

## Original article (Orijinal araştırma)

# Evaluation of the insecticidal activity of some Turkish entomopathogenic fungal isolates against second larval stage of *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae)<sup>1</sup>

Bazı Türkiye entomopatojen fungus izolatlarının *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae)'nin ikinci larva dönemine karşı insektisidal etkisinin değerlendirilmesi

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## Abstract

In this study, the efficacy of two local isolates of the entomopathogenic fungus (EPF) *Beauveria bassiana* (Bals.-Criv.) Vuill. (GOPT-DYYLD, BBE2) (Hypocreales: Cordycipitaceae) and one isolate of *Metarhizium brunneum* (Petch) (ORP-13) (Hypocreales: Clavicipitaceae) against the second instar larvae of the tomato leafminer, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) was determined. The study was conducted in 2023 at the Entomology Laboratory, Department of Plant Protection, Faculty of Agriculture, Tokat Gaziosmanpaşa University. As positive controls, the commercial preparation of *B. bassiana* strain Bb-1 and 120 g/L Spinetoram were used. The EPF isolates were evaluated in dose-mortality assays at concentrations ranging from  $1 \times 10^3$  to  $1 \times 10^9$  conidia/mL<sup>-1</sup>. The EPF isolates were evaluated using two different methods: Topical application bioassays and foliar spray application bioassays. In topical application bioassays, at a dose of  $1 \times 10^8$  conidia/mL<sup>-1</sup> on the 5th day, GOPT-DYYLD caused 69.8% mortality, BBE2 45.8%, ORP-13 29.2%, while *B. bassiana* strain Bb-1 caused 72.9% mortality and 120 g/L Spinetoram caused 96.9% mortality. In foliar spray application bioassays, at a dose of  $1 \times 10^8$  conidia/mL<sup>-1</sup> on the 5th day, GOPT-DYYLD caused 76.04% mortality, BBE2 54.2%, and ORP-13 24.5%. Promising results were obtained in the control of tomato leaf miner using the GOPT-DYYLD and BBE2 isolates.

**Keywords:** *Beauveria bassiana*, entomopathogenic fungus, *Metarhizium brunneum*, tomato, tomato leafminer

## Öz

Bu çalışmada entomopatojen fungus (EPF) *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae)'nin iki yerel izolatı (GOPT-DYYLD, BBE2) ve *Metarhizium brunneum* (Petch) (Hypocreales: Clavicipitaceae)'nin bir izolatının domates güvesi, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) 2. dönem larvalarına karşı etkinlikleri belirlenmiştir. Çalışma, 2023 yılında Tokat Gaziosmanpaşa Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, Entomoloji Laboratuvarında gerçekleştirilmiştir. Pozitif kontrol olarak ticari preparat *B. bassiana* strain Bb-1 ve 120 g/L Spinetoram kullanılmıştır. EPF izolatları,  $1 \times 10^3$ -  $1 \times 10^9$  konidia/mL<sup>-1</sup> konsantrasyonlarında doz-ölüm denemelerine tabi tutulmuştur. EPF izolatları, topikal uygulama ve yaprağa püskürtme denemeleri olmak üzere iki farklı yöntemle değerlendirilmiştir. Topikal uygulama denemelerinde  $1 \times 10^8$  konidi/mL<sup>-1</sup> dozunda 5'inci günde GOPT-DYYLD 69.8%, BBE2 45.8%, ORP-13 29.2% oranında ölüme neden olurken *B. bassiana* strain Bb-1 72.9%, ve 120 G/L Spinetoram 96.9% oranında ölüm oluşturmuştur. Yaprğa püskürtme denemelerinde EPF'lar  $1 \times 10^8$  konidi/mL<sup>-1</sup> dozunda 5. gün GOPT-DYYLD 75.5%, BBE2 53.2%, ORP-13 24.5% oranında ölüm göstermiştir. GOPT-DYYLD ve BBE2 izolatlarıyla domates güvesi mücadelesinde ümit verici sonuçlar elde edilmiştir.

**Anahtar sözcükler:** *Beauveria bassiana*, entomopatojen fungus, *Metarhizium brunneum*, domates, domates güvesi

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## Introduction

Tomato, *Solanum lycopersicum* L. (Solanales: Solanaceae) is one of the most extensively cultivated and economically significant vegetable crops across the globe (Nicola et al., 2009). The top producers are China and India, Türkiye is third in the world, with a production of approximately 13.1 million tons annually. The production of tomatoes, grown both in field and greenhouse conditions, consist of 41.2% of the total vegetable production in Türkiye (Demircan, 2023). As with the other cultivated plants, one of the main factors that causes yield and quality losses in tomatoes are diseases and pests (Sora, 2018). One of the significant pests is *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), commonly referred to as the tomato leaf miner (Öztemiz, 2012). The Tomato leaf miner was first detected in Türkiye in 2009 in the provinces of İzmir, Çanakkale, and Muğla (EPPO, 2010). Subsequently, the pest exhibited rapid dispersal, establishing itself in all geographical areas where tomatoes are cultivated. In addition to tomatoes, this pest also affects eggplant, *Solanum melongena* L. (Solanales: Solanaceae) and pepper, *Capsicum annuum* L. (Solanales: Solanaceae) (Gahramanova & Mamay, 2020). Therefore, *T. absoluta* has become one of the main pests in both field and greenhouse tomato cultivation (Öztemiz, 2012). Depending on climate conditions, *T. absoluta* can produce more than one generation per year. The adult forms can migrate several kilometers and are easily carried by the wind (Van Deventer, 2009). The larvae feed on the leaves, stems, and fruits, and can significantly decrease both yield and fruit quality. The pest can cause losses up to 80-100% in greenhouse and field conditions (Terzidis et al., 2014).

In the control of *T. absoluta*, synthetic chemical pesticides have been used widely in recent years. Since the larvae nourish themselves in the galleries they generate under the leaf epidermis, the effectiveness of insecticides in the control of the pest is restricted (Cabello et al., 2009). In addition, it was revealed that the pest developed resistance to insecticides (Öztemiz, 2012). Due to the resistance developed from the intensive use of insecticides, some pest control compounds have been found to be ineffective in achieving the expected results. Resistance development against a broad spectrum of insecticides including spinosyns, organophosphates, oxadiazines, pyrethroids, benzoylureas, avermectins, semicarbazones, and diamides has been recorded in pest species around the world (Siqueira et al., 2001; Silva et al., 2016). Due to the reduced effectiveness of chemical insecticides against this pest, the potential of entomopathogenic fungi as biological control agents has gained significant attention (Vega & Kaya, 2012).

