Entomopathogenic fungi tested in planta on pepper and in field on sorghum, to control commercially important species of aphids

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Abstract

Given the aphids high reproductive capacity, assessing their biocontrol by using entomopathogenic fungi is crucial; to determine their potential, fungi were tested in planta and in field conditions. Significant decrease of Myzus persicae (Sulzer) population was observed in planta after applying Beauveria bassiana (strain 7R), Trichoderma gamsii (strain Z) or Metarhizium brunneum (strain Meta Br1) at \(1 \times 10^7\) or \(1 \times 10^8\) conidia/mL on pepper plants. Significant differences of aphids’ populations were detected between fungus concentration and control (\(F = 68.743, \text{df} = 6.980, P < 0.001\)), where M. brunneum at \(1 \times 10^8\) conidia/mL reduced aphids population close to zero. At 20 °C, dead aphids’ mycosis by B. bassiana and T. gamsii was 78% and 84%; at 25 °C was 83% and 88%; and at 30 °C was 75% and 79%, respectively. In field conditions, Mexican PTG4 and commercial GHA B. bassiana strains were tested [(\(1 \times 10^6\) conidia/mL + corn starch) seed treatments] against the Melanaphis sacchari (Zehntner) aphid populations, on naturally infested sorghum plants. Results showed that plant germination and emergence were not affected, whereas yield (grams of sugar/plant) was significantly higher among treated compared with untreated plants. The aphid population decreased in plants from PTG4 treated seeds; indeed, this treatment had a significant positive effect on the flowering index, whereas the stem fresh weight and juice volume was significantly increased among plants from GHA treated seeds. Taken together, tested strains can be used as a tool to control aphids’ population on several crops such as pepper and even increase the yield in sorghum.

Keywords Aphid’s biocontrol · Beauveria bassiana · Metarhizium brunneum · Trichoderma gamsii · Pepper · Sorghum production

Introduction

Aphids are among the most serious pests in agriculture and horticulture. They produce damage by being efficient vectors of plant viruses; by removing plant sap, which weaken the plants resulting in lower quantity and quality of fruits; and by producing “honeydew”, a carbohydrate-rich exudate that is a suitable medium for the growth of pathogenic fungi (Wakil et al., 2017). Aphid control is predominantly achieved with chemical insecticides. However, this practice causes environmental pollution and may result in aphid’s population resistance development. Indeed, several insect pests have developed resistant to chemical pesticides, to the point that certain insecticides groups have been banned, leading the growers for non-chemical plant protection methods and products. Pest management through biological control by using different predators, parasites and pathogens, is reliable. Entomopathogenic fungi (EPF) can be used for pest
control and do not have effects on other non-target organisms (Mantzoukas and Eliopoulos 2020). EPF are natural regulators to many insects, including aphids, and their dissemination among infected insects may result in epizootics (Fan et al., 2007; De Faria and Wraight, 2007). EPF insect’s infection is a result of the fungus conidia contact to the susceptible insect cuticle. The process by which insect pathogens penetrate the insect cuticle is influenced by internal and external factors, which ultimately determine whether the pathogen will rupture the host’s cuticle or not. EPF have a biological cycle that is synchronized with the host but also with the prevailing environmental conditions (Shahid et al., 2012). Infection can be inhibited by low relative humidity, the inability to use available nutrients of the insect’s cuticle, and the absence of host recognition factors (Sierotzki et al., 2000; Shaw et al., 2002). The infection depends on the EPF genetic competence to infect, the insect capacity to defend itself, and many biological and abiotic factors and interactions (Shahid et al., 2012).

