Efficacy of some Entomopathogenic Fungi Against Tomato Leaf Miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Iraq

Efficacy of some Entomopathogenic Fungi Against Tomato Leaf Miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Iraq

Jaafar A. Issa[1], Abdulnabi A. Matrood[2] and Alaa H. Al-Farttoosy[3]

[1-3]Department of Plant Protection, College of Agriculture, University of Basrah, Basrah, Iraq.

[1]E-mail: studies.amc@gmail.com
[2]E-mail: abdul_nabi.matrwod@uobasrah.edu.iq
[3]E-mail: alaa.hassan@uobasrah.edu.iq

Abstract. The most important tool for biological management of several insect pests is entomopathogenic fungi. The purpose of this study was to compare the effectiveness of *Beauveria bassiana* and Chaetomium globosum as biological agents against *Tuta absoluta*. According to the findings, *B. bassiana* and *C. globosium* may be able to influence *T. absoluta* larvae's eggs and third instar. By extending the duration and concentration, the fungus became more active. *B. bassiana* has had the greatest death rates after 7 days in the eggs and the third instar of the insect by 83.86% and 68.3% at 2 $\times 10^7$ conidia mL^{-1}, respectively.. This score was significantly differs to the *C. globosium* reached 60.09%and 31.7% in the destruction both eggs and the 3rd larvae- instar respectively. The outcomes also confirmed that the fungal colonies of both fungi isolated from leaves after 10 days of the application. The highest colony percentage belongs to the *C. globosium* 32.29% with a significant difference to the *B. bassiana* by 23.26%. The presence of both fungal colonies had a significant effect on the eggs, larvae, and pupae period development. Consequently, the weight of the pupal was influenced compared to the control. The *B. bassiana* has supremacy in comparison to the *C. globosium* and the control treatment. A gradual decrease has been shown in the proportion of the two fungal colonies tomato leaves after 30^{th} days. The lowest level score was4.17% and 9.37% for both *B. bassiana* and *C. globosium* respectively.

Keywords. Tomato, Vegetable crops, Fungi Against, Beauveria bassiana.

1. Introduction

Tomatoes are widely cultivated and consumed as one of the most important vegetable crops around the world. The tomato growers have faced the seriously losing in the production due to the *Tuta absoluta* (Meyrick) [1]. The tomato yield loss was estimated at 80% in the greenhouses and 100% in the open fields [2-5].

One of the main characteristics with this pest is a short life cycle 28-29 days. During this period, it can have between 10-12 generations a year [6]. Many control methods have applied to decrease *T. absoluta* populations for instance the pheromone traps. These traps were effective in capturing lots of adults resulting in reducing their population [7,8]. Chemical control has also been employed. It has

proved it is insufficient to prevent the damage caused by this pest [9]. However, although insecticides are effective in reducing the *T. absoluta* population, it also can be harmful to the natural enemies, human health and most important the environmental parts [10]. Furthermore, previous studies have indicated the susceptibility decreasing of the tomato leafminer, leading to failure of the certain synthetic insecticides application [11]. The excessive and extreme use of insecticides not only increases the production costs, but also poses environmental risks [10]. Hence, the pesticide residue is another concern, particularly for tomato exports [12]. On the other hand, owing to the drawbacks associated with the synthetic pesticides used, biological control methods have important gains [13]. Therefore the natural enemies have been used like the entomopathogenic fungi (EPF). Meanwhile many fungi were employed against *T. absoluta* [14]. The most commonly used EPF species are *Beauveria bassiana* and *Cheatomium globosum*. Extensive researches have shown that the *Paecilomyces lilacinus* was applied as a bioinsecticides against various agricultural and forest insect pests[15, 16,17,18,19] . It has previously been evaluated its efficacy against the tomato leafminer. The application of blastospores of a specific strain significantly reduced the number of pest larvae. Similarly, *Metarhizium anisopliae* var. *anisopliae* and *B. bassiana* have shown high mortality rates on *T. absoluta* eggs [20,21] .Another study was carried out by [22] conducted using that the *B. bassiana, Verticillium lecanii* and *M. anisopliae against* eggs- larvae *T. absoluta* tomato leafminer. They concluded that the fungi efficacy scored 48.3 ± 2.8%, 44.8 ± 4.8% and 41.3 ± 6.8% respectively against larva, while the selected bio-pesticides significantly reduced the *T. absoluta* egg counts compared to the untreated control. In view of the spread the economic pest *T. absoluta* on the tomato crop in i9Basra province and it resistance to many chemical pesticides and the environmental and health risks caused by these pesticides, this study was aimed to evaluate some entomopathogenic fungi effectiveness in controlling this serious pest.

2. Materials and Method

2.1. Entomopathogenic Fungi

This investigation utilises *Beauveria bassiana* and *Cheatomum globosum*. The isolate *B. bassiana* was obtained from Mycology laboratory, department of Plant Protection, College of Agriculture, University of Basrah. Meanwhile the *Cheatomum globosum* was isolated from the tomato plant tissue. After that, both isolates were activated and reproduced using Potato Dextrose Agar (PDA). To separate the fungal mycelia, the spores were transferred into a sterile glass bottle after washing by sterile distilled water containing 0.02% Tween 20 and scraping it using a sterile scalpel. Then, the concentration suspension was adjusted to 2×10^8 conidia by either adding some more spores or sterile water.