Studies conducted worldwide have shown an increasing interest in the use of EPF against the *T. absoluta* (Tomato leaf miner) pest. These studies aim to utilize entomopathogenic fungi as an effective alternative for pest control within biological control methods. Specifically, fungal entomopathogens including *Metarhizium anisopliae* (Metschn.) Sorokin (Hypocreales: Clavicipitaceae), *Isaria fumosorosea* Wize (Hypocreales: Cordycipitaceae) and *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae) are recognized as effective microbial agents for the control of *T. absoluta* (Karaca et al., 2022). Several indigenous entomopathogenic fungal isolates have been reported to exhibit promising efficacy against *T. absoluta* larvae under Sudanese conditions (Hammad et al., 2022). Similarly, the susceptibility of *T. absoluta* to soil-applied entomopathogenic fungal biopesticides was evaluated, and certain fungal formulations were found to significantly reduce *T. absoluta* survival; this highlights the potential of entomopathogenic fungi as a biological control agent in integrated pest management strategies (Erasmus et al., 2021). Previous studies have reported that *B. bassiana* and *Metarhizium brunneum* (Petch) (Hypocreales: Clavicipitaceae) isolates have been identified and shown high efficacy against various insect pests. A study conducted on *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae) larvae demonstrated that *B. bassiana* was most effective at the highest dose, causing the greatest mortality (Koca, 2025). Furthermore, under laboratory conditions, *B. bassiana* and *M. brunneum* isolates were found to be virulent against the pine processionary moth, *Thaumetopoea wilkinsoni* Tams, 1925 (Lepidoptera: Notodontidae), indicating their potential for biological control (Yanar et al., 2023). These findings support

that the isolates possess a broad host spectrum and can be incorporated into integrated pest management programs. These biological control agents are intended to decrease dependency on chemical pesticides, thereby fostering environmentally sustainable agricultural practices (Shaili et al., 2025).

The primary objective of this research was to evaluate the efficacy of two indigenous isolates of the entomopathogenic fungus *B. bassiana* (GOPT-DYYLD, BBE2) and one isolate of *M. brunneum* (ORP-13) against the second stage larvae of the tomato leaf miner, *T. absoluta*. These isolates, obtained from Tokat and Ordu provinces, were tested for the first time against *T. absoluta* using both topical application and foliar spray application methods, thereby providing an original contribution to the literature.

## Materials and Methods

### Mass rearing of *Tuta absoluta*

The larvae of *T. absoluta* were collected from tomato-producing greenhouses in Tokat province and maintained on potted tomato plants kept under controlled environmental conditions ( $27\pm 1^\circ\text{C}$ ,  $75\pm 5\%$  RH, and a 16:8 h light:dark photoperiod). The adults emerging from the pupae were transferred to tomato plants grown in cages (30x50 cm in size). Cotton soaked in sugar solution was placed inside the cages to encourage egg-laying and feeding by the adults. To maintain the continuity of the stock culture, adult individuals were transferred onto healthy tomato seedlings cultivated in a separate cage (Taşçı & Muştu, 2023).

### Entomopathogenic fungal isolates used in experimental bioassays

Isolates of *Beauveria bassiana* (GOPT-DYYLD and BBE2) from Tokat and Ordu provinces, Türkiye, along with the *M. brunneum* ORP-13 isolate and a commercial formulation of *B. bassiana* strain Bb-1 (T. Stanes and Company Limited, Coimbatore, India) were tested against 2nd instar *T. absoluta* larvae (Table 1).

Table 1. Entomopathogenic fungal isolates used in the study

| Isolates       | Species                               | Source of Isolation                              | Accession Number |
|----------------|---------------------------------------|--|------------------|
| GOPT-DYYLD     | <i>Beauveria bassiana</i>             | Insect ( <i>Leptinotarsa decemlineata</i> )      | MK411548         |
| ORP-13         | <i>Metarhizium brunneum</i>           | Soil   | MW410195.1       |
| BBE2           | <i>Beauveria bassiana</i>             | Pepper   | MK411544         |
| NOSTALGİST BL® | <i>Beauveria bassiana</i> strain Bb-1 | T. Stanes and Company Limited, Coimbatore, India |                  |

Indigenous entomopathogenic fungal isolates derived from soil and insect sources were subcultured on potato dextrose agar (PDA) and maintained at  $25^\circ\text{C}$  for a duration of four weeks. The concentration of conidia within the resulting suspension was quantified using Thoma hemocytometer under a light microscope.

### Determination of the viability of conidiospores

Entomopathogenic fungus spores were collected from the petri dish using a spatula, transferred to 10 mL tubes containing sterile dH<sub>2</sub>O with 0.02% Tween 80, mixed, and spore count in the suspension was performed using Thoma hemocytometer (ISOLAB Laborgeräte GmbH, Eschau, Germany) under a light microscope. After dilution ( $1\times 10^5$  conidia/mL<sup>-1</sup>), 100 µl of the suspension was taken and spread onto Petri dishes containing PDA (prepared with 1.5% agar). To determine the germination rates, the petri dishes were incubated for 24 hours in an incubator at  $25\pm 2^\circ\text{C}$  with a 16:8-hour light/dark cycle. After incubation, the germination status of the spores was determined through counts under a microscope. This procedure was repeated three times, with 100 spores counted in each replicate.

### Topical application bioassays single dose screening tests on *Tuta absoluta* larvae

In the single-dose tests, the second stage larvae of *T. absoluta* were used. In the trials, larvae were treated using the topical application method. Fungal suspensions prepared at a concentration of  $1\times 10^8$  conidia/mL<sup>-1</sup>,

were applied dorsally at a volume of 1 µl per insect with a micro-applicator (Hamilton-Repeating Dispenser). The treated insects were introduced into 90 mm diameter glass petri dishes containing tomato leaves. Strain for comparison, *B. bassiana* strain Bb-1 (at a dose of  $1 \times 10^8$  conidia/mL<sup>-1</sup>), 120 g/L Spinetoram (50 mL/100 L water) and for negative control 0.02% Tween 80 solution were applied. All larvae used in the experiment were incubated at  $25 \pm 2^\circ\text{C}$ , with a 16:8 hour light/dark cycle and an average humidity of  $65 \pm 5\%$  for 10 days. Observations were made on the 1st, 3rd, 5th, 7th, and 9th days. Following the completion of the incubation phase, deceased larvae were collected and transferred into sterile 90 mm glass petri dishes containing moistened filter paper to maintain humidity. The dishes were then incubated at  $25 \pm 2^\circ\text{C}$  for 9 days. At the end of the incubation period, mycosis was observed. In the experiments, 10 larvae were used per replicate, with 5 replications and 2 repetitions for each treatment.

