

Research Article

Compatibility and efficacy of *Beauveria bassiana* with selected insecticides and fungicides for managing *Thrips tabaci* (Thysanoptera: Thripidae)

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Abstract: Onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), is a major pest in Iran, causing significant agricultural losses. Biological control methods are crucial for reducing reliance on chemical insecticides and fungicides, minimizing environmental pollution, and mitigating insect resistance. This study evaluated the toxicity of the insecticides flonicamid and thiodiazinon, the pathogenicity of *Beauveria bassiana*, the compatibility of the fungicides iprodione and carbendazim, and famoxadone and cymoxanil with *B. bassiana*, and the efficacy of insecticide-fungus combinations against second-instar onion thrips. A completely randomized design with four replications was conducted under laboratory conditions. Results showed that LC₅₀ values for thiodiazinon, flonicamid, and *B. bassiana* were 4.3 mg L⁻¹, 2.3 mg L⁻¹, and 5.4 × 10⁷ conidia mL⁻¹, respectively. Thrips mortality increased with higher concentrations and longer exposure to insecticides and *B. bassiana*. Flonicamid did not affect the germination or mycelial growth of *B. bassiana*, making it a compatible insecticide. However, iprodione + carbendazim and famoxadone + cymoxanil severely inhibited the biological activity of *B. bassiana*, making them unsuitable for integrated pest management (IPM). The combination of *B. bassiana* (6 × 10⁷ conidia mL⁻¹) and flonicamid (1.2 mg L⁻¹) was the most effective treatment against onion thrips. This combination could serve as an alternative to chemical insecticides in IPM programs, pending field validation.

Keywords: entomopathogenic fungus, fungicide, combined application, pesticide, onion thrips

Introduction

Onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), is a significant polyphagous pest that poses a considerable threat to agricultural production worldwide. (Kachot *et al.*, 2021). It is a severe pest of Alliaceae plants, such as onions and leeks

(McKenzie *et al.*, 2000), as well as Brassicaceae plants like cabbage (Shelton *et al.*, 1998). This pest has a global distribution due to its adaptation to various environmental conditions (Loredo Varela and Fail 2022).

Its larvae and adult insects cause great damage to plants by feeding on green leaves and reducing the photosynthetic rate worldwide.

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Additionally, the most significant damage caused by this pest is a reduction in yield and tuber size (Kansagara *et al.*, 2018; Kachot *et al.*, 2021). This pest reduces the marketability of onions and serves as a carrier of plant viruses, such as yellow spot viruses (Gulzar *et al.*, 2021).

Chemical insecticides are a common method for producing agricultural products due to their immediate effects on controlling pests, especially onion thrips (Iglesias *et al.*, 2021). However, these insecticides have become a serious threat due to the concerning increase in insect resistance, environmental pollution, and adverse effects on nontarget organisms (Dannon *et al.*, 2020). In the face of this challenge, using integrated management programs and non-chemical strategies to protect plants against pests, notably thrips, is an attractive and environmentally friendly option. Applying natural enemies of insects, especially entomopathogenic agents (e.g., fungi), is a useful alternative strategy (Dannon *et al.*, 2020; Gulzar *et al.*, 2021; Iglesias *et al.*, 2021).

B. bassiana fungus was very effective against onion thrips, causing 90% thrips mortality on onion plants (Gulzar *et al.*, 2021). By investigating the effect of two entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, on onion thrips populations in different varieties of onion plants, the researchers concluded that *B. bassiana* fungus can significantly reduce thrips populations and provide better long-term management against onion thrips on onion plants. They also stated that the entomopathogenic fungus *B. bassiana* had a highly toxic effect on the production of onion thrips progeny in field conditions compared to the entomopathogenic fungus *M. anisopliae* (Ain *et al.*, 2021). The application of the entomopathogenic fungus *B. bassiana* significantly controlled onion thrips in laboratory conditions (Gulzar *et al.*, 2021). It has been determined that the infection of western flower thrips by entomopathogenic fungus *B. bassiana* strain BbYT12 using a concentration of 1×10^8 conidia/mL leads to 81.48% mortality of adult insects in 6 days after its application (Zhang *et al.*, 2021). Bioassays of several *B.*

bassiana strains in laboratory conditions revealed that two strains, JEF-350 and JEF-341, significantly impacted the control of the melon thrips population (Li *et al.*, 2021).

The compatible combination of chemical insecticides and entomopathogenic fungi may act synergistically to enhance pest control efficiency, allowing for the use of lower concentrations of chemical insecticides and the preservation of natural enemies. This issue can reduce resistance in insects and environmental pollution (Sain *et al.*, 2019; Kachot *et al.*, 2021). Therefore, it is possible to develop pest management programs that identify fungal genotypes compatible with specific insecticides (Kumar and Gupta, 2019; Sain *et al.*, 2019).

Flonicamid is a new systemic insecticide in the pyridine carboxamide group. Its mechanism is starvation caused by inhibiting stylet penetration into plant tissues (Arthur *et al.*, 2022). Thiocyclam insecticide is a gastrointestinal contact insecticide with penetrative properties from the nereistoxin group (Ghosal, 2018). In research, it was found that the application of thiocyclam and acephate insecticides significantly controlled onion thrips in onion crops (Aguilar Carpio *et al.*, 2017). In a study on the use of flonicamid, spinetoram, and tolfenpyrad insecticides to control western flower thrips, it was found that flonicamid was the most effective insecticide in reducing the thrips population (Bilbo *et al.*, 2020). The application of flonicamid and spinosad insecticides in the form of foliar spraying on mung bean plants, which host various types of thrips, including onion thrips, demonstrated satisfactory control of thrips (Kansagara *et al.*, 2018). It has also been reported that applying flonicamid insecticide results in mortality rates of western flower thrips ranging from 40% to 91% (Radosevich *et al.*, 2020). It has been reported that the application of a half dose of flonicamid increased the growth of the *B. bassiana* fungus strains Bb-409, Bb-4543, Ij-102, and Bb-4565, more effectively than the control treatment (Sain *et al.*, 2019). The synergistic effect of the insecticide flonicamid with the entomopathogenic fungus *B. bassiana*

in controlling insects and their compatibility with each other has been reported in some studies (Sain *et al.*, 2019; Wari *et al.*, 2020; Hiremath *et al.*, 2020).

