process of TSSM on soybean plants under field conditions.

INTRODUCTION

Spider mites are serious pests for many crops around the world by causing noticeable economic losses in their yields. The two-spotted spider mite (TSSM), *Tetranychus urticae* Koch is one of the most cosmopolitan and polyphagous mites and can attack and feed on around 1110 plant species that related to more than 150 plant families, including economical and vital crops under field and greenhouse conditions (Seiedy *et al.*, 2012; Dogan *et al.*, 2017). TSSM can be responsible for chlorosis, leaf and fruit deformation and stunted plant growth with high reductions in marketable values and yields (Bugeme *et al.*, 2015). Chemical acaricides are still widely used to control TSSM but many of them are not always effective due to the rapid development and resistance, short generation time and high fecundity of TSSM (Amjad *et al.*, 2012). Besides the poor management with acaricides, there is also a great concern about their residual effect and toxicity for humans and animals (Gatarayiha *et al.,* 2010). The extensive use of acaricides also causes high reductions in the natural enemies (predators) of TSSM and this leads to noticeable increases in its population (Hassan *et al.,* 2017). So it is necessary to search for alternatives to conventional acaricides through using bio-control agents. Application of bio-pesticides is characterized by low toxicity, short environmental persistence, undesirable side effects and safety to beneficial and non-target organisms. Moreover, it is highly recommended as a sustainable way to diminish the use of acaricides to control TSSM (Maniania *et al.,* 2016; Dogan *et al.*, 2017; Zhang *et al.* 2018). Predatory mites of family Phytoseiidae such as *Neoseiulus californicus, Typhlodromips* (*Amblyseius*) *swirski* and *Phytoseiulus persimilis* are effective species with marked impacts on TSSM. They also are more selective to TSSM and safer to the environment compared to chemical pesticides and this makes them more compatible and important elements with other control strategies in the integrated management program of spider mites (Muştu *et al.*, 2016; Wu *et al.,* 2018). Alongside the use of predatory mites, farmers have recently adopted using commercial microbial products such as entomopathogenic fungi to control populations of TSSM.

Entomopathogenic fungi (EPF) are vital organisms for governing of spider mites under laboratory and field conditions (Geroh *et al.*, 2015). Many investigators around the world evaluated the influence of different isolates of EPF (*Lecanicillium* spp., *Isaria* spp., *Metarhizium* spp., *Beauveria* spp.) in controlling of TSSM (Draganova and Simova, 2010; Ullah and Lim, 2015; El-Kawas *et al.*, 2017). In recent years, these EPF have gained noticeable importance for the management of TSSM because they are relatively easy and inexpensive to mass-produce. Moreover, they do not have hazard impacts on the environment and human health. The impact of EPF as biological control agents on TSSM does not depend only on their high efficacy against harmful pests, but also on their influence on beneficial arthropods such as predators and parasitoids (Seiedy and Moezipour 2017). Also, the efficacy of EPF in controlling TSSM depends on the strain, dose, formulation, environmental factors and compatibility with pesticides (Ullah and Lim, 2015; El-Kawas *et al.*, 2017).

Combined application of predatory mites and EPF has become an increasingly popular option in suppressing the population of TSSM and also has considered as a good strategy and an alternative method instead of using pesticides (traditional management) to minimize the economic loss in the quality and yield of crops. There are several predatory mites, such as *Phytoseiulus persimilis* and *Neoseiulus californicus* (Acari: Phytoseiidae) and EPF such as *Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillium lecanii* have been used and show marked successes to control TSSM and other spider mites (Maniania et al., 2008 and 2016). Thus, in the present study, the impact of the entomopathogenic fungus (*Isaria fumosorosea*) alone or in combination with *Phytoseiulus persimilis* will be evaluated against *Tetranychus urticae* (Acari: Tetranychidae) on soybean plants, which are grown under field conditions.