### Foliar spray application bioassays

In foliar spray application bioassays, fungal suspensions were prepared at a dose of  $1 \times 10^8$  conidia/mL<sup>-1</sup>, were applied to both surfaces of tomato leaves using a hand sprayer, with 300 µl (3 sprays) of the solution containing 0.02% Tween 80. Strain for comparison, *B. bassiana* strain Bb-1 (at a dose of  $1 \times 10^8$  conidia/mL<sup>-1</sup>), 120 g/L Spinetoram (50 mL/100 L water) and for negative control 0.02% Tween 80 solution were applied. In foliar spray application bioassays, the second-stage larvae of *T. absoluta* were used. Counts were made on the 1st, 3rd, 5th, 7th, and 9th day. The dead larvae were collected and incubated at  $25 \pm 2^\circ\text{C}$  and  $65 \pm 5\%$  relative humidity for 7 days. At the end of the incubation period, signs of mycosis were observed in the larvae. In the experiments, 10 larvae were used per replicate, with 5 replications and 2 repetitions for each treatment.

### Dose-mortality bioassays

In the study, solutions were prepared with concentrations of  $1 \times 10^3$ ,  $1 \times 10^5$ ,  $1 \times 10^7$ ,  $1 \times 10^8$  and  $1 \times 10^9$  conidia/mL<sup>-1</sup> using EPF isolates obtained from Tokat and Ordu provinces GOPT-DYYLD and BBE2 (*B. bassiana*). Dose mortality studies were conducted using the EPF isolates with both methods described above.

### Statistical analysis

Corrections to the *T. absoluta* mortality percentages were made using the method proposed by Abbott (1925). Before analysis data is converted by using sqrt (Square Root). Statistical analyses were conducted using one-way ANOVA in SPSS software (version 17), and differences between treatment means were assessed using Tukey's multiple comparison test with a confidence level of 95% (SPSS Inc., 2008). Data from concentration-response experiments were analyzed using the Polo-PC program (LeOra, 1994) to estimate Lethal Concentration and Lethal Time values.

## Results

### Topical application bioassays

*Beauveria bassiana* isolates caused immobility and color changes in the infected *T. absoluta* larvae. Data obtained from the laboratory experiments on the contact effect of topical application bioassays are presented in Table 2. In topical application bioassays, the mortality percentages started to show significant differences on the 3rd and 5th days following the treatment. It is observed that the positive control, 120 g/L Spinetoram, exhibited the highest lethal effect from the first day onwards, while ORP-13 *M. brunneum* showed the least effect (Table 2).

When the mortality rates of *T. absoluta* second instar larvae following the application of the positive controls, *B. bassiana* strain Bb-1 and 120 g/L Spinetoram, at the recommended doses were examined, it was observed that on the 5th day, the mortality rates reached 72.9% and 96.9%, respectively ( $F=470.23$ ;  $df=5, 54$ ;  $p<0.05$ ), and by the end of the 7th day, they reached 88.1% and 97.8% ( $F=811.43$ ;  $df=5, 54$ ;  $p<0.05$ ). *Metarhizium brunneum* ORP-13 isolate caused 29,16% larval mortality by 5th day ( $F=470.23$ ;

df=5, 54;  $p<0.05$ ), 33.9% on 7th day ( $F=811.43$ ; df=5, 54;  $p<0.05$ ), and 58.4% on 9th day ( $F=1684.93$ ; df=5, 54;  $p<0.05$ ), showing the lowest effect among all tested isolates ( $F=154.73$ ; df=5, 54;  $p<0.05$ ), (Table 2). In topical application bioassays, at a concentration of  $1 \times 10^8$  conidia/mL<sup>-1</sup>, the mean mortality rates of the *B. bassiana* isolates GOPT-DYYLD and BBE2 against the larvae, it was noted that by the 3rd day post-treatment, the GOPT-DYYLD isolate achieved a mortality rate of 53.61%, whereas the BBE2 isolate resulted in a comparatively lower mortality of 23.7% ( $F=172.97$ ; df=5, 54;  $p<0.05$ ), and by the 5th day, they reached 69.8% and 45.8% respectively ( $F=470.23$ ; df=5, 54;  $p<0.05$ ). By the end of the 7th day, the larval mortality rates of GOPT-DYYLD and BBE2 isolates reached 82.6 % and 75.01%, respectively ( $F=811.43$ ; df=5, 54;  $p<0.05$ ). GOPT-DYYLD and commercial *B. bassiana* Bb-1 mortality rates are at similar category statistically by the 5rd day post-treatment 69.8%, 72.9% ( $F=470.23$ ; df=5, 54;  $p<0.05$ ), by the 7th day post-treatment 82.6%, 88.1% ( $F=811.43$ ; df=5, 54;  $p<0.05$ ) and by the 9th day post-treatment 97.8%, 96.6% average mortality ( $F=1684.93$ ; df=5, 54;  $p<0.05$ ) respectively (Table 2). Results show that GOPT-DYYLD is a compatible isolate when comparing with the commercial one.

Table 2. Single-dose screening in topical application bioassays

| Treatment                      | Average percentage mortality rates (Corrected data) (%±S.E.) |                          |                          |                          |
|--------------------------------|--|--------------------------|--------------------------|--------------------------|
|                                | Day 3  | Day 5                    | Day 7                    | Day 9                    |
| GOPT- DYYLD                    | 53.61±4.14 <sup>ca</sup>                                     | 69.79±2.43 <sup>cd</sup> | 82.64±2.39 <sup>cd</sup> | 97.75±1.49 <sup>cd</sup> |
| BBE2                           | 23.72±2.75 <sup>b</sup>                                      | 45.83±3.02 <sup>c</sup>  | 75.05±2.31 <sup>c</sup>  | 92.13±2.39 <sup>c</sup>  |
| <i>B. bassiana</i> strain Bb-1 | 59.79±3.59 <sup>c</sup>                                      | 72.91±2.39 <sup>d</sup>  | 88.07±2.53 <sup>de</sup> | 96.62±1.71 <sup>cd</sup> |
| 120 G/L Spinetoram             | 81.44±2.99 <sup>d</sup>                                      | 96.87±5.09 <sup>e</sup>  | 97.83±1.44 <sup>e</sup>  | 100.00±0.00 <sup>d</sup> |
| ORP-13                         | 16.49±2.41 <sup>b</sup>                                      | 29.16±2.59 <sup>b</sup>  | 33.85±2.53 <sup>b</sup>  | 58.42±2.39 <sup>b</sup>  |
| Control                        | 0.00±0.00 <sup>a</sup>                                       | 0.00±0.00 <sup>a</sup>   | 0.00±0.00 <sup>a</sup>   | 0.00±0.00 <sup>a</sup>   |

\* As determined by the Tukey multiple comparison analysis ( $p \leq 0.05$ ), means sharing a common letter within a column do not differ statistically.

