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RESEARCH

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Efficacy of endophytic wild strains of entomopathogenic fungi against the tomato leafminer *Tuta absoluta* Meyrick) (Lepidoptera: Gelechiidae) in tomato plants

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Abstract

Background Tomato (*Solanum lycopersicum* L.) is a vegetable of great economic value. The tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a serious pest of tomato. Its control is difficult due to the protection of the larvae in the galleries they create its fast resistance development too many active ingredients and due to its many generations per year. Many entomopathogenic fungi (EPF) have been reported as endophytes for various plant pathogens and pests. In this study, the endophyticity of *Beauveria bassiana* Balsamo (Vuillemin) (Hypocreales: Cordycipitaceae) and *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) isolated from the soils of Crete against larvae of *T. absoluta* in tomato plants was examined.

Results As a result, the EPF isolates of Crete decreased both the distance of larval travel from the egg to the opening of the gallery and the weight of the *T. absoluta* larvae, but also colonized the tomato plants. The entomopathogenic fungi were isolated from tomatoes again. Mycelium began to appear 7 days later and had developed completely after 12 days at 25 ± 2 °C and in 95% RH. Interestingly, the period between the hatched egg and the emerged first instar *T. absoluta* larvae was affected significantly by the different treatments, but not by "repetition" or "plant". Interestingly, the distance was further significantly shortened after treatment with *M. anisopliae* isolate Crete and *B. bassiana* isolate Crete and the commercial strain. The development period of *T. absoluta* larvae varied significantly among the treatments, but not by repetition in time or plant. The treatments caused a significant variation in the weight of *T. absoluta* pupae. The lowest weight was recorded in the plants colonized by *M. anisopliae* isolate Crete.

Conclusions The study demonstrated the ability of the *B. bassiana and M. anisopliae* from Crete to colonize the tomato plants and the wild strains of Crete affect the distance of larvae. With the presence of endophytic EPF, the period between the hatched egg and the emerged first instar T. absoluta larvae was decreased, as does the weight of the *T. absoluta* pupae.

Keywords *Tuta absoluta*, Entomopathogenic fungi, Endophytes, *Beauveria bassiana*, *Metarhizium anisopliae*

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Background

Tomato (*Solanum lycopersicum* L.) (Solanales: Solanaceae) is one of the most popular vegetable crops in the world. Tomato crop is infested by various pests such as aphids, thrips, leafminers, lepidopteran larvae and mites (Mukwa et al. 2020). The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is one of the most serious pests of tomatoes (Han et al. 2019). This is due to its rapid resistance development and the adverse effects of insecticides on natural enemies (Guedes et al. 2019). There is a need for alternative control methods to suppress *T. absoluta* infestation. Among them, EPF are a useful tool for effective and environmentally friendly management of *T. absoluta*. Klieber and Reineke (2015) reported that *Beauveria bassiana* Balsamo (Vuillemin) (Hypocreales: Cordycipitaceae) caused high mortality rates and significantly reduced the longevity of *T. absoluta* larvae when fed on treated leaves. Application of *B. bassiana* by spraying adversely affected the life table parameters of *T. absoluta* (Younes et al. 2018). Commercial biopesticides based on *B. bassiana* and *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) were effective against larvae of *T. absoluta* (Ndereyimana et al. 2019).

Besides their efficacy against *T. absoluta* when used as epiphytes on the surface of tomato leaves, evidence existed showing that entomopathogens may offer benefits to plant protection by their ability to endophytically colonize plants. This can be particularly relevant in the cases of cryptic pests such as *T. absoluta* whose larvae develop by feeding inside leaf tissues. Endophytes are microorganisms that spend at least part of their lives in a non-parasitic association with plants (Mantzoukas et al. 2021). As endophytes, entomopathogens are protected from adverse environmental conditions such as ultraviolent radiation or unfavorable climatic conditions (Dash et al. 2018) offering protection for long periods (Sword et al. 2017). In addition, endophytes may promote plant growth (Barelli et al. 2016). *B. bassiana* and *M. anisopliae,* when artificially established as endophytes on tomato plants, reduced symptoms of several fungal pathogens and caused adverse effects on tomato insect pests including *T. absoluta* (Mwamburi 2021). In addition, the endophytic nature of *B. bassiana* and *M. anisopliae* has not been reported as negatively affect the development and fruiting processes of colonized host plants (Tefera and Vidal 2009). Therefore, the endophytic establishment of EPF in tomato plants may consist of a valuable alternative strategy to suppress populations *T. absoluta*.