However, fungicides pose the greatest risk to the successful introduction of entomopathogenic fungi into integrated management programs for crop protection (Khun *et al.*, 2021). Some studies have reported the negative effect of fungicides on *B. bassiana*. For example, fungicides frupica and prosper 300 CS reduced the colony growth of *B. bassiana* by 9.7% and 6.9%, respectively. Also, boscalid, pyraclostrobin, cyprodinil, and fludioxonil, individually and in the mixture (boscalid+pyraclostrobin and cyprodinil+fludioxonil) reduced the fungal colony growth, conidia germination, germ tube elongation, and conidia survival (Roberti *et al.*, 2017). Fungicides mepanipyrim, polyoxin, and chlorothalonil negatively affected the conidia germination of *B. bassiana* GHA strain five days after treatment (Wari *et al.*, 2020). The two fungicides, carbendazim and pyraclostrobin, were toxic to *B. bassiana* at all tested concentrations (Khun *et al.*, 2021). Only limited information is available on the compatibility of *B. bassiana* strains with the new fungicides iprodione + carbendazim and famoxadone + cymoxanil. This research has focused on investigating the pathogenicity of *B. bassiana* on onion thrips in laboratory conditions, specifically examining the compatibility of fungicides and *B. bassiana* with each other, determining the compatibility of insecticides and *B. bassiana* with each other, and studying the efficiency of *B. bassiana* in combined applications with pesticides.

Materials and Methods

Collection and rearing of onion thrips

Onion thrips were collected from infected cucumber plants in the vicinity of Zabol city, Iran. They were transferred to the entomology laboratory of the Faculty of Agriculture at the University of Zabol, Zabol, Iran, to isolate the adult insects. To rear onion thrips, primarily the

petioles of the first two leaves of the common bean *Phaseolus vulgaris*, which served as a host plant (three weeks after planting), were placed in plastic containers containing 20 mL of water. Adults were placed on the leaves for 24 h. Every other day, the containers were filled with water. The rearing containers were placed in an incubator with a temperature of 25 ± 1 °C, a relative humidity of $60 \pm 5\%$, and a photoperiod of 16:8 h (L: D).

Preparation of *B. bassiana*

The *B. bassiana* strain Saravan (IRAN 441C) was obtained from the collection of the Department of Agricultural Entomology Research (Iranian Research Institute of Plant Protection, Tehran, Iran). For the culture of this isolate, first, the larvae of the greater wax moth, *Galleria mellonella* (L.), were infected with the fungus. Then, the conidia that appeared on the surface of the larval carcass were used for culture in Sabouraud dextrose agar medium with 1% yeast extract (SDA + Y). After 10-14 days, the surface of the culture medium was scraped with a sterile scalpel, and the resulting liquid was poured into bottles with metal lids. In this way, the resulting suspension was passed through the dual muslin cloth to separate the mycelial pieces. To separate the conidia from each other and prevent them from forming a chain or lump when counting, the suspension was poured into a tube containing glass beads and shaken vigorously for a few minutes. One drop of Tween 80 (0.05 %) was added to 10 mL of this stock solution, and a uniform suspension was prepared. The suspension concentration was determined by counting the conidia using a hemocytometer slide. The following equation was used to prepare other required concentrations of conidia per volume unit:

$$\frac{\text{Required volume of concentration} \times \text{Calculated concentration}}{\text{Required concentration}} = \text{Required volume}$$

A stock solution was used in the experiments. The viability test previously determined conidial viability. In other words, after one day, this index (germination of more than 85% of conidia) can be determined by observing the symptoms of

germination (germination tube formation) under the light microscope. For this purpose, the day before the experiment, 1 mL of the desired suspension with a concentration of 10^7 conidia mL^{-1} was sterilely suspended in a 0.05% Tween 80 solution and stirred by vortexing. Then, it was cultured on SDA+Y medium, which was maintained in an incubator at 25 °C. After 24 hours, the conidia were counted under a light microscope (x40) from 100 conidia from each culture medium. Conidia whose germination tube length was longer than the conidia diameter were counted as germinated conidia. The germination percentage of conidia was calculated as follows. The next day, the culture with more than 85% germination was used. The above suspension was maintained at a temperature of 4 °C until the experiments were conducted (a maximum of 5 days).

Germination percentage of conidia = (The number of germinated conidia / Number of counted conidia) \times 100

Preparing used insecticides

The insecticides flonicamid (Teppeki® (WG 50%), Ishihara Sangyo Kaisha, Japan) and thiocyclam (Evisect® (SP 50%), Arysta Life Science, Japan) were used in this research. First, a concentration of 2000 mg/L was prepared from the formulation of each insecticide. Then, concentrations of 0.05, 0.5, 5, 10, 100, and 1000 mg/L were prepared from the formulation of insecticides using the serial dilution method. Different concentrations (based on the amount of effective substance) were tested for each insecticide. It should be noted that the mentioned concentration was the recommended concentration of the insecticides. First, a series of preliminary experiments was conducted to determine the minimum and maximum concentrations of each insecticide on onion thrips using the repeated dilution method. Then, based on the results obtained, the concentration that caused more than 25% mortality was chosen as the lowest concentration, and the concentration that caused about 75% mortality was selected as the highest effective concentration for conducting the main experiments. Afterward, four

concentrations were considered between these two concentrations, and the final experiments were conducted with six concentrations for each insecticide, along with the control treatment (Walker, 1994).

Preparing used fungicides

The fungicides iprodione + carbendazim (Rovral-TS® (52.5% WP)) and famoxadone+cymoxanil (Equation pro® (52.5% WDG)) were used in this research. These fungicides were purchased from the Agricultural Eksir Company in Yazd, Iran. First, a concentration of 2000 mg L^{-1} was prepared from the formulation of each of the fungicides. Then, concentrations of 0.05, 0.5, 5, 10, 100, and 1000 mg/L were prepared from the fungicide formulation using the serial dilution method. Different concentrations (based on the amount of effective substance) were tested for each insecticide. It should be noted that the mentioned concentration was the recommended concentration of the fungicides (Walker, 1994).

Evaluating the *B. bassiana* and insecticides on onion thrips

The five concentrations of fungal isolate and the control treatment were considered for bioassay. First, the preliminary experiments determined the maximum and minimum lethal concentrations (75 and 25%) of the fungus to obtain the LC_{50} .