Aphids have been a EPF target, since they are susceptible to natural fungal epizootics (Milner, 1997). In nature, the most successful EPF for aphids’ control, the Entomophthorales, have proven difficult to mass-produce and to formulate for achieving active ingredient shelf-life (Leite et al., 2005). Thus, most pests have focused on more production-friendly species, primarily ascomycetous species of the Beauveria, Metarhizium and Isaria anamorphic genera. Studies conducted to evaluate the EPF potential control of aphids are numerous. Kim and Kim (2008) tested the efficacy of six EPF isolates collected in Korea, including Beauveria bassiana (Bals.-Crv.) Vuill., Isaria fumosorosea Wize and Lecanicillium attenuatum Zare & W. Gams against the cotton aphid, Aphis gossypii Glover. Various EPF isolates such as Lecanicillium lecanii Zare & W. Gams, Isaria farinosa, B. bassiana, Metarhizium anisopliae, Cordyceps scarabaeicola Kobayasi and Shimizu, and Metarhizium rileyi Kepler S.A. Rehner & Humber, were screened for controlling A. gossypii and M. persicae. Among the tested ones, L. lecanii showed the highest virulent pathogenicity for both A. gossypii and Myzus persicae (Sulzer) (Vu et al., 2007). In addition, B. bassiana and I. fumosorosea showed high efficacy against Phorodon humuli (Schrank) (Dorschncher et al., 1991), Aphis craccivora Koch, and Bemesia tabaci (Gennadius) (Zaki 1998). Mesquita et al. (1996) reported that different EPF species/isolates induced similar mortalities in the Russian wheat aphid (Diuraphis noxia Kurdjumov) even though these isolates were from different host insects. In contrast, Vandenberg (1996) found that isolates of any individual fungal species had different efficacy against the same host aphid cohort. In addition, an emerging approach where the infection mechanism is through indirect contact between the aphid and the plant, appears to be an efficient alternative. This approach takes advantage of the EPF ability to colonize within plant tissues as endophytes, being the colonization more effective through seed treatments (Lopez et al., 2014). Therefore, this study first objective was to document the aphid mortality and their population regulation in planta, to offer a better understanding of the EPF-aphids relationship, and their potential as alternative to control a devastating pest such as M. persicae. Our second objective was to evaluate the efficacy of two B. bassiana strains against naturally M. sacchari populations infesting sorghum in field conditions after seed treatments, and their effect on several physiology parameters of sorghum plants.

Materials and methods

Entomopathogenic fungal strains source

Entomopathogenic B. bassiana (strain 7R), Trichoderma gansii Samuels & Druzhin (Hypocreales: Hypocreaceae) (strain Z) and M. brunneum (strain Meta Br1) isolated from the Achaia region, Greece, were used for the in planta bioassays on pepper. In addition, the commercial B. bassiana GHA strain (Botanigard® 22WP, BioWorks Inc., Victor, NY) and the Mexican B. bassiana PTG4 strain (GenBank accession number KC759730.1), isolated from Periplaneta americana, were used to treat sorghum seeds, for the field experiment in Mexico.

Fungi production for in planta bioassays

This part of the work was done in Greece following their specific procedures based on their previous reports (Mantzoukas and Lagogiannis 2019). To achieve the fungi growth, strains were cultured on Petri dishes with Sabouraud dextrose agar (SDA) culture medium (Oxoid Ltd., Pavlos Arnaoutis SA, Athens, Greece), incubated at 25 ± 2 °C temperature in dark conditions. After each fungus grew until covering the dish surface, each one was isolated once more, in order to avoid contamination and to achieve pure cultures of each one. Isolates were maintained in Petri dishes with SDA medium, incubated at 25 ± 1 °C and renewed every month. The conidia were retrieved from these cultures maintained on SDA. Fresh conidia were collected from the SDA cultures after 15 d 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 4 layers of sterile cloth to remove hyphal debris and prepared by mixing the solution with a magnetic stirrer for 5 min (Dorschner et al., 1991).
**In planta bioassays performed on pepper plants**