Toward the further developments in the use of endophytes and focusing on the practical applications of this new pest management approach, the selection of the most effective and, at the same time, easy to apply plant inoculation technique is one of the most urgent needs. Allegrucci et al. (2017) compared the endophytic establishment rate of *B. bassiana* in tomato plants when inoculated by leaf spraying, root dipping or seed inoculation and the most effective technique was the leaf spraying, followed by root dipping, whereas seed inoculation gave poor results. However, leaf spraying may not be the most selective approach since *B. bassiana* may harm natural enemies of insect pests, whereas root dipping may be too laborious for practical application. In another study, seed inoculation was again tested for *B. bassiana* but it showed a low colonization rate of tomato leaves, although it showed a high colonization rate in roots. In the same study, *M. anisopliae* isolates failed to colonize tomato (Agbessenou et al. 2020). Therefore, an alternative approach to inoculate tomato plants would be through irrigation. This technique can be easily applied without additional cost whereas does not directly affect natural enemies. Hammad et al. (2022) showed that soil drenching was effective in the endophytic colonization of tomato plants with *B. bassiana*. However, this technique has not been evaluated against *T. absoluta* using either *B. bassiana* or *M. anisopliae*. Inoculation through irrigation is simple but can be a challenging issue if considering that generally the inoculation efficacy of plant tissues by EPF depends on several interacting factors such as plant tissue, plant genotype and entomopathogen isolate type (Hardoim et al. 2015). Therefore, to tackle with these complexities, the exploration of more than one isolates of each EPN may be a valuable research strategy.

The aim of this study was to examine: 1. the potential of local isolates of *B. bassiana* and *M. anisopliae* and commercial *B. bassiana* to endophytically colonize tomato plants when introduced by root drenching and 2. evaluate the potential of the inoculated tomato plants to become less suitable for larval development of *T. absoluta.*

Methods

Plant material

In this study, tomato plants (ELPIDA F1, Spirou S.A., Athens, Greece) were developed from seeds in plastic seed trays with compost (Bas Van Burren B.V, The Netherlands). At the stage of 2–3 leaves, the seedlings were transplanted into plastic pots (12 X 10 cm) filled with the same substrate. The plants grew in entomological cages ($100 \times 80 \times 70$ cm) kept under natural lighting conditions in an air-conditioned greenhouse of the Laboratory of Agricultural Zoology and Entomology (Agricultural University of Athens, Greece) at 25 ± 3 °C and 65 ± 10% RH. No pesticide was applied to the plants. The plants regularly checked from any pests or diseases.

Tuta absoluta laboratory rearing

Tuta absoluta rearing was kept in the insectary of Laboratory of Agricultural Zoology and Entomology in cages with tomato plants. *T. absoluta* was originated from a tomato crop located in Marathon, Greece (38.15°N, 23.96° E). Breeding conditions were 25 ± 1° C, 65 ± 5% and photoperiod 16L: 8D. The plants were checked daily to exclude natural enemies of *T. absoluta* or other pests were removed.

Entomopathogenic fungi (Sampling-isolation-identification-preparation)

Soil sampling of the collection of EPF was carried out in the wider area of Messara Heraklion, Crete, in March 2020. Specifically, 8 soil samples were collected from each of the following sites: Kouses, Vagionia, Galia and Moires in Heraklion (Table 1). The samples were collected from organic olive groves. In eight random locations in each olive grove and at about 1 m distance from the trunk of the nearest tree, a soil sample of 300 g was collected starting from the soil surface to a depth of 10 cm. Then, each sample was placed in plastic bag and stored at 4 °C until processing. The location of each sampling site was recorded via GPS mobile application (Map Coordinates) (Table 1).

According to Mantzoukas et al. (2020), the entrapment of EPF was performed using the *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) and *Tribolium confusum* (Jacquelin du Val) (Coleoptera: Tenebrionidae) baits trap method. These samples were kept in dark chambers at 25 ± 1 °C and they were daily observed under a stereoscope to determine possible infestation by EPF. Fungal isolation was performed by culturing fungal conidia, which had sprouted on the infected adults or larvae on nutrient Sabouraud Dextrose Agar (SDA) substrate. The fungal conidia dishes were placed in chambers at 25 ± 1 °C until the growth of the fungi. To prevent infections when mycelium growth was detected, the individuals were re-isolated (Mantzoukas et al. 2020). The fungal morphology was initially examined macroscopically by noticing the colony features (shape, color, hyphae and size).