MATERIALS AND METHODS

Predatory Mite:

Phytoseiulus persimilis was chosen as a predatory mite in the current study and it was collected from a citrus farm at the Faculty of Agriculture, Benha University, Egypt. The stocks of *P. persimilis* were maintained (reared) on different stages of *T. urticae* on discs of bean leaves, which were placed in plastic Petri dishes (90 mm diameter and 20 mm depth) at 25 °C±1, 70% ±5 RH, and photoperiod of a 16L: 8D h. The discs of bean leaves were changed regularly as necessary. The edges of Petri dishes were covered by a band of tissue paper (1 cm wide) to prevent the mites from escaping.

Acaricide:

A synthetic acaricide, ORTUS SUPER 5% EC (Fenpyroximate) was obtained from Shoura Chemicals Company, Cairo, Egypt.

Preparation of Conidia:

A stock culture of the fungus, *Isaria fumosorosea* was obtained from Plant Pathology Research Institute, Agricultural Research Centre, Egypt. Sabouraud dextrose agar media (SDA), which was consisted of peptone: 10 g/L; Dextrose: 40 g/L and Agar: 15 g/L with yeast extract 20 g/L was used to promote the used fungus, *I. fumosorosea*. The media was autoclaved at 121 °C for 15 min, cool to 50 °C and pour into Petri dishes for the cultivation process of *I. fumosorosea*. The plates were horizontally shaken on the surface to spread the suspension and left until solidification of agar. After that, plates were incubated in darkness at 25 ± 2°C and 60-70 % RH for 12 days to get the conidia. At the end of the incubation period, the plates were examined to check the fungal growth. Each plate was replicated four times. Conidial germination of over 85% was accepted. Conidia were harvested by adding 10 ml sterile distilled water containing 0.02% Tween 80 by scrubbing the culture surface with a glass bar. The conidia suspension was filtered through moist filter paper (Whatmann No.1) and shaken for 15 min to produce a homogenous suspension. The spores of *I. fumosorosea* were counted using a haemocytometer.

Field Experiments:

Field experiments were conducted in the summer seasons of 2016 (from May 15, 2016 to September 13, 2016) and 2017 (from May 25, 2017 to September 24, 2017) at the Agronomy Farm, Faculty of Agriculture, Benha University, Egypt. The average of high temperature was 33.5, 34.5, 35.1, 36.3 and 33.6 °C, while the average of low temperature was 17.8, 21.5, 22.4, 22.8 and 20.2 °C during 4 experimental periods in May, June, July, August, and September, respectively. The experimental work was arranged in a randomized complete block design with four replicates. Nine treatments with three replicates were performed as fellow: CK= control, F1= 10^4, F2=10^6, F3=10^8, PM=predatory mite, F1+PM, F2+PM, F3+PM and Ortus=acaricide to study their effects on the population of TSSM eggs and motile stages. On the other hand, four treatments (PM=predatory mite, F1+PM, F2+PM, F3+PM) were chosen to determine the influence of the entomopathogenic fungus on the density of PM eggs and motile stages. The size of each plot was 10.5 m^2 (3 m length x 3.5 m width) and the distance between the plots was 1m. Soybean (*Glycine max* L.) variety Giza 21 was used as an indicator plant in this experiment. The soil of the field had a clay-loam texture, pH=7.65, electrical conductivity (EC) =1.98 dS/m, organic matter=2.15%. Seeds of soybean were kindly supplied by Department of Leguminous Crops, Sakha Agricultural Research Station, Kafr El-Sheikh Governorate, Egypt. Fifteen days before sowing the seeds, the soil was fertilized with superphosphate at a rate of 150 kg/fed. The seeds were treated with bacterial inoculum (Okadeen at a rate of 400 g/fed) one hour before the sowing process to enhance the production of root nodules. After the sowing process, the soil received 15 kg N in the form of urea (32.3 kg) as an activation dose. The thinning process was conducted two weeks after sowing the soybean seeds. Potassium sulfate at a dose of 50 kg/fed was applied two times before the second and third irrigations as recommended by the Ministry of Agricultural and Land Reclamation, Egypt.