In order to assess the impact by the three EPF on the *M. persicae* populations, *in planta* bioassays were settled up. *M. persicae* was obtained from the Plant Protection Institute of Patras, ELGO Dimitra and maintained on potted pepper plants variety Staurus Peloponnesus (*Capsicum annuum* L., Solanaceae: Solanaceae). Pepper plants were pre-germinated in 2 × 2 cm pots (one seed per pot at a depth of about 1 cm) with Pindstrum plus peat substrate and then they were transplanted into one-liter pots with Pindstrum plus type peat substrate. Aphids were maintained at 25 ± 5 °C and a 14:10 h light (L):darkness (D) regime. For this bioassay, insects were kept in a room with pepper plants throughout their developmental cycle, at a constant 25 ± 1 °C temperature, 60–70% relative humidity (RH), and 16:8 h light (L):darkness (D) photoperiod. Experimental pepper leaves were sprayed with 5 mL of the desired conidial concentration suspension (from 1 × 10^7 or 1 × 10^8 conidia/mL) of either *B. bassiana* strain 7R, *T. ganssi* strain Z, or *M. brunneum* strain Meta Br1, using a Badger 100 artist’s airbrush (Badger Air-Brush Company, Franklin Park, IL). Control treatments were sprayed with 5 mL of sterile distilled water containing 0.05% Tergitol NP9. After spraying suspensions of the selected fungus and concentration, plants were covered for 24 h with large diameter black bags to maintain high moisture on the plant surface. After this, one apterous *M. persicae* aphid was placed on a randomized leaf after two-hour starvation. Ten aphids were used per treatment (n = 10), and each experiment (n = 40) was replicated five times (n = 200). Well-developed pepper leaves were used for each out of three treatments, plus one for the untreated control, in a block designed assay with four blocks. Therefore, each block consisted of three treatments plus the control, which were replicated five times, thus producing a total of 200 plants for the entire experiment. Each aphid-infected leaf was covered with an organdie 10 × 30 cage, to prevent dispersion into the experimental area. The aphid’s population was recorded on each leaf after 3, 6 and 9 d. The experiment, organized in a completely randomized design, was not analyzed as a factorial (since there was only one aphid species). Dead insects were removed and placed on 1.5% agar plates at 25 °C for an additional 2 d to detect if aerial mycelium was developed.

In order to assess how the temperature affected the EPF infection of the *M. persicae* exposed population and its potential dissemination, dead aphids were separated by treatment and maintained at 16:8 h D:L photoperiod at three different temperatures: 20 °C, 25 °C, and 30 °C, and at 90 ± 2% RH. Humidity was maintained putting a 500 mL beaker filled with a saturated solution of Na_2SO_4 into the larger plastic box that contained the stacks of Petri dishes that were put into the incubator (MLR-352-PE Climate Chamber, PHC Europe BV, Athens, Greece).

**Fungi production for in field experiments**

In order to test for the use of more progressive approaches, we started a collaboration Greece-Mexico. Due to biological material movement restrictions, the commercial GHA and Mexican PTG4 *B. bassiana* strains were used and growth using different culture conditions than those used for fungicide production in Greece. This was due to the specific procedures followed by seed treatments, based on previous reports to control cotton aphids in field conditions (Lopez et al. 2014). In Mexico, the strains were activated by plating frozen stock cultures onto potato dextrose agar (PDA, BD Difco, Mexico City, Mexico) and incubating in darkness at 25 ± 2 °C for a week. Next, to prepare the main stock culture, a single selected colony was inoculated onto another PDA plate and incubated at 25 ± 2 °C for a week. Fresh conidia were obtained from PDA plates started from this main stock culture that were incubated for 7 d in darkness at 25 ± 2 °C. Conidial suspensions were prepared by scraping gently the top of the fungal cultures with a spatula and dissolving the spores and hyphae mixture in sterile distilled water. This suspension was filtered in 4 layers of sterile mesh-cloth to remove hyphal debris. Conidia were counted in a Neubauer chamber, and each treatment was adjusted with distilled water to the indicated concentration.