2.2. Rearing of Tuta Absoluta

The T. absoluta larvae were collected from infested tomatoes in the glasshouses that are located in the Agricultural Research Station at the University of Basrah campus. After the larva converted into adults, 10 adult insects were released on the young tomato cultivar Yasmeen seedlings that are planted in plastic pots 12 x 13 cm. Then the seedlings were kept in insect-proof rearing cages in a growth chamber at 25 °C and 65% RH and 16:8 hours light: dark photoperiod. During two days, the adults were fed with 10% sugar solution. The adult insects were then taken by using a mechanical aspirator. After the egg hatching, the larvae were allowed to feed on the potted tomato plants until they reached the third larvae instar stage. After 4-5 days of the eggs hatching, the larvae started feeding. After obtaining two generations, the bioassay of the 3rd–instar larvae has been performed according to their mean head capsule width 0.39-0.43mm [23].

2.3. Effect Entomopathogenic Fungi on T.absoluta Egg

In order to study the effect of pathogens, petri dishes measuring 9 cm were used. All plates were inside covered with a filter paper for filtration purposes. Then the dishes were divided into two groups. Each group consists of 12 dishes. Eggs were collected using a soft brush made of insect breeding papers. After that 20 eggs were placed in each dish with a piece of moist cotton for providing humidity. Both

groups were sprayed with 3 mL of a suspension containing *B.bassiana* and *C.globosum* fungi at concentrations of 10^4,10^5,10^6,10^7 spores mL^{-1}. The control group was sprayed with sterile distilled water containing 0.03% Tween 80. The results were evaluated after 3, 5, and 7 days of application. This evaluation has been carried out according to Abbott's formula [24].

2.4. Effect Entomopathogenic Fungi on T.absoluta Leavra

The *B. bassiana* and *C. globosum* at 10^4,10^5,10^6,10^7 conidia mL^{-1} were evaluated using the 3rd-instar larvae stage. The larvae were dipped in each concentration of both fungus suspension for five seconds. After that, the larvae were placed on a filter paper to dry. The control treatment was done by dipping larvae with a sterile distilled water, containing 0.03% Tween 80[25] .Then, the larvae were transported inside a transparent plastic bottle, containing tomato's leaves. The plastic bottles were put in a growth chamber at 30ºC± 2, 55%±5 a relative humidity, and 10-14 lighting periods (Light: Dark). The results were evaluated according to the Abbott's formula [24] at 3, 5, and 7 days after treatment.

2.5. Preparation of Fungal Vaccine

The *B. bassiana* and *C. globosum* inocula were prepared and reactivated on a PDA (Potato Dextrose Agar) medium. This was done using an appropriate amount of local millet seeds, *Panicum miliaceum*. The seeds were washed to remove impurities and dust, then soaked in water for six hours. The excess water was drained, and the seeds were placed on absorbent paper to dry them. Next, the seeds were distributed into 250 ml glass flasks, with 100 grams of seeds in each flask. A small amount of water was added to moisten the seeds, and the flasks were sealed with cotton plugs. The seeds were sterilised by autoclaving at a 121°C, 1 bar for one hour. After sterilisation, the flasks were allowed to cool and left for six hours. Subsequently, the sterilised flasks containing the millet seeds were inoculated with the individual fungal strains, *B.bassiana* and *C.globosum*. Each flask received five 0.5 cm diameter fungal colonies that were grown on PDA medium for seven days. The flasks were then incubated at a 25±2°C for two weeks. It was ensured that the flasks were shaken every 2-3 days to ensure even distribution of the inocula onto all the seeds[26].

2.6. Endophytic Colonization

Two fungal isolates, *B.bassiana* and *C.globosum*, were individually added after their growth on millet seeds using the previously mentioned method. A 10 grams of fungal inoculum were added to the soil near the roots of each tomato plant. Four replicates of the Yassamin tomato varieties that are, produced by Syngenta in Switzerland and distributed in Iraq by Green Gold Company, were used. The fungal growth rate was measured on tomato plant leaves after 10, 20, and 30 days of the fungal inoculum addition.

Four randomly leaves were selected from different positions, involving the lower, middle, and upper parts of each plant. These leaves were placed in plastic bags and stored at a temperature of 4°C to be ready in the isolation. The samples were washed under tap water to remove any dirt. Subsequently, the leaves were cut into small, equal-sized pieces of 0.5 cm after surface sterilisation with 70% ethanol for 30 seconds. They were then washed with distilled water for one minute and transferred to a 3% sodium hypochlorite solution for one minute, followed by rinsing with sterile distilled water. The leaf pieces were finally transferred to Petri dishes, containing Potato Dextrose Agar (PDA) medium supplemented with chloramphenicol to prevent bacterial growth. After that, the dishes were incubated at 25°C for five days, during which fungal colonies started to grow. The fungal isolates were then transferred to separate Petri dishes, containing PDA medium for further cultivation. The number of samples showing fungal growth was calculated using the following equation mention in [27,28] .