In the following, three concentrations were selected within the range of the maximum and minimum lethal concentrations, and ultimately, the final experiments were performed with concentrations of 10^3 , 10^4 , 10^5 , 10^6 , and 10^7 conidia mL^{-1} . Then, cucumber leaf disks were immersed in experimental concentrations for five seconds. After drying the cucumber leaf discs in the air, ten 2nd-instar nymphs of thrips were separated from the colony by an aspirator under laboratory conditions and transferred to Petri dishes containing the leaf disc soaked in the solution. Around the Petri dishes were covered with parafilm tape and placed in an incubator with a temperature of 25 ± 1 °C, RH $60 \pm 5\%$ and LD 16: 8 h. Distilled water and Tween 80 (0.05

%) were used in the control treatment. After 24 h, the loss rate was recorded by a stereomicroscope (Martin *et al.*, 2003).

Biological factors of *B. bassiana*

First, *B. bassiana* was cultivated in several Petri dishes containing an SDA culture medium. After autoclaving, flasks containing SDA culture medium were placed at room temperature until they reached a temperature of $45 \pm 5^\circ\text{C}$. Using the method of mixing poisons (insecticides and fungicides) with the culture medium, the concentrations of half average (0.5 MC, Mean Concentration), average (MC, Mean Concentration), and twice average (2 MC, Mean Concentration) of poisons were added to these flasks (De Olivera. and Neves, 2004). The average concentration was obtained from the mathematical mean of the recommended concentrations of insecticides. In the experiments where a series of concentrations was prepared, the aim was to determine the LD_{50} and subsequently characterize one of the insecticides on onion thrips. Then, under sterile conditions, a 5 mm ring with a height of 3 mm was taken from the growing margin of a seven-day colony of fungus, which was kept at 25°C , along with the fungus. Afterward, it was placed upside down in the center of the culture medium alone (Control) and the culture medium with poisons, and kept in an incubator at 25°C , with a 12-hour light and 12-hour dark cycle. Each treatment had four replications. After three days, a ruler was used to measure the growth line of the fungus from four points of the ring for 15 days, and the resulting average was used to perform statistical calculations (Irigaray *et al.*, 2003).

Mixing poisons with the culture medium was used to determine the germination percentage of conidia. The control was the culture medium without insecticide. To count conidia, a circle with a diameter of 10 mm was cut from the Petri dish and placed inside a small glass. Then, 10 mL of sterile distilled water containing Tween 80 (0.05% concentration) was added and vortexed for 5 min to separate the conidia chain. The suspension concentration was determined by Neobar lam. After 24 hours, the number of conidia, ranging from 50 to 100, was counted

using a microscope (X40 eyepiece). The conidia whose length of the germ tube was more than half of the length of the conidium were considered germinated (Irigaray *et al.*, 2003). The following formula was used to calculate the inhibition percentage of fungal mycelium vegetative growth in different treatments, in which I is the inhibition percentage of the vegetative growth, C is the vegetative growth of the colony in the control in cm, and T is the vegetative growth of the colony in the treatments in cm (Vincent 1947).

$$I = [(C-T)/C] \times 100$$

After 24 h, the percentage of non-germinated conidia compared to the control was calculated using the following formula:

$$T = (20 (VG) + 80 (SP))/100$$

In this formula, T represents the corrected value of fungal vegetative and reproductive growth, while VG and SP denote the percentages of vegetative growth and sporulation compared to the Control (Irigaray *et al.*, 2003).

Mortality rate of onion thrips

This experiment was conducted based on the compatibility evaluation test of pesticides (insecticides and fungicides) with *B. bassiana* fungus. Based on this, the pesticide compatible with the *B. bassiana* fungus was selected, and the bioassay tests of the mixture of the fungal isolate and the insecticide were performed. In this experiment, 1 mL of suspension obtained from three concentrations of *B. bassiana* fungus [including LC_{50} and two concentrations less than LC_{50} (5.5×10^7 and 6×10^7 conidia mL^{-1})] was considered and mixed with the LC_{15} amount of insecticide in the test tube. The control treatment consisted of Tween 80 (0.05%) without the addition of fungal conidia. The leaf immersion method was used as before. For each treatment, 10 nymphs (2nd instar) of thrips were used. Mortality was recorded daily for 10 days, and the percentage of cumulative losses was calculated (Trisyono and Whalon, 1999). If mortality was observed in the control, it was corrected with Abbott's formula (Abbott 1925).

Statistical analysis

The values of lethal and sublethal concentrations were calculated by probit analysis in SPSS software (version 16). Statistical analysis was performed by one-way ANOVA with four replications. Means were compared with Duncan's multi-range test at the 5% probability level with SPSS software (version 16). Since there were no interaction effects in this analysis and the effects were fixed, Duncan's multiple range test was used. Before analysis, the normality of the data was confirmed by the Kolmogorov-Smirnov test ($P < 0.05$).

Results

Evaluating the insecticides and *B. bassiana* on onion thrips

Based on tables 1-4, flonicamid insecticide significantly affects ($P < 0.05$) the mortality of the 2nd instar nymph of thrips within 24 to 72 h. Additionally, there was a significant difference

($P < 0.05$) in the effectiveness of various concentrations of entomopathogenic fungus in controlling this pest. The highest mortality rate of thrips was observed at a concentration of 3 mg L⁻¹ of flonicamid, and further increasing it did not affect the mortality rate. The concentrations of 8 and 10 mg L⁻¹ of thiocyclam resulted in the highest thrips mortality, with no significant difference. As the concentration increased, the mortality rate also increased. The maximum lethal effect was 24 h after thiocyclam application. According to the probit analysis results, the LC₅₀ values for flonicamid and thiocyclam were 2.3 and 4.3 mg/L, respectively. The LC₅₀ value for *B. bassiana* was 5.4×10^7 conidia mL⁻¹, which caused 50% mortality in the thrips. The minimum mortality rate of thrips was obtained by the concentration of 3×10^7 conidia mL⁻¹ fungus after 48 h, and the highest mortality percentage (77%) was achieved by the concentration of 6×10^7 conidia mL⁻¹ in 144 h.

Table 1 Probit analysis and LC₅₀ values of flonicamid and thiocyclamine and *Beauveria bassiana* on 2nd instar nymph of *Thrips tabaci*.