Application of Acaricide, Entomopathogenic Fungus, and Predatory Mite:

The acaricide (ORTUS SUPER 5% EC) was added at the recommended rate (300 ml/fed = 0.5 ml/1000 ml water). Three doses of *Isaria fumosorosea* (1×10^4, 1×10^6 and 1×10^8 conidia/ml) were added directly after the thinning process of soybean plants (average 600 ml/fed) as mentioned in Erler et al. (2013). The distilled water was used in the control treatment. Predatory mite (*Phytoseiulus persimilis*) was released at a rate of 4 individuals / m^2 after one week from the application of fungus to avoid the repellence effect of the fungus.

Numbers of *T. urticae* eggs and mobile stages per leaf were recorded 25, 50, 75 and 100 days after the thinning process using hand lens from both upper and lower surface of leaves. It was easy to distinguish live mites from dead individuals by observing their movement. The live mites were mobile, whereas immobile mites (failing to respond with leg movements after being tightly nudged with a fine bird feather pick) were considered dead. Care was taken to avoid spray drifting to neighbouring plots by holding polythene sheets.

Statistical analysis:

The statistical significance of data was assessed by one factorial analysis of variance (ANOVA). Means were then compared using Duncan's multiple range test (P≤ 0.05).

RESULTS AND DISCUSSION

Effect of Different Treatments on Eggs and Motile Stages of TSSM:

Application of entomopathogenic fungus (F= *I. fumosorosea*) and predatory mite (PM= *P. persimilis*) individually or in a combination status caused marked reductions in eggs of TSSM (Tables 1 and 2) and motile stages of TSSM (Tables 3 and 4) in 2016 and 2017 at all investigated periods [25, 50, 75 and 100 days after thinning (DAT) of soybean plants]. Numbers of TSSM eggs and motile stages in CK treatment increased with the increase of the growth periods from 25 DAT to 75 DAT and then decreased only at 100 DAT but at other treatments, these numbers decreased when the growth period of soybean changed from 25 DAT to 50, 75 and 100 DAT. The effect of *P. persimilis* in controlling of TSSM (eggs and motile stages) was generally higher than that of *I. fumosorosea*. The combination of *P. persimilis* with *I. fumosorosea* led to greater reductions in eggs and motile stages of TSSM than their using in an individual case. The highest numbers of TSSM eggs were recorded in CK treatment (8.87, 10.31, 15.39 and 7.62 in 2016, and 7.21, 8.36, 12.05 and 6.27 in 2017), whereas the lowest values were found in PM+ F3 treatment (2.51, 2.13, 1.11 and 0.64 in 2016, and 1.83, 1.42, 0.46 and 0.25 in 2017) and in Ortus treatment (2.16, 1.65, 0.89 and 0.48 in 2016, and 1.35, 1.27, 0.18 and 0.16 in 2017) at 25, 50, 75 and 100 DAT, respectively. Mean numbers of TSSM eggs in 2016 declined from 10.55 in CK to 4.35, 3.08, 1.60 and 1.30 with reduction percentages of 58.81%, 70.82%, 84.85% and 87.72% due to F3, PM, PM+ F3 and Ortus additions, respectively. In 2017, using F3, PM, PM+ F3 and Ortus treatments diminished mean values of TSSM eggs from 8.47 in CK to 2.68, 2.42, 0.99 and 0.74 with 68.37%, 71.50%, 88.32% and 91.27% decreases, respectively.

Table 1. Numbers of TSSM eggs on soybean leaves as affected by *I. fumosorosea* (F) at different levels and *P. persimilis* (PM) alone or in combination case