Identified fungal isolations were subcultured several times on plates with SDA to ensure purity and monosporic cultures. Following the method outlined by Rogers and Bendich (1985), the genomic DNA (gDNA) was extracted. Applying universal primer sets ITS4 (5′-TCC TCCGCTTATTGATATGC-3′) and ITS5 (5′-GGAAGT AAAAGTCGTAAC AAGG-3′), a fragment of the ITS spacer region was expanded. PCR reactions (30 μl) included 50 ng of template gDNA, 1.25 μl of each 10 pM oligonucleotide, 1 μl of 10 mM dNTPs, 1 μl of 2 U/μl Taq DNA polymerase (Minotech), 1.5 μl of MgCl2 and 2.5 μl of 10 × PCR buffer. The PCR protocol for amplification of ITS regions includes 31cycles, at 94 °C for 60 s, 55 °C for 60 s and 72 °C for 90 s, followed by a final elongation at 72 °C for 5 min. PCR products were kept at 4 °C. The quantity and quality of PCR products were determined by gel electrophoresis, using 2% agarose gel, which was stained with SYBR Safe DNA Gel Stain (Invitrogen) and visualized under UV light (BIO-RAD, Molecular Imager Gel Doc XR System). The purification and the sequencing of the amplified products took place at Eurofins Genomics, Ebersberg, Germany. The similarity of the fungal DNA sequences in the present work with homologous sequences was matched using the Basic Local Alignment Search Tool (NCBI BLAST). After 15 days, fresh conidia were collected from the SDA cultures and transferred to a 500 ml glass beaker with 100 ml sterile distilled water containing 0,05% Tergitol NP9. The conidial suspension was filtered across 10–12 layers of sterile cloth to remove hyphal debris and then homogenized by mixing on a magnetic stirrer for 5 min. The concentration of conidia was adjusted to 10^8 conidia/ml using a Neubauer haemocytometer under a phase contrast microscope at 400 × magnification (Axioplan; Zeiss, Oberkochen, Germany). The assessment of conidia viability pointed to a germination exceeding 95%. This was established by examining conidia at 40 × magnification after they had been incubated for 24 h on SDA (Mantzoukas et al. 2021).

Table 1 Details of the locations from where soil samples had been collected at Crete and the entomopathogenic fungi identified per location

Soil samples	Longitude	Latitude	Entomopathogenic Fungi	Blast ID Number
Kouses	35°00′37.7"**B** N	24°49′06.9"**A** E	*Metarhizium anisopliae*	E7JJ49D9899
Vagionia	34°59′54.4"**B**	25°00′46.0"**A**	*Beauveria bassiana*	G9DJ98N9016
Galia	35°03′59.1"**B**	24°51′08.2"**A**	*Metarhizium anisopliae*	D9JJ45D9301
Moires	35°02′49.1"**B**	24°52′25.9"**A**	*Beauveria bassiana*	H9WJ96N9013

Effect of endophytic entomopathogenic fungi on *T. absoluta*

The fungi isolated from the soil samples and used in the experimental design were *B. bassiana* Crete isolate and *M. anisopliae* Crete isolate. Their action against *T. absoluta* was compared with the commercial *B. bassiana* strain GHA 10.735% (Botanigard 10,7SC, K&N Efthymiadis Single Member S.A., Thessaloniki, Greece) isolated in SDA plate on Laboratory of Productive Agriculture and Plant Health at University of Ioannina. As a positive control treatment, the insecticide Minecto alpha 10/1.25 SC, (10% cyantraniliprole + 1.25% acibenzolar-S-methyl, Syngenta Hellas) and as a negative control deionized water (control) were used. The commercial strain and the insecticide have been registered for the control of *T. absoluta* and used in the recommended concentration according to their label (125 cc/lt, 75 cc/lt, respectively).