Treatments	Number of insects	LC ₅₀ (mg L ⁻¹) (conidia mL ⁻¹)	χ^2 df = 1	P	Slope \pm SE	y-intercept
Flonicamid	280	2.3	2.79	0.99	0.17 \pm 0.06	0.04
Thiocyclam	280	4.3	3.87	0.99	0.24 \pm 0.02	0.01
<i>B. bassiana</i>	240	5.4×10^7	1.30	0.99	0.46 \pm 0.35	-2.59

Table 2 The effect of flonicamid insecticide on the biological factors of *Beauveria bassiana*.

Concentrations (MC ²)	Germination rate (%)	Germination reduction (%)	Mycelial growth (mm)	Mycelium reduction (%)	Number of conidia ¹	Conidia reduction (%)
0.5	75.60 \pm 1.71 c	20.10 \pm 0.47 c	41.00 \pm 1.2 b	24.1 \pm 1.2 c	1.50 \pm 0.10 b	53.10 \pm 1.75 c
0.5	76.10 \pm 1.50 c	20.00 \pm 0.50 c	40.00 \pm 1.1 b	25.9 \pm 1.3 c	1.40 \pm 0.09 bc	56.30 \pm 1.88 bc
0.5	79.90 \pm 1.22 bc	20.80 \pm 0.58 c	40.00 \pm 1.3 b	25.9 \pm 1.1 c	1.30 \pm 0.08 c	59.40 \pm 1.90 b
0.5	77.40 \pm 1.41 bc	21.90 \pm 0.65 c	42.00 \pm 1.0 b	22.2 \pm 1.4 c	1.60 \pm 0.11 b	50.00 \pm 2.00 c
1	69.20 \pm 1.63 d	27.40 \pm 0.47 b	38.00 \pm 1.4 bc	29.6 \pm 1.5 c	1.20 \pm 0.07 cd	62.50 \pm 1.65 ab
1	70.30 \pm 1.58 d	26.30 \pm 0.55 b	38.00 \pm 1.3 bc	29.6 \pm 1.3 c	1.10 \pm 0.06 d	65.60 \pm 1.60 a
1	69.90 \pm 1.63d	27.40 \pm 0.47 b	36.00 \pm 1.5 bc	33.3 \pm 1.2 b	1.00 \pm 0.05 d	68.80 \pm 1.50 a
1	68.10 \pm 1.67 d	25.40 \pm 0.65 b	39.00 \pm 1.1 bc	27.8 \pm 1.4 c	1.20 \pm 0.08 cd	62.50 \pm 1.65 ab
2	68.60 \pm 1.22 d	28.40 \pm 0.58 a	35.00 \pm 1.6 c	35.2 \pm 1.6 ab	1.00 \pm 0.05 d	68.80 \pm 1.50 a
2	66.70 \pm 1.50 d	26.90 \pm 0.75 a	34.00 \pm 1.7 c	37.0 \pm 1.7 a	1.00 \pm 0.05 d	68.80 \pm 1.50 a
2	69.10 \pm 1.32 d	27.40 \pm 0.47 a	30.00 \pm 1.8 d	44.4 \pm 1.5 a	1.10 \pm 0.06 d	65.60 \pm 1.60 a
2	69.30 \pm 1.41 d	27.20 \pm 0.50 a	32.00 \pm 1.4 d	40.7 \pm 1.4 a	0.90 \pm 0.04 e	71.90 \pm 1.40 a
0	95.90 \pm 0.00 a	0.00 \pm 0.00 d	54.00 \pm 0.9 a	0.00 \pm 0.00 d	3.20 \pm 0.15 a	0.00 \pm 0.00 d

In each column, Means with the same letters are not significantly different (Duncan, $P < 0.05$).

¹ Number $\times 10^7$ conidia mL⁻¹; ² Mean Concentration

Table 3 The effect of thiocyclam insecticide on the biological factors of *Beauveria bassiana*.

Concentration (MC) ²	Germination rate (%)	Germination reduction (%)	Mycelial growth (mm)	Mycelium reduction (%)	Number of conidia ¹	Conidia reduction (%)
0.5	61.10 ± 1.22 c	35.80 ± 1.55 c	32.00 ± 1.49 c	40.70 ± 1.54 c	1.00 ± 0.05 b	68.80 ± 1.10 b
0.5	60.30 ± 1.31 c	36.80 ± 1.47 c	33.00 ± 1.62 c	38.90 ± 1.49 c	1.10 ± 0.06 b	65.60 ± 1.20 b
0.5	58.80 ± 1.43 c	38.90 ± 1.39 c	35.00 ± 1.56 c	35.20 ± 1.63 c	1.00 ± 0.05 b	68.80 ± 1.15 b
0.5	60.10 ± 1.14 c	36.80 ± 1.51 c	34.00 ± 1.45 c	37.00 ± 1.58 c	1.20 ± 0.06 b	62.50 ± 1.30 b
1	48.60 ± 2.14 b	49.50 ± 1.68 b	28.00 ± 1.34 cb	48.10 ± 1.39 cb	1.00 ± 0.05 b	68.80 ± 1.05 b
1	45.50 ± 1.99 a	52.60 ± 1.56 a	29.00 ± 1.29 c	46.30 ± 1.47 c	0.70 ± 0.04 c	78.10 ± 0.90 a
1	47.00 ± 1.68 ab	50.50 ± 1.54 ab	26.00 ± 1.39 b	51.90 ± 1.52 b	0.80 ± 0.05 bc	75.00 ± 1.00 a
1	49.10 ± 2.12 b	48.40 ± 1.43 b	25.00 ± 1.46 ab	53.70 ± 1.61 ab	0.90 ± 0.04 bc	71.90 ± 1.10 ab
2	42.80 ± 2.02 a	55.80 ± 1.59 a	26.00 ± 1.87 b	51.90 ± 1.44 b	0.70 ± 0.05 c	78.10 ± 0.85 a
2	40.10 ± 2.23 a	57.90 ± 1.44 a	24.00 ± 1.43 ab	55.60 ± 1.59 ab	0.70 ± 0.04 c	78.10 ± 0.90 a
2	43.90 ± 2.34 a	54.70 ± 1.35 a	23.00 ± 1.34 a	57.40 ± 1.39 a	0.60 ± 0.05 c	81.30 ± 0.80 a
2	44.30 ± 2.16 a	53.70 ± 1.64 a	20.00 ± 1.29 a	63.00 ± 1.24 a	0.70 ± 0.04 c	78.10 ± 0.85 a
0	95.10 ± 0.00 d	0.00 ± 0.00 d	54.00 ± 1.87 d	0.00 ± 0.00	3.20 ± 0.10 a	0.00 ± 0.00 c

In each column, Means with the same letters are not significantly different (Duncan, $P < 0.05$).