Treatments	2016				2017			
	25 DAT	50 DAT	75 DAT	100 DAT	25 DAT	50 DAT	75 DAT	100 DAT
CK	8.87 a	10.31 a	15.39 a	7.62 a	7.21 a	8.36 a	12.05 a	6.27 a
F1	6.24 b	6.00 b	4.88 b	3.91 b	4.58 b	4.23 b	2.63 b	2.24 b
F2	5.83 c	5.49 c	3.40 c	3.16 c	4.19 c	3.76 c	2.14 c	1.76 c
F3	5.75 c	5.42 d	3.17 c	3.04 c	3.84 c	3.23 d	1.96 c	1.69 c
PM	4.59 d	4.17 e	2.20 d	1.35 d	3.40 d	3.01 d	1.89 c	1.40 d
PM+F1	3.27 e	2.98 f	2.01 d	1.08 e	2.31 e	1.98 e	0.75 d	0.43 e
PM+F2	2.94 e	2.40 g	1.38 e	0.93 e	2.24 e	1.68 f	0.53 e	0.37 e
PM+F3	2.51 f	2.13 g	1.11 f	0.64 f	1.83 f	1.42 g	0.46 e	0.25 f
Ortus	2.16 g	1.65 h	0.89 f	0.48 f	1.35 g	1.27 g	0.18 f	0.16 f

CK= Control, F= *I. fumosorosea*, F1=10^4 conidia/ml, F2=10^6 conidia/ml, F3=10^8 conidia/ml, PM= *P. persimilis*, and DAT= days after thinning. Different letters within the same column are significantly different.

Table 2. Mean numbers and decrease percentages of TSSM eggs on soybean leaves treated with different doses of *I. fumosorosea* (F) and *P. persimilis* (PM) individually or grouping together

Treatments	2016		2017	
	Mean numbers	Mean reductions (%)	Mean numbers	Mean reductions (%)
CK	10.55	0.00	8.47	0.00
F1	5.26	50.15	3.42	59.63
F2	4.47	57.62	2.96	65.03
F3	4.35	58.81	2.68	68.37
PM	3.08	70.82	2.42	71.50
PM+F1	2.34	77.86	1.37	83.86
PM+F2	1.91	81.87	1.21	85.78
PM+F3	1.60	84.85	0.99	88.32
Ortus	1.30	87.72	0.74	91.27

CK= Control, F= *I. fumosorosea*, F1=10^4 conidia/ml, F2=10^6 conidia/ml, F3=10^8 conidia/ml, PM= *P. persimilis*.

At control treatment, numbers of motile stages of TSSM at 25, 50, 75 and 100 DAT were 15.46, 20.31, 28.25 and 10.71 in 2016 and were 13.75, 18.26, 25.1 and 9.94 in 2017 and reached their lowest values after the addition of PM+ F3 treatment (2.76, 1.91, 1.22 and 0.89 in 2016, and 2.39, 1.64, 0.83 and 0.59 in 2017) and ortus treatment (2.44, 1.76, 1.05 and 0.75 in 2016, and 1.76, 1.51, 0.35 and 0.29 in 2017), respectively. Mean numbers of TSSM motile stages changed from 18.68 and 16.76 at CK treatment to 4.74 and 4.14, to 4.39 and 3.89, to 1.70 and 1.35, and to 1.50 and 0.98 in F3, PM, PM+ F3 and Ortus treatments with decrease percentages of 74.66-75.32%, 76.50-76.82%, 90.93-91.95% and 91.87- 94.17% in 2016 and 2017, respectively.

Table 3. Numbers of TSSM motile stages on soybean leaves as affected by *I. fumosorosea* (F) at different levels and *P. persimilis* (PM) alone or in combination case

Treatments	2016				2017			
	25 DAT	50 DAT	75 DAT	100 DAT	25 DAT	50 DAT	75 DAT	100 DAT
CK	15.46 a	20.31 a	28.25 a	10.71a	13.75 a	18.26 a	25.1 a	9.94 a
F1	9.94 b	8.05 b	5.76 b	4.19 b	7.39 b	5.63 b	4.45 b	3.67 b
F2	7.78 c	6.16 c	4.91 c	3.27 c	6.24 c	4.79 c	3.68 c	2.93 c
F3	6.75 d	5.28 d	3.80 d	3.11 c	6.01 c	4.61 c	3.28 d	2.65 d
PM	6.24 d	4.75 d	3.55 d	3.02 c	5.77 c	4.11 d	3.15 d	2.51 d
PM+F1	5.12 e	3.39 e	2.18 e	1.74 d	4.21 d	2.61 e	1.93 e	1.45 e
PM+F2	3.87 f	2.26 f	1.53 f	1.39 d	3.16 e	2.02 f	1.89 e	0.78 f
PM+F3	2.76 g	1.91 e	1.22 e	0.89 e	2.39 f	1.64 g	0.83 f	0.59 f
Ortus	2.44 g	1.76 e	1.05 e	0.75 e	1.76 g	1.51 g	0.35 g	0.29 g

CK= Control, F= *I. fumosorosea*, F1=10^4 conidia/ml, F2=10^6 conidia/ml, F3=10^8 conidia/ml, PM= *P. persimilis*, and DAT= days after thinning. Different letters within the same column are significantly different.