Fungal isolates presenting ≥ 95% viability were used in the insect bioassays. Conidial viability was calculated based on the formula: Viability (%) = [G1/(G1 + G2)] × 100, where G1 refers to the number of germinated conidia, G2 are the number of non- germinated conidia, while the sum of G1 and G2 is equal to 100. The viable conidia percentage was determined by counting a total of 100 conidia per fungal isolate. The concentration of suspensions used was 1×10^8 conidia/ml. The experimental process initiated with the addition of 20 ml suspension in glass beaker, which were then stirred for 10 min. Then, tomato plants with eight leaves were watered and then were irrigated with 3 ml fungal suspension. After irrigation with fungal suspensions, the surface of the pot of each plant was covered with aluminum foil for 48 h aiming to maintain moisture at high levels to enhance the root colonization by fungi. After irrigation, the plants were transferred to entomological cages (116 X 94 X 60 cm) and kept at 25 ± 1 °C and 65 ± 5% RH and 16:8 L: D in the insectary of the Laboratory of Agricultural Zoology and Entomology of AUA. The plants were watered twice a week. On the 15th day after application of the fungal suspension (i.e., 15 days post-treatment, 15dpt), one ready to hatch *T. absoluta* egg (4d-old) was placed on each of the third and fourth leaf (counting from the top) of each treated plant. The distance between the egg and the mine of each newly emerged larva was recorded. Then, each leaf with a larva was enclosed in an organdy bag (12 × 15 cm) and each plant was placed individually in a cage. The larval developmental period and pupal weight were recorded per larva. Each pupa was weighed using an analytical balance (ACS 80–4, KERN & Sohn GmbH, Balingen, Germany). Ten replications (plants) were used per treatment or control. Each block therefore consisted of four treatments plus the control which were replicated three times, thus producing a total of 48 plants for the entire experiment. The pupal weight was recorded in two of the replications. Experiments were repeated four times.

Endophytic colonization

Aiming to investigate the presence of endophytic stage EPF on the tomato plants, in each block, 1 similarly treated tomato plant had been added to be used for this purpose. Fourteen days after irrigation of the plants with the suspension, each leaf of this plant was separately collected and cut into 1 cm diameter and 0.5 cm thick disks in a laminar flow chamber. The samples were surface sterilized by immersion in 96% ethanol solution for one min, in 6% sodium hypochlorite solution for five min and finally, in 96% ethanol solution for 30 s (Mantzoukas et al. 2015). Then, sterile leaf samples were inoculated into SDA substrate using a sterile metal hook. The cultures on the SDA substrate samples were incubated in the dark at 25 °C ± 2 and 80% RH. Conidial growth sequence lasted 14 days. The germination of fungal conidia on the leaves was evaluated using an optical microscope (40 x). The number of samples which displayed fungal growth was calculated using the following formula: number of tomato leaves samples with fungal growth/total number of samples (Mantzoukas et al. 2015). The above-mentioned process was completed inside a laminar flow chamber (Equip Vertical Air Laminar Flow Cabinet Clean Bench, Mechanical Application LTD, Athens, Greece).

Statistical analysis

The distance between the hatched egg and the mine of the larva, the period of larval development and the pupal weight of each *T. absoluta* larva were compared among the isolated fungi, the commercial strain, the insecticide and the deionized water by one-way ANOVA with treatment as fixed factor. "Repetition in time" and "Plant" were included as factors. The data of distance and development were log transformed prior to the analysis. The means were compared using the Tukey's HSD test ($\alpha = 0.05$). All the analyses were performed by the statistical package JMP 14.1.0. (SAS Institute 2016).

Results

The entomopathogenic fungi were isolated from tomato leaves once again. Mycelium began to appear 7 days later and had developed completely after 12 days at 25 ± 2 °C and in 95% RH. Successful re-isolations of the three fungi were obtained from leaves of plants from corresponding treatments (Fig. 1). Decline of colonization was observed after 14 days, in leaves of plants treated with endophytes ($F = 2.156$, d$f = 3, 70$, $P = 0017$).