¹ Number $\times 10^7$ conidia ml⁻¹; ² Mean Concentration. M

Table 4 The effect of iprodione + carbendazim and famoxadone + cymoxanil fungicides on the biological factors of *Beauveria bassiana*.

Treatments	Concen. (mg L ⁻¹)	Germination rate (%)	Germination reduction (%)	Mycelial growth (mm)	Mycelium reduction (%)	Number of conidia *	Conidia reduction (%)
Iprodione + Carbendazim	0.5	5.00 ± 0.50 c	94.70 ± 0.45 c	1.80 ± 0.12 b	96.70 ± 0.25 a	0.40 ± 0.05 b	87.50 ± 0.45 b
	0.5	5.00 ± 0.45 c	94.70 ± 0.40 c	2.00 ± 0.15 b	96.30 ± 0.20 a	0.30 ± 0.05 b	90.60 ± 0.35 ab
	0.5	9.00 ± 0.60 c	90.50 ± 0.50 c	1.90 ± 0.14 b	96.50 ± 0.30 a	0.50 ± 0.05 b	84.40 ± 0.50 b
	0.5	6.00 ± 0.55 c	93.70 ± 0.48 c	2.00 ± 0.13 b	96.30 ± 0.22 a	0.30 ± 0.05 b	90.60 ± 0.40 a
	1	0.00 ± 0.00 d	100.00 ± 0.00 d	0.00 ± 0.00 c	100.00 ± 0.00 a	0.00 ± 0.00 a	100.00 ± 0.00 a
	1	0.00 ± 0.00 d	100.00 ± 0.00 d	0.00 ± 0.00 c	100.00 ± 0.00 a	0.00 ± 0.00 a	100.00 ± 0.00 a
	1	0.00 ± 0.00 d	100.00 ± 0.00 d	0.00 ± 0.00 c	100.00 ± 0.00 a	0.00 ± 0.00 a	100.00 ± 0.00 a
	1	0.00 ± 0.00 d	100.00 ± 0.00 d	0.00 ± 0.00 c	100.00 ± 0.00 a	0.00 ± 0.00 a	100.00 ± 0.00 a
	2	0.00 ± 0.00 d	100.00 ± 0.00 d	0.00 ± 0.00 c	100.00 ± 0.00 a	0.00 ± 0.00 a	100.00 ± 0.00 a
	2	0.00 ± 0.00 d	100.00 ± 0.00 d	0.00 ± 0.00 c	100.00 ± 0.00 a	0.00 ± 0.00 a	100.00 ± 0.00 a
	2	0.00 ± 0.00 d	100.00 ± 0.00 d	0.00 ± 0.00 c	100.00 ± 0.00 a	0.00 ± 0.00 a	100.00 ± 0.00 a
	2	0.00 ± 0.00 d	100.00 ± 0.00 d	0.00 ± 0.00 c	100.00 ± 0.00 a	0.00 ± 0.00 a	100.00 ± 0.00 a
Famoxadone + Cymoxanil	0.5	2.00 ± 0.40 b	97.90 ± 0.35 b	4.00 ± 0.20 a	92.60 ± 0.18 b	0.20 ± 0.05 b	93.80 ± 0.25 ab
	0.5	1.00 ± 0.35 b	98.90 ± 0.38 b	3.00 ± 0.18 a	94.40 ± 0.22 b	0.30 ± 0.05 b	90.60 ± 0.30 ab
	0.5	2.00 ± 0.38 b	97.90 ± 0.40 b	2.00 ± 0.15 b	96.30 ± 0.15 a	0.20 ± 0.05 b	93.80 ± 0.28 a
	0.5	3.00 ± 0.45 b	96.80 ± 0.42 b	2.00 ± 0.14 b	96.30 ± 0.18 a	0.20 ± 0.05 b	93.80 ± 0.25 ab
	1	0.00 ± 0.00 d	100.00 ± 0.00 d	0.00 ± 0.00 c	100.00 ± 0.00 a	0.00 ± 0.00 a	100.00 ± 0.00 a
	1	0.00 ± 0.00 d	100.00 ± 0.00 d	0.00 ± 0.00 c	100.00 ± 0.00 a	0.00 ± 0.00 a	100.00 ± 0.00 a
	1	0.00 ± 0.00 d	100.00 ± 0.00 d	0.00 ± 0.00 c	100.00 ± 0.00 a	0.00 ± 0.00 a	100.00 ± 0.00 a
	1	0.00 ± 0.00 d	100.00 ± 0.00 d	0.00 ± 0.00 c	100.00 ± 0.00 a	0.00 ± 0.00 a	100.00 ± 0.00 a
	2	0.00 ± 0.00 d	100.00 ± 0.00 d	0.00 ± 0.00 c	100.00 ± 0.00 a	0.00 ± 0.00 a	100.00 ± 0.00 a
	2	0.00 ± 0.00 d	100.00 ± 0.00 d	0.00 ± 0.00 c	100.00 ± 0.00 a	0.00 ± 0.00 a	100.00 ± 0.00 a
	2	0.00 ± 0.00 d	100.00 ± 0.00 d	0.00 ± 0.00 c	100.00 ± 0.00 a	0.00 ± 0.00 a	100.00 ± 0.00 a
	2	0.00 ± 0.00 d	100.00 ± 0.00 d	0.00 ± 0.00 c	100.00 ± 0.00 a	0.00 ± 0.00 a	100.00 ± 0.00 a
Control		95.00 ± 0.00 a	0.00 ± 0.00 a	54.00 ± 0.50 a	0.00 ± 0.00 c	3.20 ± 0.10 c	3.20 ± 0.10

In each column, Means with the same letters are not significantly different (Duncan, $P < 0.05$).

* Number $\times 10^7$ conidia ml⁻¹.