Table 4. Mean numbers and decrease percentages of TSSM motile stages on soybean leaves treated with different doses of *I. fumosorosea* (F) and *P. persimilis* (PM) individually or grouping together

Treatments	2016		2017	
	Mean numbers	Mean reductions (%)	Mean numbers	Mean reductions (%)
CK	18.68	0.00	16.76	0.00
F1	6.99	62.61	5.29	68.47
F2	5.53	70.40	4.41	73.69
F3	4.74	74.66	4.14	75.32
PM	4.39	76.50	3.89	76.82
PM+F1	3.11	83.37	2.55	84.79
PM+F2	2.26	87.92	1.96	88.29
PM+F3	1.70	90.93	1.35	91.87
Ortus	1.50	91.97	0.98	94.17

CK= Control, F= *I. fumosorosea*, F1=10^4 conidia/ml, F2=10^6 conidia/ml, F3=10^8 conidia/ml, PM= *P. persimilis*.

The virulence of entomopathogenic fungi strains could result from their ability to produce some extracellular enzymes such as protease that could penetrate the cuticle of arthropods to control them. Different isolates of *M. anisopliae* and *B. bassiana* were tested by Wekesa *et al.* (2005) to determine their pathogenicity for tobacco spider mite, *T. evansi*. Their results showed that all chosen isolates were pathogenic to adult females of *T. evansi* with mortality percentages ranged from 22.1 to 82.6% under laboratory conditions. Moreover, when tomato plants artificially infected with *T. evansi*, both *B. bassiana* (GPK) and *M. anisopliae* (ICIPE78) declined its population mass in comparison to the control treatment under greenhouse settings and this highlighted the important role of the used pathogenic fungi in the management *T. evansi*. Shi *et al.*, (2008) studied the potential of two isolates of *I. fumosorosea* as biocontrol agents against adult female of carmine spider mite (*T. cinnabarinus)* and found that the isolates of *I. fumosorosea* had virulence effect on this mite and the highest mortality percentage was 77.7% due to application of the high conidial concentration. Amjad *et al.* (2012) evaluated the influence of some entomopathogenic fungi (*Verticillium lecanii Metarhizium anisopliae* and *Paecilomycis fumosoroseus*) at three doses ($1x10^6$, $1x10^7$ and $1x\ 10^8$ conidia ml^{-1}) as biological control agents against eggs and adult females of *T. urticae* on cotton. They found that all chosen fungi were pathogenic to eggs and adult females of *T. urticae* and the mortality of *T. urticae* female enlarged with the rise of the conidial levels. Erler *et al.* (2013) found in a greenhouse experiment that *B. bassiana* caused 81.7 and 78.1%, while *M. anisopliae* led to 69.8 and 66.7% mortalities in eggs of *T. cinnabarinus* in 2010 and 2011, respectively. For motile forms of *T. cinnabarinus, M. anisopliae* and *B. bassiana* were responsible for 80.6-82.1% and 74.3-71.7% deaths in years of 2010 and 2011, respectively. So, both fungi had significant capability in the infection of eggs and motile forms of *T. cinnabarinus*, and their effects on the mortality depended basically on the sprayed conidial doses. It was shown by Tehri *et al.* (2015) that addition of *B. bassiana* at concentrations of 0.3×10^9, 0.3×10^8 and 0.3×10^7 conidia ml^{-1} caused mortalities in *T. urticae* on okra plants by 59.64, 48.23 and 37.92% in 2010 and by 65.18, 55.44 and 43.07% in 2011 as compared with the control treatment. Ullah and Kim (2015) evaluated the effect of mycopathogen (*Beauveria bassiana*) as a plant defense approach at four doses of conidia ($1x10^5$, $1x10^6$, $1x10^7$, or $1x10^8$ conidia/ml) for adult and nymphal of *T. urticae* Koch on the bean. Their results indicated that the populations of *T. urticae* adult and nymphal per plant were condensed to zero after 20 days of *B. bassiana* spraying and $1x10^8$ spores/ml was the most operative dosage could be suggested to device *T. urticae*. The combination of *M. anisopliae* and the predatory mite *Phytoseiulus longipes* led to notable reduction in *T. evansi* numbers in the greenhouse and field, but application of *M. anisopliae* alone had no significant effect in controlling of this mite on tomato (Maniania *et al.*, 2016). In a laboratory experiment, Zhang *et al.* (2016) showed that using *I. cateniannulata* (strain 08XS-1) in a suspension form at a dose of 2×10^7 conidia ml^{-1} led to largest mortalities by 100, 100 and 70% respectively for females, eggs and larvae of *T. urticae* at 25 °C and 100% relative humidity, while application of 2×10^8 conidia ml^{-1} of *I. cateniannulata* caused marked reductions and with high efficacy against the populations of *T. urticae* in bean, eggplant and cucumber fields 83, 83.8 and 88.6%, respectively 10 days after the treatment. Seiedy and Moezipour (2017) found that the fit eggs of *T. urticae* were suave, colourless and shining after putting on the bean leaves but their surfaces became distorted after one week's revelation to high conidial levels of the entomopathogenic fungus, *B. bassiana*.