$$\text{Colonization} = \frac{\text{number of tomato leaf samples with fungal growth}}{\text{Ttotal number of samples}} \text{ x } 100$$

2.7. Effect Endophytic in T.absoluta

The Yassamin variety tomato seedlings were transferred to the pre-prepared foam plates and planted in the plastic pots containing 5 kg soil. The pots were placed in a plastic greenhouse. After one month of plant growth, the fungal inoculum for *B. bassiana* and *C. globosum* was added. The pots were placed

in an incubator at 25°C ± 1 and a relative humidity 65% ± 5. Fifteen days after adding the fungal inoculum, the newly hatched larvae were placed on the treated tomato plants and the control plants. After that the larvae were placed in a cage consisting of a Petri dish with a side hole allowing the passage of the leaf petiole, and multiple holes on the top covered with a piece of mesh fabric for the ventilation. Three to four leaves were placed in the plate, and three larvae were placed inside. Once the leaves were consumed, the dish was transferred by cutting the petiole to provide new leaves for larval feeding. The developmental period of the larval stage, pupal stage, and weight of the pupae were recorded using a sensitive balance. Five replicates were used for each treatment, including the control[29].

3. Results

3.1. Efficiency of Two Fungal Isolates on T.absoluta Egg

The results obtained from the fungal isolates evaluation of *B. bassiana* and *C. globosium* are summarized in Table 1.A. It can be seen that both isolates have potential effectiveness as a result of time-concentration increased. From this data, we can see that the **2×**10^7 conidia mL^{-1} have significantly effect on the eggs hatchability after 3rd days. The hatchability proportions were reached 51.0% and 44.3% in *B. bassiana* and *C. globosium* respectively without a significant difference at $p<0.01$. On the other hand, no significant differences can be shown when using the low concentration **2×**10^4 conidia mL^{-1} for both isolates *B. bassiana* and *C. globosium.* They achieved 38.7 % and 21.7% respectively at the 3rd days of application. However both *B. bassiana* and *C. globosium* have a significant differences effectiveness in 5th days. The 2×10^7 conidia/ml concentration scored 71.8% and 51.7 respectively as shown in (Table1.B). Moreover the eggs hatchability was continues declined. The statistical analysis illustrated no significant differences after treating by 2×10^7 conidia *B. bassiana* and *C. globosium*, achieving 83.86% and 60.09% respectively at 7th days (Table1.C).

Table 1.A. Effect of fungus suspension on *T. absoluta* eggs after 3 days.

Fungi	Concentration conidia/ml				Mean of the fungi effect
	2×10^4	**2×10^5**	**2×10^6**	**2×10^7**	
B. bassiana	38.7	44.3	44.3	51.0	44.6
C. globosium	21.7	27.3	27.3	44.3	30.2
Mean of the Concentration effect	30.2	35.8	35.8	47.6	

For fungi, LSD $_{0.01}$= 7.33, for the concentration, LSD $_{0.01}$= 10.44, for the interaction between the fungi vs the concentrations LSD $_{0.01}$= 14.76

Table 1.B. Effect of fungus suspension on *T. absoluta* eggs after 5 days.

Fungi	Concentration conidia/ml				Mean of the fungi effect
	2×10^4	**2×10^5**	**2×10^6**	**2×10^7**	
B. bassiana	48.3	55.3	74.3	71.8	62.4
C. globosium	26.0	30.5	38.3	51.7	36.6
Mean of the Concentration effect	37.2	42.9	56.3	61.7	

For fungi, LSD $_{0.01}$= 6.04, for the concentration, LSD $_{0.01}$= 8.54, for the interaction between the fungi vs the concentrations LSD $_{0.01}$= 12.07

Table 1.C. Effect of fungus suspension on *T. absoluta* eggs after 7 days.

Fungi	Concentration conidia/ml				Mean of the fungi effect
	2×10^4	**2×10^5**	**2×10^6**	**2×10^7**	
B. bassiana	53.94	69.83	75.86	83.86	70.87
C. globosium	32.14	35.84	51.72	60.09	44.95
Mean of the Concentration effect	43.04	52.83	63.79	71.97	

For fungi, LSD $_{0.01}$= 4.162, for the concentration, LSD $_{0.01}$= 5.886, for the interaction between the fungi vs the concentrations LSD $_{0.01}$= 8.324.

3.2. Effectiveness Fungi on T.absoluta Larva

Table (2: A, B, C) presents the mortality data on 3rd instar of *T. absoluta* larvae. The *B. bassiana* accomplished the highest mortality at 2×10^4, 2×10^5, 2×10^6, 2×10^7 conidia mL^{-1} reached 31.0%,42.1% ,57.1% and 68.3% at 7th days after treatment respectively. This data discovered a high significant difference to the fungus *C. globosium*, similarly to the time and the concentration at 2×10^4, 2×10^5, 2×10^6, 2×10^7 conidia mL^{-1}. The mortality has recorded 15.9%, 20.6% 26.2% and 31.7% respectively. Additionally, no significant differences between *B. bassiana* and *C. globosium* effectiveness in 3rd days after larva treating at 2×10^4 conidia mL^{-1}. The mortality reached 16.8% and 14.5% respectively (Table 2: A).