**Field experiment on sorghum**

Survival of native *Melanaphis sacchari* ([Zehntner] (Hemiptera: Aphididae)] populations, potentially affected by the seed treatments with EPF, was recorded at the Experimental Field Unit of the Autonomous University of Nuevo Leon-School of Agronomy, located in Marín, Nuevo Leon, with a geographical location of 25°52’24.0” N 100°03’03.0” W. Tested sorghum was sweet sorghum (*Sorghum bicolor* L.) (Moench) variety ‘Roger’, which germplasm is deposited in the UANL Plant Varieties National Catalog (registration number SOG-261-050315) (López-Sandin et al. 2021). Sorghum seeds used in this work were directly obtained from this facility. *B. bassiana* GHA or PTG4 strains were used at the final concentration of 1 × 10^6 conidia/mL in each sorghum seed treatment. Corn starch (CS) (Unilever Manufacturera, S. de R.L. de C.V., Mexico City, Mexico) was mixed with conidia for adequate attachment to the seeds. Conidia + CS (4% CS final concentration) was prepared by first dissolving CS in boiling distilled water to a pre-gelatinized state. When the sticker suspension was at room temperature, conidia were then added until a homogeneous suspension was obtained (both the CS and conidia suspension were
first prepared as 2X, then diluted twice when preparing the final conidia + CS suspension; next, seeds [5,000 seeds/treatment] were added into 150 mL volume of conidia + CS, mixed gently for 5 min and then air-dried at 25 °C for 24 h.

Sorghum field trials consisted in 4 treatments and 4 replicated randomized plots. Treatments were PTG4 + CS treated seeds (PTG4), GHA + CS treated seeds (GHA), non-treated control seeds (CONTROL) and chemically treated control plants (CHEM) (Imidaclopid/Betaciflutrin). A treatment with only CS was not included. All seeds of each treatment were sown in the field and regular agricultural practices were followed under rainfed farming conditions. In addition, one irrigation was applied at 45 d after sowing, and no fertilizers nor herbicides were applied throughout the cultivation cycle.

**Melanaphis sacchari survival during the sorghum crop cycle**

After sorghum germination, the presence of *M. sacchari* was monitored every week, until detecting 50–125 or higher aphids’ population per plant in the untreated control plants. After this, only the chemical control was applied, following the Imidaclopid/Betaciflutrin application guidelines (Muralla Max®, Bayer CropScience, Mexico City, Mexico) using a backpack applicator and a flexible hose, to avoid movement of the insecticide to the randomized control and EPF treated plots. After two weeks, the flowering percentage was recorded as the number of panicles present in 100 plants/treatment and was reported as the flowering index [flowering percentage transformed to a decimal value (0 to 1) per treatment]. At the same time point, aphid’s population survival analysis was done, focused on their relative abundance per treatment, among 100 plants chosen randomly. To express the aphid’s relative abundance values per plant, a scale of A-F (A = 1–25 aphids, B = 26–50 aphids, C = 51–100 aphids, D = 101–500 aphids, E = 501–1000 aphids and F = > of 1000 aphids, was used (Bauer, 2015; Bowling et al. 2015). The scale F damage percentage was calculated as index F damage [percentage of scale F aphid’s relative abundance transformed to a decimal value (0 to 1) per treatment] and was chosen as indicative of sorghum damage.

**Sorghum yield as stem fresh weight per plant, juice and sugar content per plant**

After the sorghum crop cycle was ended, 10 plants per treatment/replicate (a total of 40 plants per treatment) were randomly selected and their fresh stems weight were recorded, using an analytical scale (L-EQ, Torrey, Mexico City, Mexico). Juice from those stems was extracted using a juice extractor (QJH-L100A, Ban Hing Holding Sdn Bhd, Kuala Lumpur, Malaysia), collected in a graduated plastic cylinder, its volume measured in mL and weighted in an analytical scale. A digital refractometer (PAL-1, Atago USA, Bellevue, Washington, USA) was used to determine sugar content as Brix-degree percentage (one-degree Brix corresponded to 1 g of sucrose in 100 g of sorghum juice) and with this data, the amount of sugar per plant was calculated.