### Foliar spray application bioassays

The results have shown that the EPF species isolates significantly affected the mortality of *T. absoluta*. It has been shown that there are significant differences between the *B. bassiana* and *M. brunneum* isolates in causing the mortality. In foliar spray application bioassays as in topical application method, the mortality percentages started to show significant differences on the 3rd and 5th days following the treatment. It is observed that the positive control, 120 g/L Spinetoram, exhibited the highest lethal effect from the first day onwards, while ORP-13 *M. brunneum* showed the least effect (Table 3).

Table 3. Foliar spray application bioassays

| Treatment                      | Average percentage mortality rates (Corrected data) (%±S.E.) |                         |                          |                          |
|--------------------------------|--|-------------------------|--------------------------|--------------------------|
|                                | Day 3  | Day 5                   | Day 7                    | Day 9                    |
| GOPT- DYYLD                    | 65.31±4.08 <sup>de*</sup>                                    | 76.04±2.22 <sup>d</sup> | 92.55±1.63 <sup>de</sup> | 97.80±1.47 <sup>d</sup>  |
| BBE2                           | 43.88±4.09 <sup>c</sup>                                      | 54.17±2.78 <sup>c</sup> | 68.09±2.75 <sup>c</sup>  | 81.32±2.86 <sup>c</sup>  |
| <i>B. bassiana</i> strain Bb-1 | 54.08±3.49 <sup>cd</sup>                                     | 69.79±3.27 <sup>d</sup> | 87.23±3.09 <sup>d</sup>  | 95.60±2.43 <sup>d</sup>  |
| 120 G/L Spinetoram             | 81.63±3.66 <sup>e</sup>                                      | 93.75±1.70 <sup>e</sup> | 100.00±0.00 <sup>d</sup> | 100.00±0.00 <sup>d</sup> |
| ORP-13                         | 15.31±2.66 <sup>b</sup>                                      | 26.04±2.88 <sup>b</sup> | 37.23±2.48 <sup>b</sup>  | 65.93±3.46 <sup>b</sup>  |
| Control                        | 0.00±0.00 <sup>a</sup>                                       | 0.00±0.00 <sup>a</sup>  | 0.00±0.00 <sup>a</sup>   | 0.00±0.00 <sup>a</sup>   |

\* As determined by the Tukey multiple comparison analysis ( $p \leq 0.05$ ), means sharing a common letter within a column do not differ statistically.

In the results obtained from the foliar spray application bioassays, at a concentration of  $1 \times 10^8$  conidia/mL<sup>-1</sup>, the *B. bassiana* GOPT-DYYLD isolate achieved a larval mortality effect of 76.04% on the 5th day (F=424.99; df=5, 54;  $p < 0.05$ ), 92.6% by the end of the 7th day (F=817.47; df=5, 54;  $p < 0.05$ ) and 97.8% by the end of 9th day (F=1012.71; df=5, 54;  $p < 0.05$ ). When examining the average percentage mortality rates of *T. absoluta* larvae caused by the *B. bassiana* BBE2 isolate, the mortality effects were observed 54.2% mortality on the 5th day (F=424.99; df=5, 54;  $p < 0.05$ ), 68.1% on the 7th day (F=817.47; df=5, 54;  $p < 0.05$ ) and 81.3% by the end of 9th day (F=1012.71; df=5, 54;  $p < 0.05$ ). When examining the mortality rates of *T. absoluta* larvae following the application of the positive controls, *B. bassiana* strain Bb-1 and 120 g/L Spinetoram at the recommended doses, it was observed that by the end of the 3rd day, the mortality rates reached 54.1% and 81.6%, respectively (F=170.88; df=5, 54;  $p < 0.05$ ), and by the 5th day, they reached 69.8% and 93.8% mortality (F=424.99; df=5, 54;  $p < 0.05$ ) (Table 3). As in topical application method, GOPT-DYYLD and commercial *B. bassiana* Bb-1 mortality rates are at similar category statistically by the 5rd day post-treatment 76.04%, 69.8% (F=424.99; df=5, 54;  $p < 0.05$ ), by the 7th day post-treatment 92.6%, 87.2% (F=817.47; df=5, 54;  $p < 0.05$ ) and by the 9th day post-treatment 97.8%, 95.6% average mortality (F=1012.71; df=5, 54;  $p < 0.05$ ) respectively (Table 3). In foliar spray application also results show that GOPT-DYYLD is a compatible isolate when comparing with the commercial one.

### Dose-mortality bioassays

The data obtained from applications of the local EPF isolates GOPT-DYYLD and BBE2 (*B. bassiana*) at different doses on *T. absoluta* larvae were statistically analyzed, and the results are presented in Table 4.

Table 4. Mortality percentage of *Tuta absoluta* larvae treated with a range of doses of a *Beauveria bassiana* isolate in topical application bioassays