Table 2.A. Effect of fungus suspension on *T. absoluta* larva after 3 days.

Fungi	Concentration conidia/ml				Mean of the fungi effect
	2×10^4	2×10^5	2×10^6	2×10^7	
B. bassiana	16.8	20.5	25.3	29.2	22.9
C. globosium	14.5	8.5	11.6	12.6	10.1
Mean of the Concentration effect	12.3	14.5	18.4	20.8	

For fungi, LSD $_{0.01}$= 7.79, for the concentrations, LSD $_{0.01}$= 11.01, for the interaction between the fungi vs the concentrations LSD $_{0.01}$= 15.85

Table 2.B. Effect of fungus suspension on *T. absoluta* larva after 5 days.

Fungi	Concentration conidia/ml				Mean of the fungi effect
	2×10^4	2×10^5	2×10^6	2×10^7	
B. bassiana	29.0	32.3	37.3	43.5	35.5
C. globosium	9.7	14.5	19.2	22.8	16.6
Mean of the Concentration effect	19.3	23.4	28.3	33.1	

For fungi, LSD $_{0.01}$= 11.05, for the concentrations, LSD $_{0.\ 0.01}$= 15.63, for the interaction between the fungi vs the concentrations LSD $_{0.01}$= 22.10.

Table 2.C. Effect of fungus suspension on *T. absoluta* larva after 7 days.

Fungi	Concentration conidia/ml				Mean of the fungi effect
	2×10^4	2×10^5	2×10^6	2×10^7	
B. bassiana	31.0	42.1	57.1	68.3	49.62
C. globosium	15.9	20.6	26.2	31.7	23.6
Mean of the Concentration effect	23.4	31.3	41.7	50.0	

For fungi, LSD $_{0.01}$= 9.77, for the concentrations, LSD $_{0.01}$= 13.81, for the interaction between the fungi vs the concentrations LSD $_{0.01}$= 19.53.

3.3. Endophytic Colonization of Tomato Plant

Figure 1 there is a successful attempt to isolate the *B. bassiana and C. globosium* from tomato leaves after 10 days of their isolates adding. Their colonisation rates were scored 23.26% and 32.29% respectively. Also, we can see the colonisation ratio decreased in the tomato leaves after 20th days for both *B. bassiana* and *C. globosium* which were reached 14.58% and 23.96% respectively. At the 30th day, the colonisation percentage of *B. bassiana* and *C. globosium* tomato levees decreased to reach the lowest level were 4.17% and 9.37% respectively. Eventually, the outcomes revealed the significant differences between those two isolates in particular at the 10, 20, and 30th day. Meanwhile no fungus has been isolated from the leaves of the control treatment.

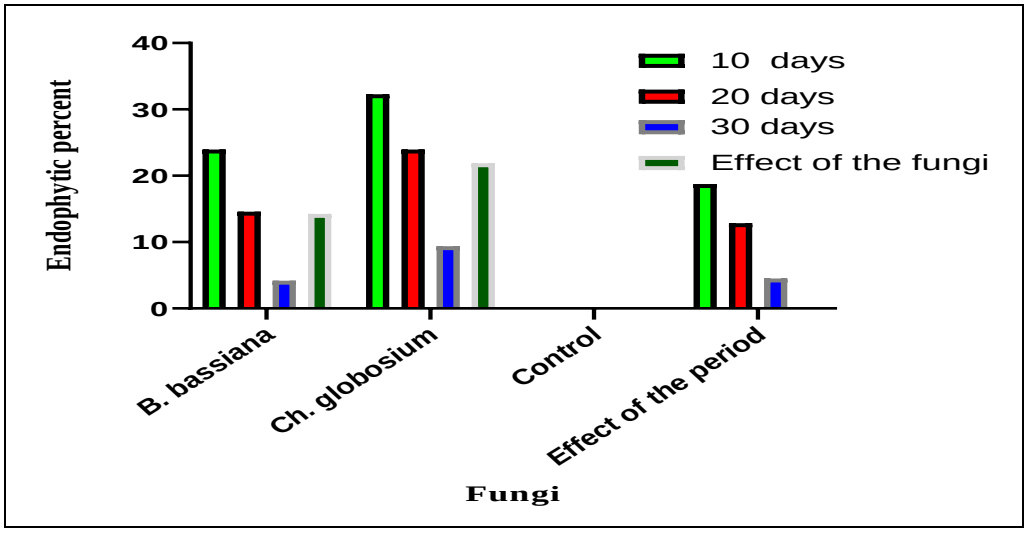

Figure 1. The effect of fungi and time on the endophytic%. L.S.D. $_{0.01}$ for the Fungi and the time = 4.625, L.S.D $_{0.01}$ for the interaction between the fungi vs the time = 8.011.