**Statistical analysis**

Prior to analysis, the *M. persicae* mortality values were arcsine transformed. Mortality data were then analyzed by univariate ANOVA means, using the IBM general linear model (version 25.0, SPSS Inc., Armonk, NY, USA). The significance level was set at *P* < 0.05. In case of significant *F* values, means were compared using the Bonferroni test. Kaplan–Meier analysis was also selected to determine the *M. persicae* population median survival time, following to the EPF exposure. Median survival time comparison was performed using one-way ANOVA (treatment as factor) (SPSS v.25.0). Flowering index (normalized by arcsine transformation) and the effect of *M. sacchari* presence in sorghum, reported as index F damage (arcsine transformed), were analyzed by non-parametric Kruskal-Wallis means. Comparison of means was done using Bonferroni test. To determine the seed treatments global effect on this sorghum variety production, all collected data from fresh stem weight (in g/plant), juice volume (in mL/plant) and yield (g of sugar/plant) were analyzed altogether using Principal Component Analysis (PCA) by the Spearman method settings by the XLSTAT statistical package (V.2021 for Windows, Addinsoft, NY, USA). PCA correlations were considered significant when the Bartlett’s sphericity test *p* value was ≤ 0.05. Variables with a correlation coefficient ≥ 0.6 were considered relevant.

**Results**

*M. persicae survival after EPF exposure using in planta bioassays**

EPF tested were extremely pathogenic against the *M. persicae* aphid population on pepper plants. Significant differences were detected between concentration and untreated control (*F* = 68.743, df = 6.980, *P* < 0.001). A significant decrease in the aphid’s population was observed by all EPF when applied 1 × 10⁷ and 1 × 10⁸ conidia/mL; and especially by *M. brunneum* treatment, where aphids’ population on a 1 × 10⁸ concentration treated leaf was almost zero.

The aphid population average after three days exposure to *B. bassiana* treatment resulted in 38 (10³ conidia/mL) and
24 (10^8 conidia/mL); after *M. brunneum* exposure, 45 (10^3 conidia/mL) and 21 aphids (10^8 conidia/mL); aphids average, after *T. gamsii* exposure, 35 (10^3 conidia/mL) and 20 (10^8 conidia/mL) aphids, whereas for control (H_2O + Tergitol

| Treatment          | Concentration (conidia/mL) | 3 days Median Survival Time (days) | Std. Deviation | 95% Confidence Interval |
|--------------------|---------------------------|-----------------------------------|----------------|-------------------------|
| **Beauveria bassiana** (strain 7R) | 10^3 | 8.5 | 0.217 | 8.076 | 8.924 |
|                    | 10^4 | 8.3 | 0.241 | 7.827 | 8.773 |
|                    | 10^5 | 8.0 | 0.265 | 7.481 | 8.519 |
|                    | 10^6 | 7.7 | 0.313 | 7.087 | 8.313 |
|                    | 10^7 | 6.5 | 0.325 | 5.864 | 7.136 |
|                    | 10^8 | 5.8 | 0.351 | 5.113 | 6.487 |
| **Metarhizium brunneum** (strain Meta Br1) | 10^3 | 8.6 | 0.148 | 8.511 | 8.889 |
|                    | 10^4 | 8.1 | 0.299 | 7.514 | 8.686 |
|                    | 10^5 | 7.9 | 0.309 | 7.295 | 8.505 |
|                    | 10^6 | 7.6 | 0.315 | 6.983 | 8.217 |
|                    | 10^7 | 6.7 | 0.344 | 6.026 | 7.374 |
|                    | 10^8 | 5.5 | 0.300 | 5.112 | 6.288 |
| **Trichoderma gamsii** (strain Z) | 10^3 | 8.5 | 0.184 | 8.340 | 9.060 |
|                    | 10^4 | 8.0 | 0.407 | 7.002 | 8.598 |
|                    | 10^5 | 7.9 | 0.345 | 7.224 | 8.576 |
|                    | 10^6 | 7.5 | 0.376 | 6.762 | 8.238 |
|                    | 10^7 | 6.2 | 0.288 | 5.636 | 6.764 |
|                    | 10^8 | 5.9 | 0.337 | 5.239 | 6.561 |
| **Control**        | 8.9 | 0.098 | 8.707 | 9.093 |