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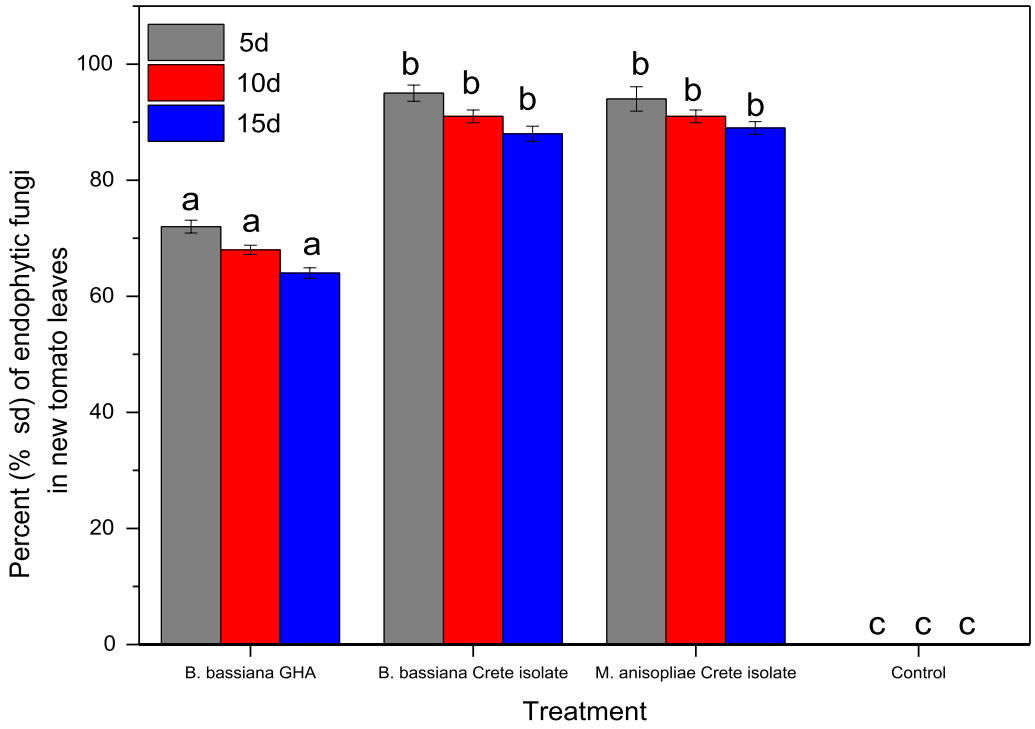

Fig. 1 Mean (± sd; $n = 24$) colonization of tomato leaf parts by *Beauveria bassiana* Crete isolate and *Metarhizium anisopliae* Crete isolate and *B. bassiana* GHA strain at 5 days, 10 days and 15 days after exposure. Mean ± sd values with the same superscript letter are not different in a significant way (Bonferroni's test: $P < 0.05$)

developed completely after 12 days at 25 ± 2 °C and in 95% RH. Successful re-isolations of the three fungi were obtained from leaves of plants from corresponding treatments (Fig. 1). Decline of colonization was observed after 14 days, in leaves of plants treated with endophytes ($F = 2.156$, d$f = 3, 70$, $P = 0.150$).

The period between the hatched egg and the mine of the emerged first instar *T. absoluta* larvae was significantly affected by the different treatments (F = 49.84, df = 4,163, $P < 0.001$) but not by the "repetition in time" and "plant" ($P > 0.67$ and $P > 0.58$, respectively). It was the longest in case of the negative control (deionized water), followed by a significantly short one following the application of the positive control (insecticide) (Fig. 2). Interestingly, the distance was further significantly shortened after the treatment with *M. anisopliae* isolate Crete and *B. bassiana* isolate Crete and the commercial strain. Among them, the distance recorded in the plants treated with the *M. anisopliae* isolate Crete and *B. bassiana* isolate Crete were the shortest ones, significantly differing to that recorded in the case of the commercial *B. bassiana* strain.

The developmental period of *T. absoluta* larvae was significantly different among the treatments ($F = 3.29$, d$f = 3, 121$, $P < 0.023$) but not by the "repetition in time" and "plant" ($P > 0.07$ and $P > 0.92$, respectively). It was significantly longer in the treatment with *B. bassiana* commercial strain than the negative control (deionized water) (Fig. 3). Among the other treatments, non-significant differences were recorded. In plants, the fungal pathogens' treatments caused the larvae not to complete its development.

The treatments caused a significant variation on the weight of *T. absoluta* pupae ($F = 46.34$, d$f = 3.73$, $P < 0.001$). The lowest weight was recorded in the plants colonized by *M. anisopliae* isolate Crete (Fig. 4.). It was significantly increased in the plants treated with *B. bassiana* isolate Crete which was non-significantly different in comparison to that recorded after the application of the *B. bassiana* commercial strain. The pupal weight recorded in the negative control was significantly higher than all the other treatments.