| Isolate number             | Average percentage mortality rates (Corrected data) (%±S.E.) |                            |                          |                           |                           |
|----------------------------|--|----------------------------|--------------------------|---------------------------|---------------------------|
|                            | Day 1  | Day 3                      | Day 5                    | Day 7                     | Day 9                     |
| GOPT-DYYLD $1 \times 10^3$ | 22.0±2.91 <sup>cd*</sup>                                     | 32.65±3.47 <sup>cd</sup>   | 48.45±3.76 <sup>bc</sup> | 57.89±3.84 <sup>b</sup>   | 85.11±3.62 <sup>b</sup>   |
| GOPT-DYYLD $1 \times 10^5$ | 27.0±3.34 <sup>d</sup>                                       | 37.76±4.68 <sup>cde</sup>  | 52.58±3.83 <sup>bc</sup> | 61.05±3.53 <sup>bc</sup>  | 89.36±3.55 <sup>bcd</sup> |
| GOPT-DYYLD $1 \times 10^7$ | 31.0±4.06 <sup>d</sup>                                       | 47.96±3.86 <sup>def</sup>  | 58.76±3.43 <sup>de</sup> | 66.31±2.63 <sup>bcd</sup> | 94.68±1.77 <sup>bcd</sup> |
| GOPT-DYYLD $1 \times 10^8$ | 30.0±2.98 <sup>d</sup>                                       | 54.08±4.11 <sup>ef</sup>   | 70.1±2.41 <sup>e</sup>   | 85.26±2.81 <sup>f</sup>   | 97.87±1.42 <sup>cd</sup>  |
| GOPT-DYYLD $1 \times 10^9$ | 35.0±2.69 <sup>d</sup>                                       | 56.12±3.06 <sup>f</sup>    | 73.19±3.15 <sup>e</sup>  | 88.42±3.31 <sup>f</sup>   | 100.0±0.00 <sup>d</sup>   |
| BBE2 $1 \times 10^3$       | 9.0±1.79 <sup>b</sup>  | 18.37±2.63 <sup>b</sup>    | 38.14±3.44 <sup>b</sup>  | 66.67±3.03 <sup>bcd</sup> | 88.29±2.48 <sup>bc</sup>  |
| BBE2 $1 \times 10^5$       | 10.0±2.11 <sup>b</sup>                                       | 26.53±4.25 <sup>bc</sup>   | 40.21±3.7 <sup>b</sup>   | 68.75±2.69 <sup>bcd</sup> | 88.29±2.48 <sup>bc</sup>  |
| BBE2 $1 \times 10^7$       | 12.0±2.00 <sup>bc</sup>                                      | 38.78±4.02 <sup>cdef</sup> | 44.33±4.4 <sup>b</sup>   | 73.96±3.56 <sup>cde</sup> | 90.42±2.48 <sup>cd</sup>  |
| BBE2 $1 \times 10^8$       | 14.0±3.06 <sup>bc</sup>                                      | 39.79±3.21 <sup>cdef</sup> | 46.39±2.99 <sup>bc</sup> | 76.04±2.22 <sup>de</sup>  | 92.55±2.27 <sup>bcd</sup> |
| BBE2 $1 \times 10^9$       | 20.0±2.98 <sup>bcd</sup>                                     | 43.88±3.21 <sup>def</sup>  | 50.51±4.01 <sup>bc</sup> | 80.21±2.43 <sup>de</sup>  | 95.74±1.74 <sup>cd</sup>  |
| Control                    | 0.00±0.00 <sup>a</sup>                                       | 0.00±0.00 <sup>a</sup>     | 0.00±0.00 <sup>a</sup>   | 0.00±0.00 <sup>a</sup>    | 0.00±0.00 <sup>a</sup>    |

\* As determined by the Tukey multiple comparison analysis ( $p \leq 0.05$ ), means sharing a common letter within a column do not differ statistically.

On day 5 of the topical application, the dose of  $1 \times 10^9$  (73.2%) for GOPT-DYYLD showed the highest mortality effect (F=91,92; df=10, 99;  $p < 0.05$ ). GOPT-DYYLD mortality rates on the 7th day of the application at concentrations of  $1 \times 10^3$ ,  $1 \times 10^5$ ,  $1 \times 10^7$ ,  $1 \times 10^8$ , and  $1 \times 10^9$  conidia/mL<sup>-1</sup> were 57.9%, 61.1%, 66.3%, 85.3%, and 88.4%, respectively (F=216.43; df=10, 99;  $p < 0.05$ ). It was observed that for the GOPT-DYYLD isolate, at all doses, there was a time dependent increase in the average mortality rates from the 1st day to the 9th day. In topical application bioassays with the BBE2 isolate, the 5th day data showed that at a concentration of  $1 \times 10^7$ ,  $1 \times 10^8$  and  $1 \times 10^9$  conidia/mL<sup>-1</sup>, the average mortality rate was 44.3%, 46.4% and 50.5%, respectively (F=91,92; df=10, 99;  $p < 0.05$ ). BBE2 mortality rates on the 7th day of the application at concentrations of  $1 \times 10^3$ ,  $1 \times 10^5$ ,  $1 \times 10^7$ ,  $1 \times 10^8$ , and  $1 \times 10^9$  conidia/mL<sup>-1</sup> were 66.7%, 68.8%, 73.9%, 76.04%, and 80.2%, respectively (F=216.43; df=10, 99;  $p < 0.05$ ), (Table 4).

The effect of GOPT-DYYLD foliar spray application bioassays on the second instar *T. absoluta* larvae is presented in Table 5. It was observed that the efficacy of the GOPT-DYYLD isolate increased at different doses. In the mortality data on the 7th day, the doses of  $1 \times 10^3$ ,  $1 \times 10^5$ ,  $1 \times 10^7$ ,  $1 \times 10^8$ , and  $1 \times 10^9$  showed mortality rates of 68.8%, 72.9%, 88.5%, 92.7%, 95.8% respectively ( $F=319.45$ ;  $df=10, 99$ ;  $p<0.05$ ). The BBE2 isolate on the 7th day at concentrations of  $1 \times 10^3$ ,  $1 \times 10^5$ ,  $1 \times 10^7$ ,  $1 \times 10^8$ , and  $1 \times 10^9$  conidia/mL<sup>-1</sup> caused mortality rates 63.5%, 65.6%, 78.1%, 80.2%, and 84.4%, respectively ( $F=319.45$ ;  $df=10, 99$ ;  $p<0.05$ ) (Table 5).

Table 5. Mortality percentage of *Tuta absoluta* larvae treated with a range of doses of a *Beauveria bassiana* isolate in foliar spray application bioassays