Table 1: Population (%) (% ± sd) (F = 8.396, df = 12.550, P < 0.001) of the *M. persicae* adults after application of three entomopathogenic fungi (*n* = 100) at six conidia concentration (10^3, 10^4, 10^5, 10^6, 10^7, 10^8 conidia/mL) and control treatment (H_2O + Tergitol NP9 0.05%). Mean ± sd values with the same letter within a column are not significantly different (P < 0.05).

Table 2: Survival time of adults *M. persicae* from Kaplan-Meier (Breslow test) after 9 days.
Aerial mycelium *M. persicae* on cadavers after EPF expose and incubated under three different temperatures

Following treatment with *B. bassiana* strain 7R, *T. gamssii* strain Z and *M. brunneum* strain Meta Br1, high aerial mycelium development on cadavers incubated at 25 °C was observed (*t* = 12.144, df = 2, *P* < 0.001) (Fig. 1). At 20 °C, the aerial mycelium development on dead aphids was between 78 ( *B. bassiana* ) and 84% ( *T. gamssii* ); at 25 °C was between 83 ( *B. bassiana* ) and 88% ( *T. gamssii* ); whereas at 30 °C the aerial mycelium development on dead aphids was 75% ( *B. bassiana* ) and 79% ( *T. gamssii* ).

*Melanaphis sacchari* survival after *B. bassiana* application on sorghum seeds

We designed a field trial experiment to analyze the effect of a local entomopathogen against field aphid populations. Yellow aphid is a big concern in Mexico, therefore we decided to test several treatments to control aphid population under field conditions. A Mexican *B. bassiana* isolate (PTG4) and the commercially available strain GHA (Botanigard®) were evaluated in sorghum field trials after seeds were treated with a conidia suspension prepared using an inert sticker. Results showed no differences in seed germination and plant emergence. However, with both isolates, flowering index

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**Fig. 1** Fungal growth on *M. persicae* cadavers after entomopathogenic fungi expose at different temperatures under laboratory conditions. Percentage aerial mycelium on dead aphids’ values were arcsine transformed, then analyzed by means of univariate ANOVA using the general linear model. In case of significant F values, means were compared using the Bonferroni test. The significance level was set at *p* < 0.05.
was increased compared to the values observed in the control and chemical treatments (Fig. 2).

Untreated control vs. PTG4 treatment was significantly different ($P=0.006$) as well as chemical treatment vs. PTG4 treatment ($P=0.008$). In addition, among plants from $B. bassiana$ strains treated seeds, it was observed fair $M. sacchari$ populations control, supported by the lower F damage index observed within GHA and PTG4 treated seed plants (untreated control vs. GHA treatment, $P<0.0001$; untreated control vs. PTG4 treatment $P<0.0001$). The chemical treatment showed the expected results with 0 damage index F observed (Fig. 3).