3.4. Effected Endophytic Fungi on T.absoluta

The results indicated that there were significant differences between all treatments in period of eggs development. The higher period was registrated in the case of *B. bassiana* compared to all other treatment. It reached 5.178 days. While the *C. globosium* was recorded 4.785, and the lowest period was in the control that accounted 4. 350 days (Fig2). However the development of larva has recorded the high period in *B. bassiana* 11.746 days, which significant different to the *C. globosium* 10.800 days and the control 10.193 days. In contrast, the longest pupal development period was in *B. bassiana* 7.667 days, which was significantly difference to the C. *globosium* and control that are reached 7.313 days and 7.273 days respectively.

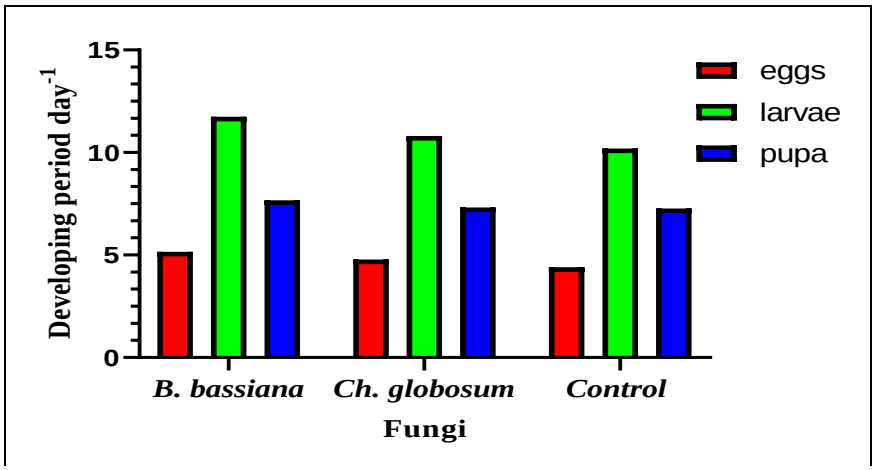

Figure 2. Effect of the fungi on the developing period of the eggs laying, larvae, and pupa. L.S.D. $_{0.01}$ for the fungi effect on eggs development= 0.3149, L.S.D. $_{0.01}$ for the fungi effect on larvae development= 0.3606, L.S.D. $_{0.01}$ for the fungi effect on pupal development= 0.2171.

The results in Figure 3 showed the significant differences between the total development periods of the insect's development, which started from the egg until the emergence of adults. It can see that the highest period was in the *B. bassiana* which reached 24.567 days followed by the *C. globosium which* scored 22.893 days. On the other hand, the lowest period was registered in the control 21.873 days.

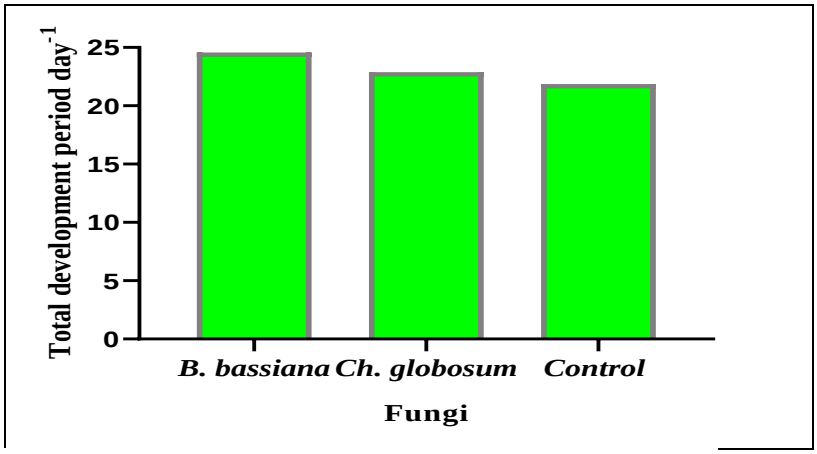

Figure 3. Effect of the fungi on the total development period on *T. absoluta*. L.S.D. $_{0.01}$ for the fungi effect on total development period= 0.5779.

From the Figure 4, it can be seen that the significant difference in the weight of the insect's pupa. The highest weight recorded in the control treatment (without fungi), which was significant difference to all other treatments. As well as, the lowest weight was reported in the case of pupil's larvae that feeding on the tomato leaves, which was treated by the endophytic fungi *B. bassiana*. It is registered 3.367 mg with significantly differences in both *C. globosium* and the control, which reported 4.031 mg and 4.367 mg respectively.

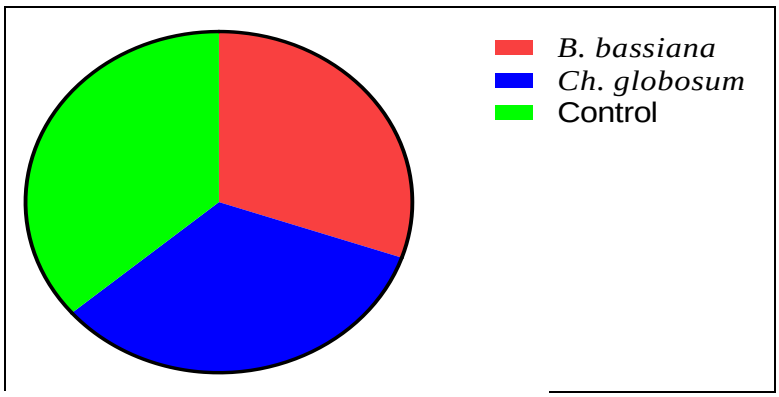

Figure 4. Effect of the fungi on the pupal weight of *T. absoluta*. L.S.D. $_{0.01}$ for the fungi effect on the weight of pupa = 0.2279.