| Isolate number             | Average percentage mortality rates (%±S.E.) |                           |                           |                          |                            |
|----------------------------|---|---------------------------|---------------------------|--------------------------|----------------------------|
|                            | Day 1                                       | Day 3                     | Day 5                     | Day 7                    | Day 9                      |
| GOPT-DYYLD $1 \times 10^3$ | 21.0±2.33 <sup>bc*</sup>                    | 33.68±3.8 <sup>bcd</sup>  | 49.48±2.85 <sup>bcd</sup> | 68.75±2.69 <sup>bc</sup> | 85.11±3.25 <sup>b</sup>    |
| GOPT-DYYLD $1 \times 10^5$ | 27.0±3.00 <sup>de</sup>                     | 37.76±3.21 <sup>bc</sup>  | 53.61±2.77 <sup>e</sup>   | 72.92±3.18 <sup>e</sup>  | 90.42±2.33 <sup>de</sup>   |
| GOPT-DYYLD $1 \times 10^7$ | 37.0±4.22 <sup>de</sup>                     | 60.2±3.21 <sup>d</sup>    | 71.13±3.7 <sup>de</sup>   | 88.54±1.87 <sup>de</sup> | 96.81±1.63 <sup>cde</sup>  |
| GOPT-DYYLD $1 \times 10^8$ | 38.0±3.59 <sup>de</sup>                     | 65.31±4.08 <sup>d</sup>   | 76.29±2.2 <sup>e</sup>    | 92.71±1.59 <sup>e</sup>  | 97.87±1.42 <sup>de</sup>   |
| GOPT-DYYLD $1 \times 10^9$ | 46.0±3.39 <sup>e</sup>                      | 69.39±2.15 <sup>d</sup>   | 79.38±2.66 <sup>e</sup>   | 95.83±1.7 <sup>e</sup>   | 100.0±0.00 <sup>e</sup>    |
| BBE2 $1 \times 10^3$       | 17.0±3.00 <sup>b</sup>                      | 28.57±2.15 <sup>b</sup>   | 40.21±3.37 <sup>b</sup>   | 63.54±2.79 <sup>b</sup>  | 87.23±2.65 <sup>bc</sup>   |
| BBE2 $1 \times 10^5$       | 21.0±3.14 <sup>bc</sup>                     | 31.63±2.66 <sup>bc</sup>  | 44.33±2.75 <sup>bc</sup>  | 65.63±3.96 <sup>b</sup>  | 89.36±2.75 <sup>bcd</sup>  |
| BBE2 $1 \times 10^7$       | 26.0±3.39 <sup>bcd</sup>                    | 40.82±4.09 <sup>bcd</sup> | 52.57±2.75 <sup>cd</sup>  | 78.13±2.88 <sup>cd</sup> | 91.49±3.09 <sup>bode</sup> |
| BBE2 $1 \times 10^8$       | 28.0±2.90 <sup>bcd</sup>                    | 43.88±4.09 <sup>cd</sup>  | 54.64±2.75 <sup>cd</sup>  | 80.21±2.43 <sup>cd</sup> | 94.68±1.77 <sup>cde</sup>  |
| BBE2 $1 \times 10^9$       | 32.0±2.00 <sup>cde</sup>                    | 45.92±3.74 <sup>de</sup>  | 58.76±2.66 <sup>de</sup>  | 84.36±2.79 <sup>de</sup> | 96.81±1.63 <sup>cde</sup>  |
| Control                    | 0.00±0.00 <sup>a</sup>                      | 0.00±0.00 <sup>a</sup>    | 0.00±0.00 <sup>a</sup>    | 0.00±0.00 <sup>a</sup>   | 0.00±0.00 <sup>a</sup>     |

\* As determined by the Tukey multiple comparison analysis ( $p \leq 0.05$ ), means sharing a common letter within a column do not differ statistically.

The slopes of the dose mortality relationship for second instar larvae of the tomato leaf miner treated by topical application of GOPT-DYYLD and BBE2 were 0.02 and 0.23, respectively, while the slopes obtained from foliar spray applications of GOPT-DYYLD and BBE2 were 0.22 and 0.10, respectively (Table 6). For the topical application, BBE2 and GOPT-DYYLD, as well as the foliar spray application of GOPT-DYYLD, exhibited the same LC<sub>50</sub> value (95% fiducial limits), estimated as 0.01 conidia mL<sup>-1</sup> (0.00–2.73). The LC<sub>90</sub> values (95% fiducial limits) for the topical application of GOPT-DYYLD and BBE2 were  $2.6 \times 10^4$  conidia mL<sup>-1</sup> ( $1.5 \times 10^3$ – $2.01 \times 10^5$ ) and  $2.6 \times 10^3$  conidia mL<sup>-1</sup> ( $0.6 \times 10^2$ – $2.4 \times 10^5$ ), respectively. LC<sub>50</sub> and LC<sub>90</sub> values for the foliar spray application of BBE2 could not be calculated (Table 6). Based on these results, the isolates GOPT-DYYLD and BBE2 can be considered highly promising in terms of their efficacy.

Table 6. LC<sub>50</sub> and LC<sub>90</sub> values of *Tuta absoluta* larvae after a 9-day incubation period

| Treatment                           | N   | df | Slope±SE  | LC <sub>50</sub> (%95 confidence interval) | LC <sub>90</sub> (%95 confidence interval) | χ <sup>2</sup> | t ratio |
|-------------------------------------|-----|----|-----------|--|--|----------------|---------|
| GOPT-DYYLD-Topical application      | 500 | 48 | 0.02±0.04 | 0.016 (0.00-2.73)                          | 25592.8 (1455.8-200484.0)                  | 22.43          | 1.46    |
| BBE2-Topical application            | 500 | 48 | 0.23±0.05 | 0.007 (0.00-1.56)                          | 2571.0 (55.54-23789)                       | 39.23          | 1.74    |
| GOPT-DYYLD-Foliar spray application | 500 | 48 | 0.22±0.05 | 0.008 (0.00-1.72)                          | 3290.1 (69.8-29182.9)                      | 36.63          | 1.69    |
| BBE2-Foliar spray application       | 500 | 48 | 0.10±0.03 | -  | -  | 17.87          | 3.38    |

In the time-mortality study, the LT<sub>50</sub> and LT<sub>90</sub> values for the second instar *T. absoluta* larvae were calculated and are presented in Table 6. The lowest LT<sub>50</sub> and LT<sub>90</sub> values were obtained in the foliar spray application bioassays with the GOPT-DYYLD *B. bassiana* isolate, which were 1.60 and 6.93 days at the end of ninth day respectively. This isolation was determined to be the most virulent against the second instar larvae (Table 7).