Effects of insecticides and fungicides on *B. bassiana*

The results obtained from the application of three concentrations (average, half the average, and twice the average) of flonicamid on the biological factors of the entomopathogenic fungus (germination, mycelial growth, and the number of conidia) showed that the negative effect and the lethal role of flonicamid on germination and mycelial growth were low. There was no significant difference between its concentrations (Table 2). However, the number of conidia decreased by more than 50% following the application of flonicamid. A significant difference was also observed between the studied concentrations of this insecticide (Table 2). The lowest germination, mycelial growth, and the number of conidia were related to the treatment of twice the LC_{50} flonicamid (Table 2).

The results obtained from the application of three concentrations of thiocyclam showed that the inhibition of mycelial growth was greater in thiocyclam insecticide than flonicamid, so that the minimum concentration decreased germination (35.4%), mycelium (32%), and conidia (65%). With the increase in the concentration of thiocyclam, the inhibition rate increased with an increasing slope, and the lowest value of germination, mycelium, and conidia was related to the concentration twice the average of thiocyclam (Table 3). The reduction rate of biological factors among the concentrations of thiocyclam showed a significant difference at the 5% level, indicating that more accuracy should be taken in determining the appropriate concentration of thiocyclam for combined application.

The results showed that due to the fungicidal nature of these fungicides, their application stops the biological factors (germination rate, mycelium rate, and the number of conidia) of the *B. bassiana* fungus almost completely, except at the concentration of 0.5MC, where little biological activity (the rate of 2-10%) was observed in this fungus. At high concentrations, the entire biological activity of this fungus

ceased, reaching zero percent (Table 4). Therefore, it can be said that the simultaneous application of these fungicides with the fungus is unreasonable and ineffective due to these effects.

Evaluating the combined effects on onion thrips

This evaluation was conducted at four time intervals: 48, 96, 144, and 240 hours. According to the analysis of variance, thrips mortality was significant at a 5% probability level in all treatments and across different periods. The highest thrips mortality was obtained in the combined treatment of 5.5×10^7 conidia mL^{-1} of *B. bassiana* + LC_{15} concentration of thiocyclam. The lowest thrips mortality was observed with the combined application of 5.5×10^7 conidia mL^{-1} of fungus and an LC_{15} concentration of flonicamid (Table 5).

Table 5 Mortality in 2nd instar nymph of *Thrips tabaci* affected by the insecticides and *Beauveria bassiana* after 48, 96, 144, and 240 h.

Insecticide	<i>B. bassiana</i> (conidia mL^{-1})	Time Interval (h)	Mortality Rate (%)
Flonicamid (1.2 mg L^{-1})	5.5×10^7	48	38 c
		96	42 c
		144	50 b
		240	52 b
	6×10^7	48	42 c
		96	55 ab
		144	66 a
		240	70 a
	6.5×10^7	48	43 c
		96	55 ab
		144	65 a
		240	69 a
Thiocyclam (2.8 mg L^{-1})	5.5×10^7	48	44 c
		96	61 ab
		144	70 a
		240	74 a
	6×10^7	48	46 c
		96	58 ab
		144	62 ab
		240	70 a
	6.5×10^7	48	45 c
		96	52 b
		144	55 ab
		240	59 a

Means with the same letters are not significantly different from each other according to Duncan's test at 5% level.

In different treatments, mortality increased over time from 48 to 240 hours. In all treatments, the rate of losses during the 96, 144, and 240 h periods was significantly higher ($P < 0.05$) compared to the 48 h period (Table 5). Considering that all treatments except the first had a high ability to control thrips, the effect of the combined treatments on the biological factors of the fungus was investigated to select the best treatment with the least inhibition of fungal growth. Considering the biological factors and the lowest reduction percentage in germination,

mycelium, and conidia, the combined treatment of 6×10^7 conidia mL^{-1} of the entomopathogenic fungus *B. bassiana* + 1.2 mg L^{-1} flonicamid was the most effective combination for controlling thrips (Table 6). Although in some treatments, the combination of thiocyclam had a more lethal effect on thrips compared to the combination of flonicamid (Table 5), for combined use with the studied pathogen, flonicamid had higher efficiency compared to thiocyclam due to the greater negative effects of thiocyclam on the *B. bassiana* (Table 6).

Table 6 Integrated treatments on the biological factors of *Beauveria bassiana*.

Insecticide	<i>B. bassiana</i> (conidia mL^{-1})	Germination rate (%)	Germination reduction (%)	Mycelial growth (mm)	Mycelium reduction (%)	Number of conidia *	Conidia reduction (%)
Flonicamid (1.2 mg L^{-1})	5.5×10^7	58.00 ± 0.50 b	34.00 ± 0.55 b	38.00 ± 0.45 c	27.00 ± 0.55 c	4.20 ± 0.10 c	24.00 ± 0.85 c
	6×10^7	59.00 ± 0.45 b	33.00 ± 0.50 b	41.00 ± 0.50 b	22.00 ± 0.50 d	5.00 ± 0.08 b	10.00 ± 0.67 e
	6.5×10^7	56.00 ± 0.60 c	36.00 ± 0.60 c	39.00 ± 0.48 bc	25.00 ± 0.52 cd	4.80 ± 0.09 b	13.00 ± 0.74 d
Thiocyclam (2.8 mg L^{-1})	5.5×10^7	58.00 ± 0.55 b	34.00 ± 0.58 b	40.00 ± 0.47 b	24.00 ± 0.50 cd	4.30 ± 0.11 c	22.00 ± 0.90 c
	6×10^7	54.00 ± 0.65 d	38.00 ± 0.65 d	39.00 ± 0.48 bc	25.00 ± 0.52 cd	4.40 ± 0.12 c	20.00 ± 0.78 cd
	6.5×10^7	58.00 ± 0.50 b	34.00 ± 0.55 b	38.00 ± 0.45 c	27.00 ± 0.55 c	4.10 ± 0.10 d	26.00 ± 0.92 b
Control		87.00 ± 0.40 a	0.00 ± 0.00 a	52.00 ± 0.30 a	0.00 ± 0.00 a	5.50 ± 0.07 a	0.00 ± 0.00 a

Means with the same letters are not significantly different from each other according to Duncan's test at 5% level.

* Number $\times 10^7$ conidia mL^{-1} .