Effect of Isaria fumosorosea on Eggs and Motile Stages of the predatory mite:

Eggs and motile stages of PM in the chosen treatments increased when the growth periods of soybean changed from 25 DAT to 75 DAT and then decreased at 100 DAT in years of 2016 and 2017 (Tables 5 and 6). Combining *I. fumosorosea* with *P. persimilis* caused small reductions in both eggs and motile stages of PM. The highest decreases in eggs and motile stages of *P. persimilis* were recorded when *I. fumosorosea* at a rate of 10^8 (F3) was mixed with PM. The values of PM eggs and motile stages were higher in 2017 than those in 2016. The highest numbers of PM eggs (3.84 and 4.59) and motile stages (5.11 and 5.95) were found at 75 DAT in PM treatment, whereas the lowest numbers of eggs (1.26 and 1.82) and motile stages (1.75 and 2.38) were shown at 25 DAT in PM+F3 treatment in 2016 and 2017, respectively.

Table 5. Effect of different doses of *I. fumosorosea* (F) on PM eggs

Treatments	2016				2017			
	25 DAT	50 DAT	75 DAT	100 DAT	25 DAT	50 DAT	75 DAT	100 DAT
PM	1.56 a	2.97 a	3.84 a	1.98 a	2.11 a	3.43 a	4.59 a	2.85 a
PM+F1	1.43 b	2.57 b	3.25 b	1.87 b	2.03 b	3.19 b	4.05 b	2.69 b
PM+F2	1.37 b	2.41 c	3.08 c	1.75 c	1.86 c	2.68 c	3.52 c	2.54 c
PM+F3	1.26 c	2.29 d	2.85 d	1.63 d	1.82 c	2.55 c	3.31 c	2.48 c

PM= *P. persimilis*, F= *I. fumosorosea*, F1=10^4 conidia/ml, F2=10^6 conidia/ml, F3=10^8 conidia/ml, and DAT= days after thinning. Different letters within the same column are significantly different.