Table 7. LT<sub>50</sub> and LT<sub>90</sub> values of *Tuta absoluta* larvae at a concentration of 1x10<sup>8</sup> conidia/ml after a 9-day incubation period

| Treatment                           | N   | df | Slope±SE  | LC <sub>50</sub> (%95 confidence interval) | LC <sub>90</sub> (%95 confidence interval) | χ <sup>2</sup> | t ratio |
|-------------------------------------|-----|----|-----------|--|--|----------------|---------|
| GOPT-DYYLD-Topical application      | 500 | 48 | 2.09±0.19 | 2.12 (1.77-2.47)                           | 8.73 (7.20-11.29)                          | 33.79          | -5.59   |
| BBE2-Topical application            | 500 | 48 | 2.35±0.20 | 3.55 (3.12-4.00)                           | 12.46 (10.22-16.28)                        | 41.65          | -9.02   |
| GOPT-DYYLD-Foliar spray application | 500 | 48 | 2.03±0.19 | 1.60 (1.28-1.91)                           | 6.93 (5.67-8.73)                           | 30.32          | -3.53   |
| BBE2-Foliar spray application       | 500 | 48 | 1.89±0.18 | 2.68 (2.24-3.11)                           | 12.68 (9.97-17.75)                         | 42.46          | -6.55   |

## Discussion

In present study, the effect of entomopathogenic fungi of the species *B. bassiana* (GOPT-DYYLD and BBE2) and *M. brunneum* (ORP-13) on tomato leaf miner larvae was evaluated under laboratory conditions. It was determined that the mortality rates caused by the *B. bassiana* isolates were higher compared to the *M. brunneum* isolate (Tables 2 and 3). The data obtained from the application of *B. bassiana* against *T. absoluta* larvae revealed that it was effective, starting from the 3rd day post inoculation. Findings indicate that *B. bassiana* (GOPT-DYYLD isolate), applied at a concentration of 1x10<sup>8</sup> conidia/mL<sup>-1</sup>, exerted a considerable lethal effect on *T. absoluta* larvae by the fifth day, resulting in mortality rates exceeding 70% (Tables 2 and 3). Numerous studies have revealed the effectiveness of entomopathogenic fungi in targeting various developmental stages of *T. absoluta* including eggs, larvae, pupae, and adults-under both controlled laboratory and field environments (Silva et al., 2020; Chouikhi et al., 2022; Hammad et al., 2022). The commercial entomopathogenic fungus (EPF) formulations, Metatech® WP [*M. anisopliae*], Beauvitech® WP [*B. bassiana*], and Botanigard ES [*B. bassiana*], were evaluated against 3rd instar *T. absoluta* larvae at a concentration of 10<sup>8</sup> spores/ml<sup>-1</sup>. Substantial differences in pathogenicity ( $p < 0.01$ ) were observed between the treatment groups between the 4th and 6th days after inoculation. Metatech® WP and Beauvitech® WP showed the highest efficacy with mortality rates of 82.8% and 60.8%, respectively, and LT<sub>50</sub> values of 3.9 and 5.2 days, respectively (Ndereyimana, 2019). İnanlı et al. (2012) examined the effectiveness of the commercial entomopathogenic fungi *B. bassiana* (Dopteril SC, 125 ml/100 L of water) and *M. anisopliae* (Met52 EC, 100 ml/100 L of water) using the dipping method against both larval and egg stages of *T. absoluta*. The effectiveness of *B. bassiana* on the egg stage was found to be 41.67% and 66.67% at the 7th and 9th days, respectively, while its effectiveness on the 1st larval stage was 4.17% and 12.50%. In contrast, the effectiveness of *M. anisopliae* on the 1st larval stage was 91.67% in both the 7th and 9th days. In our study, the efficacy of the entomopathogenic fungus *B. bassiana* against *T. absoluta* was investigated using both topical application and spraying methods. With the topical application method, the GOPT-DYYLD isolate caused 82.6% and 97.8% mortality in the second larval stage at days 7 and 9, respectively. When the same isolate was applied via foliar spraying, mortality rates were 92.6% and 97.9% at the same time points. The ORP13 isolate resulted in 33.9% and 58.4% mortality with topical application, and 37.2% and 65.9% with foliar spraying application method, on days 7 and 9, respectively. Factors such as the application method and fungus species significantly affected larval mortality. These two methods were chosen to evaluate both the theoretical and practical efficacy of the isolates. In the topical application, the conidial suspension was applied directly onto the larvae, allowing the maximum pathogenic potential to be observed through contact alone. In the foliar spray, the conidia were applied to the leaf surface, and larvae were exposed to the fungus both through contact and by ingesting the treated leaf. Thus, the two methods together allowed the assessment of both laboratory potential and field-relevant performance.

The diversity of entomopathogenic fungal (EPF) species and isolates together with conidial concentrations impacting the efficacy of EPF against *T. absoluta* larvae have been widely studied (Aynalem et al., 2021; Dabsu & Kovancı, 2022). The present study corroborates this body of work by demonstrating that both isolate identity and inoculum concentration significantly influence larval mortality. In particular, the GOPT-DYYLD and BBE2 isolates exhibited substantial pathogenicity under both topical and foliar spray

applications, with concentration-dependent increases in mortality that are consistent with the general patterns reported in the literature. In the present assay GOTP- DYYLD isolate, using topical and spray methods, a mortality rate of 85-88% was observed on the 7th day at doses of  $1 \times 10^8$  and  $1 \times 10^9$  conidia/mL<sup>-1</sup>, whereas BBE2 isolate yielded mortality rates of 76-80% under comparable conditions. Aynalem et al. (2021) applied 27 different *B. bassiana* isolates at a concentration of  $1 \times 10^7$  conidia/L<sup>-1</sup> to the 2nd and 3rd larvae stage of *T. absoluta* and reported mortality rates of 65.7%-95.7% and 68.3%-95.0%, respectively, by the 7th day. In the study by Silva et al. (2020), the application of *B. bassiana* LPP139 isolate at a dose of  $1 \times 10^8$  conidia/mL<sup>-1</sup> to leaf surfaces induced in a 90% mortality rate against the 2nd and 3rd larvae stage of *T. absoluta* within 10 days. Similarly, Dabsu & Kovancı (2022) tested the effectiveness of entomopathogenic fungi on *T. absoluta* and found that *M. anisopliae* Ak-12 achieved a mortality rate of 80.77%, while *B. bassiana* Ak-10 at the concentration of  $1 \times 10^{10}$  conidia/mL<sup>-1</sup> caused a mortality rate of 91%. Similarly, the findings of present study revealed that variations in isolate and concentration significantly affect the mortality rates of *T. absoluta* larvae. Larval mortality generally increased with higher concentrations and over time, indicating a concentration-dependent effect, with the highest mortality observed at  $1 \times 10^8$  and  $1 \times 10^9$  conidia/mL<sup>-1</sup>. Nevertheless, on certain days, no significant differences were detected between some concentrations. This phenomenon may be attributed to variation in larval susceptibility, differences in fungal infection efficiency, or environmental factors during incubation. Overall,  $1 \times 10^8$  and  $1 \times 10^9$  conidia/mL<sup>-1</sup> produced the highest mortality and can be recommended for future biological control applications. Similarly, Tadele & Emanu (2017) demonstrated that the entomopathogenic fungi *B. bassiana* (PPRC-56) and *M. anisopliae* (PPRC-2) showed significant efficacy against *T. absoluta* larvae under both laboratory and greenhouse conditions at concentrations of  $5 \times 10^7$ ,  $2.5 \times 10^8$ , and  $2.5 \times 10^9$  conidia/mL<sup>-1</sup>. Under laboratory conditions, mortality rates ranged from 79.17% to 95.83% by the 5th day, while under greenhouse conditions, mortality rates ranged from 73.0% to 84.04%. The highest mortality rate was observed with the *M. anisopliae* isolate at a concentration of  $2.5 \times 10^9$  conidia/mL<sup>-1</sup>. This finding broadens the potential applications of these pathogens in biological control. The performance of GOPT-DYYLD at  $11 \times 10^8$  and  $1 \times 10^9$  conidia/mL<sup>-1</sup> is therefore comparable to previous reports, the pathogenicity ranges described for these widely studied isolates.