Discussion

In the current research, chemical, biological, and combined chemical and biological methods were investigated to determine the most effective management strategy for onion thrips. The results showed that the sole application of flonicamid, thiocyclam, and *B. bassiana*, as well as increasing their concentration, raises thrips mortality, and flonicamid insecticide is compatible with the *B. bassiana* fungus. The compatibility of insecticides with entomopathogenic fungi is crucial for the integrated control of pests, particularly onion thrips, and for reducing the reliance on chemical insecticides. In line with these results, researchers have reported that flonicamid insecticide increases the mortality rates of western flower thrips, onion thrips, and pepper thrips (Kansagara *et al.*, 2018; Bilbo *et al.*, 2020;

Radosevich *et al.*, 2020). It has been stated that spinosad and chlorfenapyr insecticides were more effective than flonicamid, based on the mortality percentage of western flower thrips adults. All three insecticides had a remarkable effect on controlling this pest compared to the control.

Additionally, increasing the concentration of flonicamid to 10 mL increased the mortality of western flower thrips adults (Radosevich *et al.*, 2020). Three and seven days after flonicamid application, the number of western flower thrips adults was reduced (Bilbo *et al.*, 2020). The results of the present study on the effect of flonicamid in controlling onion thrips were consistent with those of the aforementioned studies. It has been determined that flonicamid is a selective feeding blocker that causes hunger in the insect and ultimately leads to its death by

inhibiting the swallow pathway (Radosevich *et al.*, 2020).

According to previous studies, *B. bassiana* has yielded better results in reducing the onion thrips population compared to other entomopathogenic fungi (Singh *et al.*, 2011). The maximum decrease in onion thrips larvae population by 86.62% was observed in onion plants sprayed with *B. bassiana* fungus (a concentration of 1×10^{11} conidia mL⁻¹) after 10 days compared to the Control (Ain *et al.*, 2021). The researchers reported that the *B. bassiana* fungus reduced onion thrips by 54-84% after 11 days (Ansari *et al.*, 2008). It has been reported that *B. bassiana* increases the mortality of onion thrips under both laboratory and field conditions (Gulzar *et al.*, 2021). The application of *B. bassiana* was very effective in controlling onion thrips (Ain *et al.*, 2021; Gulzar *et al.*, 2021), western flower thrips (Zhang *et al.*, 2021), and melon thrips (Li *et al.*, 2021). Using different strains (BbYT12, BbYT13, and BbYT14) of the *B. bassiana* fungus, as well as varying time intervals from 24 to 144 hours after application, increased the mortality rate of western flower thrips (Zhang *et al.*, 2021). Increasing the concentration of *B. bassiana* (strain JEF-350) to 108 conidia mL⁻¹ increased the mortality rate in melon thrips (Li *et al.*, 2021). It has been reported that the application of *B. bassiana* fungus for controlling the onion thrips population can reduce the use of chemical insecticides, thereby preventing or delaying the emergence of thrips-resistant generations (Maniania *et al.*, 2003; Ain *et al.*, 2021). The results of the present study on the effect of *B. bassiana* fungus against onion thrips were consistent with those of the aforementioned studies. It has been found that during the adhesion of entomopathogenic fungi to the thrips epicuticle, a dense mucilage is secreted, which, in addition to strengthening the adhesion of the fungus, can also act as a means to transfer the cuticle-degrading enzymes of the insect. Also, the expression of the protease gene *Pr1*, in *B. bassiana* fungus, which targets the host cuticle, was consistent with its

function as a cuticle-degrading enzyme (Zhang *et al.*, 2021).

Insecticides can affect different stages of growth in entomopathogenic fungi that infect insects. Therefore, it is necessary to investigate the compatibility of different strains of this group of biological agents with insecticides for use in integrated pest management programs (Sain *et al.*, 2019). The compatibility of flonicamid with *B. bassiana* fungus and the lower reduction effects of this insecticide on the colony of this fungus have been reported (Kumar and Gupta 2019). Flonicamid caused relatively moderate growth inhibition in *B. bassiana* fungus compared to other insecticides, and the highest concentration of conidia in *B. bassiana* (strain Bb-6097) was recorded with half and full doses of flonicamid insecticide (Sain *et al.*, 2019). The results of the present research on the effect of flonicamid on the biological factors of *B. bassiana* fungus and its compatibility with them were consistent with those of other researchers. According to the opinion of these researchers, the biological factors of the entomopathogenic fungus decreased with the increase in the concentration of chemicals (Sain *et al.*, 2019; Kumar and Gupta, 2019). In this study, the fungus exhibited varying degrees of survival after insecticide exposure, with flonicamid at concentrations of 0.5 MC and MC having the least effect on the germination and mycelial growth of the entomopathogenic fungus, indicating compatibility. In contrast, thiocyclam (at all three concentrations) adversely affected biological factors, even at low concentrations. In this context, it has been found that flonicamid insecticide, in half the dose, was compatible with *B. bassiana* compared to imidacloprid, fipronil, and profenophos insecticides (Sain *et al.*, 2019). The application of flonicamid insecticide was relatively compatible with *B. bassiana* compared to spirotetramat insecticide (Kumar and Gupta 2019). The importance of the germination rate in the compatibility of insecticides with entomopathogenic fungi lies in the fact that the first step in causing infection by the fungus is the germination of conidia and their penetration into

the host insect's body tissue (Dannon *et al.*, 2020). Some insecticides are incompatible with the *B. bassiana* fungus (Sain *et al.*, 2019), which is consistent with the result of this research regarding the incompatibility of thiocyclam.

Combining sub-lethal concentrations of chemical insecticides and entomopathogenic fungi can increase the negative effects on insects (Halder *et al.*, 2021). In this study, combining 6×10^7 conidia mL⁻¹ of *B. bassiana* with 1.2 mg L⁻¹ of flonicamid was able to cause losses comparable to those of the sole application of insecticide. Many researchers have reported the successful application of combined treatments of *B. bassiana* with flonicamid and other insecticides, stating that *B. bassiana* in combination with insecticides can have a synergistic effect in controlling insects (Hiremath *et al.*, 2020; Wari *et al.*, 2020). The combined application of *B. bassiana* with flonicamid reduced the mustard aphid population (Patel *et al.*, 2021). The combined application of the neonicotinoid acetamiprid insecticide in half of its recommended dose, along with *B. bassiana* caused a 50% mortality of the green peach aphid population in the shortest time (Halder *et al.*, 2021). In laboratory conditions, the combined treatment of dimethoate insecticide at a concentration of 1 mL.L⁻¹, along with a half dose (2.5 g L⁻¹) of *B. bassiana*, resulted in 95% mortality of onion thrips after 5 days (Kachot *et al.*, 2021). The results of the present study were consistent with those of the studies mentioned above.