Table 6. Effect of different doses of *I. fumosorosea* on PM motile stages

Treatments	2016				2017			
	25 DAT	50 DAT	75 DAT	100 DAT	25 DAT	50 DAT	75 DAT	100 DAT
PM	2.45 a	3.87 a	5.11 a	2.59 a	2.86 a	4.32 a	5.95 a	3.16 a
PM+F1	2.38 a	3.51 b	4.47 b	2.28 b	2.69 b	4.11 a	5.34 b	3.05 a
PM+F2	2.19 b	3.36 c	4.18 c	2.09 c	2.46 c	3.82 b	4.93 c	2.72 b
PM+F3	1.75 c	3.20 d	4.09 c	1.98 c	2.38 c	3.66 b	4.87 c	2.61 b

PM= *P. persimilis*, F= *I. fumosorosea*, F1=10^4 conidia/ml, F2=10^6 conidia/ml, F3=10^8 conidia/ml, and DAT= days after thinning. Different letters within the same column are significantly different.

Data in Tables 7 and 8 showed that application of PM without *I. fumosorosea* was responsible for the largest mean populations for its eggs (2.59 and 3.25) and motile stages (3.51and 4.07) but the use of PM+F3 treatment led to the lowest mean numbers of PM eggs (2.01 and 2.54) and motile stages (2.76 and 3.38) in 2016 and 2017, respectively. Decreases by 11.88-7.86%, 16.81-18.34%, and 22.42-21.73% were noticed in eggs of PM and by 9.84-6.75%, 15.69-14.49% and 21.33-17.00% in motile stages of PM due to the double application of PM with *I. fumosorosea* at rates of 10^4, 10^6 and 10^8 (PM+F1, PM+F2 and PM+F3) in 2016 and 2017, respectively.

Table 7. Effect of different doses of *I. fumosorosea* on mean populations and reduction percentages of PM eggs

Treatments	2016		2017	
	Mean numbers	Mean reductions (%)	Mean numbers	Mean reductions (%)
PM	2.59	0.00	3.25	0.00
PM+F1	2.28	11.88	2.99	7.86
PM+F2	2.15	16.81	2.65	18.34
PM+F3	2.01	22.42	2.54	21.73

PM= *P. persimilis*, F= *I. fumosorosea*, F1=10^4 conidia/ml, F2=10^6 conidia/ml, F3=10^8 conidia/ml.

Table 8. Effect of different doses of *I. fumosorosea* on mean populations and reduction percentages of PM motile stages

Treatments	2016		2017	
	Mean numbers	Mean reductions (%)	Mean numbers	Mean reductions (%)
PM	3.51	0.00	4.07	0.00
PM+F1	3.16	9.84	3.79	6.75
PM+F2	2.96	15.69	3.48	14.49
PM+F3	2.76	21.33	3.38	17.00

PM= *P. persimilis*, F= *I. fumosorosea*, F1=10^4 conidia/ml, F2=10^6 conidia/ml, F3=10^8 conidia/ml.

From the above-mentioned results, it could indicate that the application of *I. fumosorosea* had not high harmful effects on the population of *P. persimilis* (eggs and motile stages). Low and medium mortalities by 16.48% and 30.95% in numbers of predatory mites (*N. californicus* and *P. persimilis*), respectively were recorded 12 days after the use of *I. fumosorosea* at a concentration of 1×10^7 (Numa *et al.*, 2011). Predatory mites could remove fungal spores on the cuticles of its body by grooming behavior (using legs to clean the body); this behavior might be a defence mechanism to protect their survival (Okuno *et al.* 2012). Seiedy *et al.*, (2015) demonstrated that phytoseiids *P. longipes* Evans and *A. swirskii* (Athias-Henriot) removed most conidia attached to the body by self-grooming. Muştu et al. (2016) found that *I. farinosa* had negative effects (100% mortality) on *N. californicus* (Acari: Phytoseiidae). So, it is important to find entomopathogenic fungi with low mortality influences on populations of predatory mites to apply them in the biological control program of spider mites. On the other hand, Ullah and Lim (2017) found that *B. bassiana* and *P. persimilis* had marked effects in decreasing the populations of *T. urticae* on bean plants and these findings indicated that both *B. bassiana* and *P. persimilis* could be combined as a good strategy to govern the spider mites. It was recorded by Wu et al. (2016 and 2018) that *B. bassiana* had a virulent impact on *T. urticae* with very low pathogenic influence on the predatory mite (*P. persimilis*). Furthermore, they reported that the predatory mites had high efficiency in eliminating supreme of the fungal pathogen conidia by the behavior of self-grooming but this performance did not notice in *T. urticae*. Also, their ultra-structural experiments showed that *B. bassiana* conidia adhered to the cuticle of predatory mites but were ineffective because the conidia were either shed or shrivelled. The contagions by *I. cateniannulata* were pervasive and sweeping above the full body of *T. urticae* but in the case of *E. nicholsi*, the front legs were only concealed with the conidia. The spores of *I. cateniannulata* were firstly involved to the host cuticle, germinated and produced the impurity for *T. urticae* structure. After that, the hyphae of *I. cateniannulata* entered the cuticle and multiplied in the body of *T. urticae* (Zhang *et al.*, 2016 and 2018). Moreover, they confirmed that *I. cateniannulata* had no harmful impacts on the strength and fecundity of the predatory mite *E. nicholsi*.