4. Discussion

This study set out with the aim of assessing the importance of two fungal isolates effectiveness *B. bassiana* and *C. globosium* in controlling the eggs and larvae of T. *absoluta.* The second initial objective of the present study was to identify the capability of these isolates to form the colonies through their adding the soil. In this study, there is a significant effect on the development duration of larvae, pupae and eggs. Beside a significant effect on the weight of the pupal compared to the control, and *B. bassiana,* which were significantly superior to the control and *C. globosium*.[30] pointed out that the entophytic fungi have been gained increasing interest. This is because these fungal species can be useful to the host plant. For example, the secondary metabolites act as antimicrobials, insecticides, or plant bio stimulants in tomatoes. In addition, the plant can gain the EPF artificially from the surrounding environment, acting as the biological control agents. In this field, [31] proved the effectiveness of three different concentrations (4×10^7, 4×10^6 and 4×10^5 conidia mL^{-1}) of *Clonostachys* spp and *B. bassiana* as endophytic fungi. The fungal isolates have been shown the ability against *T. absoluta* larvae in the laboratory and the greenhouse. On the other hand, this study confirmed the fungal isolates effectiveness compared to the Emmamectin benzoate. Another study revealed that the formulation of *B. bassiana* and *Metarhizium anisopliae* succeeded in formation of

fungal colonies in the tomato leaves after adding their suspension to the plant via the irrigation. The colonization rate reached about 95% after five days of treatment, and also decreased to reach 85% after more than 15 days of treatment[29] . This study supports evidence from previous observations[32]. They were able to isolate *B. bassiana* from the leaf tissues of a wild tomato plant, and the two isolates from the soil. After tomato inoculation by three isolates through root dipping, injection, and spray the surface. They have summarised their study results that the *B. bassiana* was the most effective in formulation of the colonies compared to the two soil isolates. Furthermore they indicated that dipping the roots was the most successful method. Also it worthy noted that the increasing the colonies of *B. bassiana* increased the mortality rate of 2nd and 4th instar of *Helicoverpa armigera* larvae after one, three and five weeks of plant inoculation. On the other hand, these results indicate that the internal colonization of the fungus *B. bassiana* serves as an effective strategy to control the *H. armigera* insect on tomato.

[33] reported that the microbes and their metabolites play a crucial role in pest control strategies. Also in line with this result, many endophytic fungi were isolated from *Withania somnifera* like *C. globsum* Ef18, which showed activity against some phytopathogenic fungi, including *Sclerotinia sclerotiorum* [34]. Further study performed by[16] showed the effectiveness of three *B. bassiana* isolates against *T. absoluta*. The isolate's effectiveness has been revealed in achieving 90% mortality once spraying directly on the leaves applied. Moreover the fungus can form colonies after 30 days from the inoculation. In contrast, there are no negative effects on plant growth. Additionally, the endophytic fungi affected the survival of larva as it reached zero within seven days.

Conclusion

The purpose of the current study was to evaluate two different biological agents, including *B. bassiana* and *C. globosum* against *T. absoluta*. The results revealed that *B. bassiana and C. globosium* have potential capability in the controlling of the eggs and the3rd instar of *T. absoluta* larva. The research has also shown that the fungal activity increased by increasing time and concentration. The highest mortality percentage has been achieved by *B. bassiana* after passing 7th days in the eggs and the3rd-insect instar. The outcomes also confirmed that the fungal colonies of both fungi isolated from leaves after 10days of the application. The highest colony percentage belongs to the *C. globosium* with a significant difference to the *B. bassiana*. The presence of both fungal colonies had a significant effect on the eggs, larvae, and pupae period development. Overall, this study has shown the ability of two different fungal isolates in controlling the *T. absoluta* either eggs, pupa, larva, and the adult.

References

[1] Mansour, R., & Biondi, A. (2021). Releasing natural enemies and applying microbial and botanical pesticides for managing Tuta absoluta in the MENA region. Phytoparasitica, 49(2), 179–194. https://doi.org/10.1007/s12600-020-00849-w.

[2] Urbaneja, A., Protección Vegetal, C., Instituto, B., De Investigaciones, V., Rosa Vercher, A.), Navarro, V., & Marí, F. G. (1992). "1 ortícolas Transferencia Tecnológica La polilla del tomate, Tuta absoluta.

[3] Torres, J. B., Evangelista, W. S., Barras, R., & Guedes, R. N. C. (2002). Dispersal of Podisus nigrispinus (Het., Pentatomidae) nymphs preyino on tomato leafminer: Effect of predator release time, density and satiation level. Journal of Applied Entomology, 126(6), 326–332. https://doi.org/10.1046/j.1439-0418.2002.00653.x.