The insecticides can act as a generic stressor by attenuating the insect cuticle, decreasing the mobility of the target pest, and disordering the elimination of fungal conidia via grooming behavior, and as a result, cause the insect to be more susceptible to the attachment and entry of entomopathogenic fungi (Khun *et al.*, 2021).

This study has shown that the fungicides iprodione + carbendazim and famoxadone + cymoxanil are inherently deleterious to *B. bassiana*. The application of these fungicides to control fungal diseases of onion and other plants will substantially eradicate these entomopathogens if they have been previously

applied or are present naturally. In the mixture of iprodione and carbendazim, iprodione is a contact fungicide that inhibits glycerol synthesis and hyphal growth by disrupting signal transduction. In this mixture, carbendazim is a systemic fungicide that inhibits fungal growth by blocking the synthesis of beta-tubulin, thereby preventing mitosis (Yang *et al.*, 2011). In the mixture of famoxadone + cymoxanil, famoxadone disrupts oxygen absorption in the electron transport chain and causes the lysis of fungal cells within a few seconds (Earley *et al.*, 2011). The application of incompatible fungicides may inhibit the function of entomopathogenic fungi, thereby affecting the entire integrated pest management program. In contrast to insecticides, fungicides (regardless of formulation type) are always toxic to fungal entomopathogens (Khun *et al.*, 2021). Carbendazim 50% WP, hexaconazole 5% EC, and propiconazole 25% EC had a completely inhibitory effect on *B. bassiana* (Joshi *et al.*, 2018). Fungicides pyraclostrobin and fludioxonil completely inhibited conidia germination of *B. bassiana*. Fludioxonil alone and in a mixture with cyprodinil also completely inhibited conidia survival (Roberti *et al.*, 2017). The toxic effects of iprodione (Da Silva and Neves, 2005), carbendazim (Joshi *et al.*, 2018; Khun *et al.*, 2021), and famoxadone plus cymoxanil (Roberti *et al.*, 2002) on *B. bassiana* have been reported, which is in agreement with our results.

It is concluded that fungus *B. bassiana* (IRAN 441C) and insecticides flonicamid and thiocyclam controlled onion thrips at LC₅₀ and higher concentrations. Flonicamid at low concentrations had no inhibitory effect on the fungus and was found to be compatible. Fungal inhibition increased with the increment of thiocyclam concentration. Combining 6×10^7 conidia mL⁻¹ of *B. bassiana* with 1.2 mg L⁻¹ of flonicamid was the most effective combination for controlling onion thrips. The fungicides iprodione + carbendazim and famoxadone + cymoxanil had toxic effects on *B. bassiana* and were therefore unsuitable for integrated pest management programs. The application of

insecticides with entomopathogenic fungi can help control onion thrips. By increasing the application of this fungus and expanding its diversity, the consumption of insecticides can be greatly reduced over time. To further confirm the findings of this study and its recommendations for farms, it is suggested that field research be conducted on the effectiveness of fungus in combination with insecticides, allowing for the adoption of appropriate strategies.

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ارزیابی سازگاری و کارایی *Beauveria bassiana* با چند حشره‌کش و قارچ‌کش به‌منظور کنترل تریپس پیاز (*Thrips tabaci* (Thysanoptera: Thripidae))

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چکیده: تریپس پیاز، *Thrips tabaci* Lindeman (Thysanoptera: Thripidae)، یکی از آفات مهم در ایران است که خسارات قابل‌توجهی به محصولات زراعی وارد می‌کند. روش‌های کنترل زیستی برای کاهش وابستگی به حشره‌کش‌ها و قارچ‌کش‌های شیمیایی، به حداقل رساندن آلودگی محیط‌زیست و کاهش مقاومت حشرات ضروری هستند. این مطالعه به بررسی کشندگی حشره‌کش‌های فلونیکامید و تیوسیکلام، بیماری‌زایی *Beauveria bassiana*، سازگاری قارچ‌کش‌های ایپرودیون + کاربندازیم و فاموکسادون + سیموکسانیل با *B. bassiana*، و کارایی ترکیب حشره‌کش و قارچ بیمارگر روی لاروهای سن دوم تریپس پیاز پرداخته است. آزمایش در قالب طرح پایه بلوک‌های کامل تصادفی با چهار تکرار در شرایط آزمایشگاهی انجام شد. نتایج نشان داد که مقادیر LC_{50} برای تیوسیکلام، فلونیکامید و *B. bassiana* به‌ترتیب $4/3$ میلی‌گرم در لیتر، $2/3$ میلی‌گرم در لیتر و $10^7 \times 5/4$ کنیدی در میلی‌لیتر بود. مرگومیر تریپس با افزایش غلظت و مدت زمان قرار گرفتن در معرض حشره‌کش‌ها و *B. bassiana* افزایش یافت. فلونیکامید بر جوانه‌زنی یا رشد میسلیم *B. bassiana* تأثیری نداشت و به‌عنوان یک حشره‌کش سازگار شناخته شد. با این حال، ایپرودیون + کاربندازیم و فاموکسادون + سیموکسانیل به‌شدت فعالیت زیستی *B. bassiana* را مهار کردند و برای مدیریت تلفیقی آفت (IPM) مناسب نبودند. ترکیب *B. bassiana* ($10^7 \times 6$ کنیدی در میلی‌لیتر) و فلونیکامید ($1/2$ میلی‌گرم در لیتر) مؤثرترین تیمار علیه تریپس پیاز بود. این ترکیب، پس از تأیید در شرایط مزرعه‌ای، می‌تواند به‌عنوان جایگزینی برای حشره‌کش‌های شیمیایی در برنامه‌های IPM مورد استفاده قرار گیرد.

واژگان کلیدی: قارچ بیمارگر حشرات، قارچ‌کش، کاربرد توأم، آفت‌کش، تریپس پیاز