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ARABIC SUMMARY

توافق *Phytoseiulus persimilis* مع *Isaria fumosorosea* ضد العنكبوت الأحمر ذو البقعتين (*Tetranychus urticae*)على فول الصويا

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في هذة الدراسة تم تقييم تأثير الأكاروس المفترس (*Phytoseiulus persimilis* = PM) والفطرالممرض (*Isaria fumosorosea*= F) حيث استخدم بثلاثة مستويات ($F1=10^4$ ، $F2= 10^6$ ، $F3=10^8$ جرثومة/ملل) بصورة منفردة أو في حالة متجمعة مقارنةً مع (Ortus = acaricide) على البيض والأطوار المتحركة من العنكبوت الأحمر ذو البقعتين TSSM على فول الصويا المزروع في تربة الطينية الطينية تحت ظروف الحقل. تم تصميم تسعة معاملات وهي (CK = الكنترول، F1، F2، F3، PM، F1 + PM، F2 + PM، F3 + PM و Ortus). تم تسجيل اعداد البيض والاطوار المتحركة ل TSSM. أيضا ، تم الكشف عن تأثير الفطر الممرض المستخدم على أعداد بيض PM وأطوارو المتحركة تحت أربعة معاملات (PM، PM + F1، PM + F2 ، PM + F3) في نفس الفترات المذكورة أعلاه. وقد تسببت اضافة الفطر الممرض بجميع جرعاة التي تم اختيارها إلي إنخفاض كبير في متوسط كثافة بيض TSSM بمعدل (50.15 58.81٪ في 2016 و 59.63-68.37٪ في 2017) وفي أطوارة المتحركة بمعدل (62.61-66.74٪ في 2016 و 68.47- 75.32 ٪) بالمقارنة مع الكنترول. واظهرت النتائج أن تأثير إضافة PM على بيض TSSM كان أعلى من تأثير *I. fumosorosea* ولكن في في الأطوار المتحركة لل TSSM اختلف النمط. وأظهرت إضافة كل من *I. fumosorosea* و *P. persimilis* في صورة مجتمعة تأثيرًا أكبر على بيض TSSM واطوارة المتحركة من استخدامهم بشكل فردي. أدى رش *I. fumosorosea* مع إطلاق *P. persimilis* إلى انخفاض طفيف في البيض الخاص بة (11.88-22.42٪ في 2016 و 7.86-21.73٪ في 2017) وكذلك في أطوارة المتحركة (9.84-21.33٪ في 2016 و 6.75-17٪ في 2017). وقد أوضحت النتائج ان خلط *P. persimilis* مع *I. fumosorosea* بمعدل 10^8 جرثومة لكل ملل عادة ما يكون له نفس تأثير Ortus (المبيد الأكاروسي) على الأطوار المتحركة من TSSM ولكن على البيض الخاص بة كان التأثير مختلف. وعلية يمكن أن تستنتج من النتائج المتحصل عليها من هذة الدراسة أن مزج الفطرالممرض *I. fumosorosea* مع الأكاروس المفترس *P. persimilis* قد نجح بقوة في عملية المكافحة البيولوجية لـ TSSM على نباتات فول الصويا تحت ظروف الحقل.