This study also evaluated the effectiveness of a commercial microbial insecticide based on the *B. bassiana* strain Bb<sup>-1</sup> at a dose of  $1 \times 10^8$  against the second instar larvae of *T. absoluta*. The average mortality rates on the 5th day were found to be 72.9% and 79.2% for topical and foliar spray application bioassays, respectively. In a similar study, Erol et al. (2021) applied the *B. bassiana* Bb-1 bioinsecticide at a concentration of  $1 \times 10^8$  conidia/mL<sup>-1</sup> to the second instar *T. absoluta* larvae using a hand sprayer and found a mortality rate of 28% on the 3rd day and 40% on the 5th day. Differences in application methods, environmental factors, and population susceptibility appear to explain the higher mortality rates observed in this study. The higher efficacy observed in foliar spray applications compared to topical applications suggests that the larvae may be more effectively ingest the fungal conidia through feeding. These findings highlight the potential of *B. bassiana*-based bioinsecticides as an alternative in the control of *T. absoluta* and emphasize the decisive role of application methods and environmental factors on efficacy.

The remarkably low LC<sub>50</sub> values obtained from both topical and foliar spray applications (0.01 conidia mL<sup>-1</sup>, with overlapping 95% fiducial limits) indicate a high level of virulence. The shallow slopes obtained for the dose–mortality curves, particularly for the topical application, suggest a broad distribution of larval susceptibility to the fungal inoculum, which may be attributed to physiological heterogeneity among larvae or to variability in conidial attachment and penetration processes. Chouikhi et al. (2022) reported considerably higher median lethal concentration values for *B. bassiana* ATTC and R444LC isolates. In their study, the first method (spraying tomato leaf lets bearing active L2 mines) resulted in LC<sub>50</sub> values of  $2.63 \times 10^7$  and  $3.39 \times 10^8$  conidia mL<sup>-1</sup>, whereas the second method (transferring larvae onto treated fresh leaflets) yielded LC<sub>50</sub> values of  $4.76 \times 10^8$  and  $1.05 \times 10^7$  conidia mL<sup>-1</sup>. The second method is more analogous to the foliar application used in the present study and therefore provides an appropriate comparator. The

magnitude of difference between their LC<sub>50</sub> estimates and those of GOPT-DYYLD and BBE2 strongly suggests intrinsically greater pathogenic potential in our isolates. Methodological differences likely contribute to these contrasts. For instance, leaf mines can physically impede conidial contact with larvae, thereby reducing infection efficiency in some experimental designs. Conversely, the direct conidial deposition achieved through topical application in our study maximized exposure and may have accelerated infection dynamics. Biological variation among isolates also remains a crucial factor, as *B. bassiana* strain-level differences in virulence, sporulation capacity, cuticle-degrading enzyme activity, and host specificity are well documented.

In the present study, the time-mortality data showed LT<sub>50</sub> values for the 1x10<sup>8</sup> conidia/ml<sup>-1</sup> dose. For the topical application, GOPT-DYYLD and BBE2 had LT<sub>50</sub> values of 2.12 (1.77-2.47)/day and 3.55 (3.12-4.00)/day, respectively. In the foliar spray application method, the LT<sub>50</sub> values were 1.60 (1.28-1.91)/day for GOPT-DYYLD and 2.68 (2.24-3.11)/day for BBE2. Klieber & Reineke (2016) studied the effectiveness of a commercial microbial insecticide utilizing *B. bassiana* against all developmental stages of *T. absoluta* and found that the average mortality rates ranged from 30% to 50% within 15 days. The studies conducted indicate that different fungal species and concentrations can increase mortality rates in *T. absoluta*, and that application methods significantly influence the overall efficacy. The results obtained with the local isolates (GOPT-DYYLD and BBE2) indicate their potential effectiveness under the tested conditions. The EPFs used in this study (*M. brunneum*, *B. bassiana*) are species that have frequently been examined in previous studies on plant-pathogen interactions (Akutse et al., 2013; Woo et al., 2014; Greenfield et al., 2016; Ramakuwela et al., 2020). The local isolates used in the present study (*B. bassiana* GOPT-DYYLD and BBE2, *M. brunneum* ORP-13) were tested under laboratory conditions, with GOPT-DYYLD exhibiting the highest virulence against second instar larvae of *T. absoluta* at the highest concentration. These findings demonstrate the potential of the tested isolates as biological control agents, and it will be critical to explore the potential of these promising EPFs in field conditions.

Overall, the comparative findings position GOPT-DYYLD (and to a slightly lesser extent BBE2) as highly promising candidates for integration into *T. absoluta* management strategies. Their virulence, low LC<sub>50</sub> values, and consistent performance across application methods indicate that they are promising EPF isolates for future development as microbial control agents, potentially performing several previously characterized isolates and certain commercial formulations.

## Conclusion

This study demonstrated the potential of the GOPT-DYYLD, and BBE2 isolates as promising biological control agents against second instar larvae of *T. absoluta*. GOPT-DYYLD isolate exhibited the highest efficacy, with mortality rates of 82.6% and 92.6% at 1x10<sup>8</sup> conidia/ml<sup>-1</sup> dose in topical and foliar spray bioassays, respectively, within seven days post-inoculation. Moreover, the lower LC<sub>50</sub> and LT<sub>50</sub> values observed for GOPT-DYYLD suggest a more rapid insecticidal action compared to the other isolates. These findings highlight the considerable potential of GOPT-DYYLD for integrated pest management programs. However, further research is required to evaluate its effectiveness and feasibility under field conditions.

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