Discussion

In this study, the successful endophytic colonization and establishment of *B. bassiana* and *M. anisopliae* isolates in tomato plants using the drenching/irrigation method were demonstrated. This method had been also used successfully in the endophytic colonization of tomato plants by *B. bassiana* isolates (Hammad et al. 2022). On the contrary, according to the results of Allegrucci et al. (2017), root irrigation was not effective in the endophytic

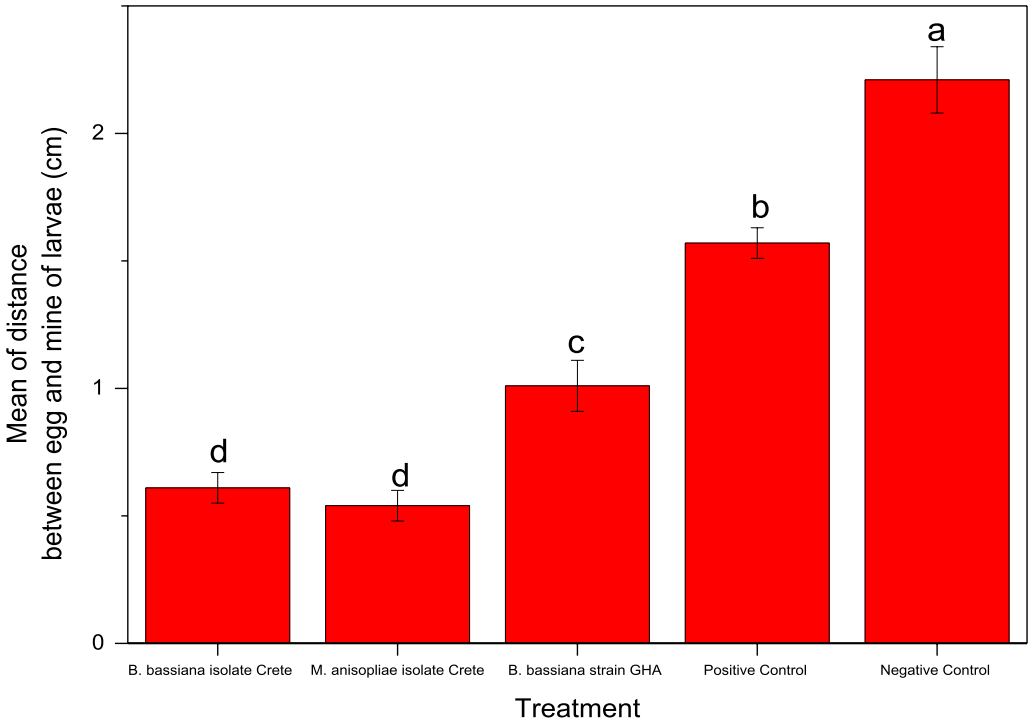

Fig. 2 Means (± se) of period between the egg and the mine of the larvae developed in fungi-free (control) plants and plants colonized by *Beauveria bassiana* isolate Crete and *Metarhizium anisopliae* isolate Crete and *B. bassiana* strain GHA after 15 DPI* (days per irrigation) or irrigated by a chemical insecticide (Positive control). Means of the same column followed by the same letter are not significantly different (HSD, $p < 0.05$)