[4] Shashank, P. R., Chandrashekar, K., Meshram, N. M., & Sreedevi, K. (2015). Occurrence of tuta absoluta (Lepidoptera: Gelechiidae) An invasive pest from India. Indian Journal of Entomology, 77(4), 323. https://doi.org/10.5958/0974-8172.2015.00070.x.

[5] Srinivasulu, A. (2017). New record of invasive South American Tomato Leaf Miner, Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) on Tomato in Andhra Pradesh. International Journal of Pure & Applied Bioscience, 5(3), 654–656. https://doi.org/10.18782/2320-7051.2940.

[6] Hameed, T. S., & Sawicka, B. (2016). LEVEL APPLICATION OF CULTIVATORS FOR SCIENTIFIC RECOMMENDATIONS TO CONTROL TOMATO LEAF MINER, TUTA ABSOLUTA. 3(5), 1–23.

[7] Ünlü, L., Ögür, E., & Uulu, T. E. (2021). Control of Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) in open field tomato crops using the mating disruption technique. Phytoparasitica, 49(3), 385–396. https://doi.org/10.1007/s12600-021-00881-4.

[8] Domínguez, A., López, S., Bernabé, A., Guerrero, Á., & Quero, C. (2019). Influence of age, host plant and mating status in pheromone production and new insights on perception plasticity in Tuta absoluta. Insects, 10(8), 1–16. https://doi.org/10.3390/insects10080256.
[9] Giorgini, M., Guerrieri, E., Cascone, P., & Gontijo, L. (2019). Current Strategies and Future Outlook for Managing the Neotropical Tomato Pest Tuta absoluta (Meyrick) in the Mediterranean Basin. Neotropical Entomology, 48(1), 1–17. https://doi.org/10.1007/s13744-018-0636-1.
[10] Mirhosseini, M. A., Sohrabi, F., Michaud, J. P., & Rezaei, M. (2019). Suitability of five tomato cultivars for Tuta absoluta (Lepidoptera : Gelechiidae): Resistance is associated with a high density of glandular trichomes. 24, 1–15.
[11] Mashtoly, T. A. (2015). Comparative Susceptibility of Tomato Leafminer, Tuta absoluta (Lepidoptera: Gelechiidae) to Common Used Chemical Synthetic Pesticides and Biopesticides in Egypt. Egyptian Academic Journal of Biological Sciences, 7(1), 81–89.
[12] Codron, J. M., Adanacioğlu, H., Aubert, M., Bouhsina, Z., El Mekki, A. A., Rousset, S., Tozanli, S., & Yercan, M. (2014). The role of market forces and food safety institutions in the adoption of sustainable farming practices: The case of the fresh tomato export sector in Morocco and Turkey. Food Policy, 49(P1), 268–280. https://doi.org/10.1016/j.foodpol.2014.09.006.
[13] Collatz, J., & Sutter, L. (2023). Models for the practitioner : Predicting the efficient biocontrol of Tuta absoluta under different management scenarios. 172(May). https://doi.org/10.1016/j.cropro.2023.106316.
[14] Magwe, M. M., Chilagane, L. A., Mulungu, L. S., & Kikuu, C. (2023). Efficacy of the Key Isolated Entomopathogenic Fungi for the Biocontrol against Tuta absoluta in Tomato Plants. 4(1), 25–37.
[15] Allegrucci, N., Velazquez, M. S., Russo, M. L., Perez, E., & Scorsetti, A. C. (2017). Endophytic colonisation of tomato by the entomopathogenic fungus Beauveria bassiana: The use of different inoculation techniques and their effects on the tomato leafminer Tuta absoluta (Lepidoptera: Gelechiidae). Journal of Plant Protection Research, 57(4), 331–337. https://doi.org/10.1515/jppr-2017-0045.
[16] Silva, A. C. L., Silva, G. A., Abib, P. H. N., Carolino, A. T., & Samuels, R. I. (2020). Endophytic colonization of tomato plants by the entomopathogenic fungus Beauveria bassiana for controlling the South American tomato pinworm, Tuta absoluta. CABI Agriculture and Bioscience 2020 1:1, 1(1), 1–9. https://doi.org/10.1186/S43170-020-00002-X.
[17] Buragohain, P., Kumar Saikia, D., Sotelo-Cardona, P., & Srinivasan, R. (2021). Evaluation of Bio-Pesticides against the South American Tomato Leaf Miner, Tuta absoluta Meyrick (Lepidoptera: Gelechiidae) in India. https://doi.org/10.3390/horticulturae7090325.
[18] Gebremariam, A., Chekol, Y., & Assefa, F. (2021). Phenotypic, molecular, and virulence characterization of entomopathogenic fungi, Beauveria bassiana (Balsam) Vuillemin, and Metarhizium anisopliae (Metschn.) Sorokin from soil samples of Ethiopia for the development of mycoinsecticide. Heliyon, 7(5), e07091. https://doi.org/10.1016/j.heliyon.2021.e07091.
[19] Sharma, A., Sharma, S., & Yadav, P. K. (2023). Entomopathogenic fungi and their relevance in sustainable agriculture: A review. Cogent Food and Agriculture, 9(1). https://doi.org/10.1080/23311932.2023.2180857.
[20] Pires, L. M., Marques, E. J., Wanderley-Teixeira, V., Teixeira, Á. A. C., Alves, L. C., & Alves, E. S. B. (2009). Ultrastructure of Tuta absoluta parasitized eggs and the reproductive potential of females after parasitism by Metarhizium anisopliae. Micron, 40(2), 255–261. https://doi.org/10.1016/j.micron.2008.07.008.
[21] HossainM. S., DAS, A. K., Akhter, S., Mian M. Y., & Muniappan, R. (2019). Development of biorational management for tomato leaf miner, Tuta absoluta. Journal of Biological Control, 33(2), 132–136. https://doi.org/10.18311/jbc/2019/23084.
[22] Mazimba, E., Nkunika, P. O. Y., Moonga, M. N., & Nyirenda, J. (2022). Efficacy of selected biopesticides against the tomato leaf miner , Tuta absoluta (Meyrick) (Lepidoptera : Gelechiidae), in Lusaka , Zambia. 37–49.
[23] Bajracharya, A. S., & Bhat., B. (2018). Geographical Distribution of South American Tomato Leaf Miner Tuta absoluta (Meyrick, 1917) (Lepidoptera: Gelechiidae) in Nepal. Journal of the Plant Protection Society, 5(1), 203–216. https://doi.org/10.3126/jpps.v5i0.47132.
[24] Abbott, W. S.(1925) .A method of computing the effectiveness of an insecticide. J. Econ. Entomol, 18(2), 265-267, 18(2), 265–267. https://doi.org/10.3109/13880209.2012.674950.
[25] Kandil, M. A.-H., Sammour, E. A., Abdel-Aziz, N. F., Agamy, E. A. E. M., El-Bakry, A. M., & Abdelmaksoud, N. M. (2020). Comparative toxicity of new insecticides generations against tomato leafminer Tuta absoluta and their biochemical effects on tomato plants. Bulletin of the National Research Centre, 44(1). https://doi.org/10.1186/s42269-020-00382-0.