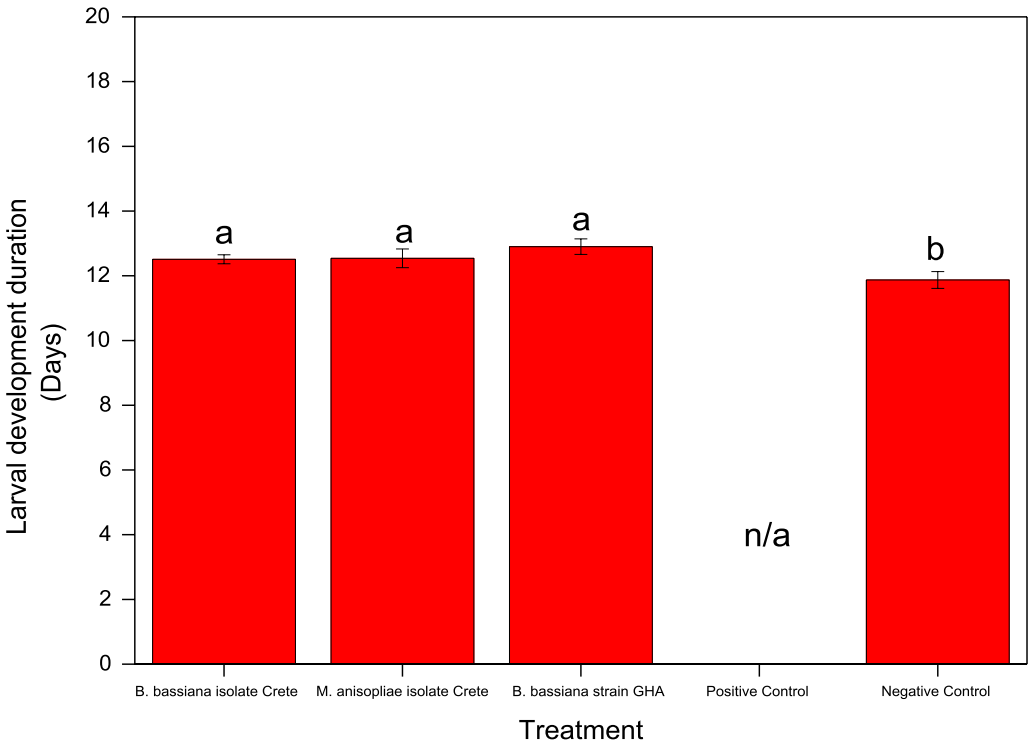

Fig. 3 Means (± se) of larval development duration in fungi-free (control) plants and plants colonized by *Beauveria bassiana* isolate Crete and *Metarhizium anisopliae* isolate Crete and *B. bassiana* strain GHA after 15 DPI* (days per irrigation). Means of the same column followed by the same letter are not significantly different (HSD, $p < 0.05$)

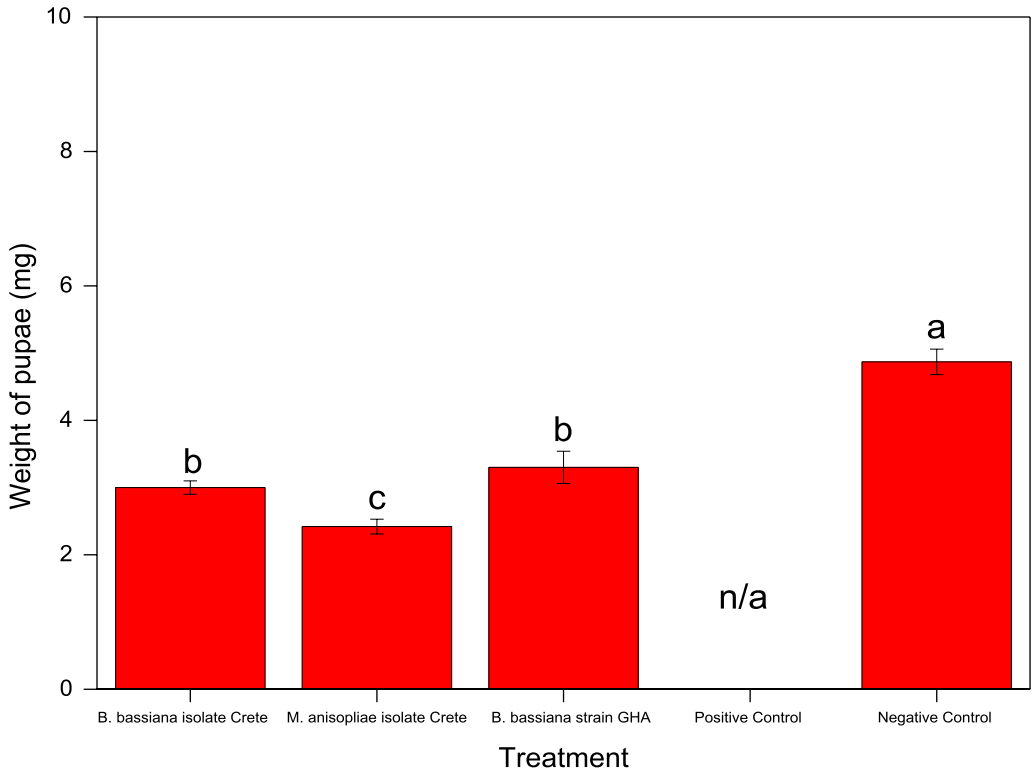

Fig. 4 Means (± se) of pupal weight of larvae developed in fungi-free (control) plants and plants colonized by *Beauveria bassiana isolate* Crete and *Metarhizium anisopliae* isolate Crete and *B. bassiana* strain GHA after 15 DPI* (days per irrigation). Means of the same column followed by the same letter are non-significantly different (HSD, $p < 0.05$)

colonization of tomato plants 14 days after treatment. This is probably due to the strain of the fungus used, the age of the plant, the concentration of the suspensions as well as the plant growth conditions (Afandhi et al. 2019). However, it may also be due to the competition of the fungus competition with saprophytic soil fungi (Tefera and Vidal 2009). The results of the present work proved that endophytic colonization through irrigation was possible and furthermore it caused negative effects on *T. absoluta*. The results introduced a promising approach for using endophytes in practice to control *T. absoluta*, since irrigation system application is a simple and easy method to apply. The local isolates outperformed the commercial one in adversely affecting the pupal weight of *T. absoluta*. Likely, the local isolates may be better adapted to colonize endophytically the plant by the irrigation method in comparison to other strains and thus local isolates may offer a valuable reservoir of unexplored material useful in further developments of this method of pest control. The developmental period was increased on plants endophytically colonized. Increased developmental period has been also reported for larvae of *T. absoluta* fed on leaves sprayed with *B. bassiana* (Younes et al. 2018). In accordance with the results of the present study in regard to the significant reduction of the pupal weight of *T. absoluta* when larvae fed on endophytically colonized tomato leaves, Mwamburi (2021) showed that the weight of the second instar larvae of *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) was significantly reduced when fed on tomato plants the roots of which had been dipped (root dipping) in a suspension of *M. anisopliae* at a concentration of 1 X 10^8 conidia/ml, 14 days before. These adverse effects show the potential of this method in the control of *T. absoluta*. Likely they are due to the production of secondary metabolites by the endophytes (Mantzoukas et al. 2015).

The period of time travelled by the neonate larva from the egg to the point of its mine creation was proved a sensitive parameter to assess the negative effects caused by the endophytes. In fact, the differences of this parameter were more evident among the treatments than in the other parameters used (i.e., larval development and pupal weight). These two factors are directly related to the development of larvae. However, path length may indicate effects of volatiles, the identity of leaf tissues, or the presence of toxic compounds produced by endophytic plant colonization. This corroborates with Agbessenou et al. (2020), who found that dead *T. absoluta* larvae did

not show any signs of fungal infection, suggesting that either feeding deterrence, or the production of secondary metabolites by endophytized tomato plants, may be responsible for their deaths. In addition, *B. bassiana* had a strong deterrent effect on *T. absoluta* females, which can also be attributed to the unfavorable volatile profile of the endophytically colonized plant (Agbessenou et al. 2020). Therefore, the assessment of this parameter may be a useful tool in assessing the effects of endophytes on *T. absoluta*. However, despite the negative effects on development caused by the endophytes used in this study, all of them, including the commercial strain, did not cause larval death in *T. absoluta* Allegrucci et al. (2017) found that *B. bassiana* applied to tomato plants by the root dipping method, caused mortality of 9.5% on *T. absoluta* larvae, while in the control zero mortality was recorded. In another study, the endophytic colonization of a commercial *B. bassiana* strain did cause mortality on larvae of *T. absoluta,* which reached to 50% on tomato plants sprayed 18 days earlier (Klieber and Reineke 2015). Likely, these differences may be associated with the different methods and strains used among the studies (Mantzoukas and Eliopoulos 2020). Hammad et al. (2022) reported that endophytic inoculation by soil drenching was a relatively slow process since the fungus was first detected in the leaves 15 days after inoculation. Likely, larval mortality could have been recorded in the study after a long period from inoculation.

Conclusions

The experiments demonstrated that native isolates of EPF can colonize tomato plants. Compared to the control, presence of endophytic EPF lowered the weight of *T. absoluta* larvae and the distance travelled of first instar larvae from the egg to its gallery. Additionally, affected the development duration of *T. absoluta* larvae. This effect was dependent on the isolate. Therefore, these isolates present a wide range of activity which can be utilized in the biological control of *T. absoluta*. The application of EPF as endophytes in crops is a new field of research with encouraging results. Therefore, it requires more investigation in the laboratory and in field conditions regarding the endophytic activity of EPF in tomato plants. Further study should be performed to investigate the effects on the lifespan of *T. absoluta* adults and their reproduction. In addition, it should be investigated whether tomato plants colonized with EPF act repulsively on *T. absoluta* under laboratory and field conditions.

Abbreviations

ITS	Internal transcribed spacer
PCR	Polymerase chain reaction
EPN	Entomopathogenic nematodes
GPS	Global positioning system
SDA	Sabouraud Dextrose Agar
BLAST	Basic Local Alignment Search Tool
NCBI	National Center for Biotechnology Information
DPI	Days post-incubation
DPT	Days post-treatment
DNA	Deoxyribonucleic acid

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Author contributions

This work was carried out in collaboration among all authors. SM and DP designed the study. EG and IL performed the laboratory experiments. EG produced the manuscript. SM and DP revised the manuscript. SD managed the literature searches. All authors contributed to revisions of the article. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this manuscript.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication.

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Competing interests

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