[26] Dewan, M. M. (1989). Identity and Frequency of Occurrence of Fungi in Roots of Wheat and Rye Grass and Their Effect on Take-all and Host Growth. Ph. D. Thesis, Univ. Western Australia., 210.
[27] Petrini, O., & Fisher, P. J. (1986). Fungal endophytes in Salicornia perennis. Transactions - British Mycological Society, 87(4), 647–651. https://doi.org/10.1016/S0007-1536(86)80109-7.
[28] Mantzoukas, S., Chondrogiannis, C., & Grammatikopoulos, G. (2015). Effects of three endophytic entomopathogens on sweet sorghum and on the larvae of the stalk borer Sesamia nonagrioides. Entomologia Experimentalis et Applicata, 154(1), 78–87. https://doi.org/10.1111/eea.12262.
[29] Giannoulakis, E., Mantzoukas, S., Lagogiannis, I., Dervisoglou, S., & Perdikis, D. (2023). Efficacy of endophytic wild strains of entomopathogenic fungi against the tomato leafminer Tuta absoluta Meyrick) (Lepidoptera: Gelechiidae) in tomato plants. Egyptian Journal of Biological Pest Control, 33(1). https://doi.org/10.1186/s41938-023-00663-6.
[30] Sinno, M., Ranesi, M., Gioia, L., D'errico, G., & Woo, S. L. (2020). Endophytic fungi of tomato and their potential applications for crop improvement. Agriculture (Switzerland), 10(12), 1–22. https://doi.org/10.3390/AGRICULTURE10120587.
[31] Mohamed Mahmoud, F., Bendebbah, R., Benssaci, B., Toudji, F., Tafifet, L., & Krimi, Z. (2021). Entomopathogenic efficacy of the endophytic fungi: Clonostachys sp. and Beauveria bassiana on Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) larvae under laboratory and greenhouse conditions. Egyptian Journal of Biological Pest Control, 31(1). https://doi.org/10.1186/s41938-021-00392-8.
[32] Qayyum, M. A., Wakil, W., Arif, M. J., Sahi, S. T., & Dunlap, C. A. (2015). Infection of Helicoverpa armigera by endophytic Beauveria bassiana colonizing tomato plants. Biological Control, 90, 200–207. https://doi.org/10.1016/J.BIOCONTROL.2015.04.005.
[33] Montesinos, E. (2003). Development, registration and commercialization of microbial pesticides for plant protection. International Microbiology, 6(4), 245–252. https://doi.org/10.1007/s10123-003-0144-x.
[34] Kumar, R., Singh, S., & Singh, O. V. (2008). Bioconversion of lignocellulosic biomass: Biochemical and molecular perspectives. Journal of Industrial Microbiology and Biotechnology, 35(5), 377–391. https://doi.org/10.1007/s10295-008-0327-8.