Yield recorded data was analyzed by PCA means (Fig. 4). The Bartlett’s sphericity test $p$ value (two-tailed)

**Fig. 2** Flowering index in the field experiment. Flowering index (normalized by arcsine transformation) values were analyzed by means of non-parametric Kruskal-Wallis setting a $p<0.05$ value as statistically significant. Comparison of means was done using Bonferroni test. Control treatment vs. $B. bassiana$ PTG4 treatment was significantly different ($P=0.006$) as well as chemical treatment vs. $B. bassiana$ PTG4 treatment ($P=0.008$)

**Fig. 3** Aphids damage in the field experiment. Damage index F (arc sine transformed) values were analyzed by means of non-parametric Kruskal-Wallis setting a $p<0.05$ value as statistically significant. Comparison of means was done using Bonferroni test. Damage index F in $B. bassiana$ GHA and $B. bassiana$ PTG4 treatments were significantly lower than the control (control treatment vs. GHA, $P<0.0001$; control vs. PTG4, $P<0.0001$). The chemical treatment showed the expected results with 0 damage index F observed.
Fig. 4 Yield parameters in the field experiment. Data was analyzed by PCA. The Bartlett’s sphericity test $p$ value (Two-tailed) was 0.007 indicating significant PCA correlations. The three variables analyzed showed correlations ≥0.6 therefore were considered relevant: Stem Fresh Weight (g/plant) = 0.826; Juice (mL/plant) = 0.902 and Yield (g of sugar/plant) = 0.741. The grouping variables indicated a tendency in the plants obtained from GHA treated seeds, to have higher fresh stem weight and juice volume. However, the yield (g of sugar/plant) did not show a clear grouping within each treatment, although it was higher among treated (GHA, PTG4 and chemical treatments) plants, compared to untreated control plants.

Discussion

The ability of virulent strains including several entomopathogenic fungi to infect different life stages of an aphid makes them potential biocontrol agents. Several physiological and morphological characteristics may explain why aphids may be less susceptible to many fungal pathogens compared to other hemipteran pests. Their fast development time and multiple nymphal stadia mean that molts are occurring every 1–2 d. Liu et al. (2003) observed that the mortality of inoculated aphid nymphs with *B. bassiana* was closely related to the interval time between inoculation and the next molting period. Specifically, the earlier the molt occurred, the lower the observed mortality. Secondly, unlike whitefly nymphs, many aphids are highly mobile, with long, stilt-like legs that minimize body contact with the leaf surface and, thus, with the more humid leaf boundary layer. This limited contact also reduces the likelihood of aphids acquiring a lethal dose of fungal conidia from treated leaf surfaces (Hall and Burges, 1979).

The very good efficacy seen in our assays is consistent with all previous studies. At the lethal concentration of more than $1 \times 10^6$ conidia/mL, *B. bassiana*, *M. brunneum* and *T. gamsii* gave appreciable mortality. Interestingly, the entomopathogenic activity of different species of *Trichoderma* against aphids is not novel (Poveda, 2021). There are reports of seed treatments with *Trichoderma atroviride* $1 \times 10^7$ conidia/mL (Coppola et al., 2019) and *Trichoderma longibrachiatum* $1 \times 10^7$ conidia/mL (Battaglia et al., 2013) to control the aphid *Macrosiphum euphorbiae* (Thomas).

Ekesi et al. (2000) reported 91 and 93% mortality of *Aphis craccivora* Koch, in the seventh day after the spraying with *B. bassiana* and *M. anisopliae*, respectively. Loureiro and Moino (2006) reported that $1 \times 10^6$, $1 \times 10^7$ and $1 \times 10^8$ conidia/mL treatments testing *B. bassiana* and *M. anisopliae* non-commercial strains resulted in 100% mortality of third instars of *A. gossypii* and *M. persicae*. Vu et al. (2007) reported control values of 100% following *B. bassiana*, *M. anisopliae*, and *I. fumosorosea* $1 \times 10^7$ conidia/mL applications on 4 d old aphids (A. gossypii). Control values were calculated upon treated insect’s population decrease, compared with the untreated ones.
Filho et al. (2011) demonstrated that the numbers of M. persicae adults and nympha per leaf were significantly reduced in plots treated with two B. bassiana isolates, ranging from 57 to 60%. Following spray applications of Lecanicillium vs. A. gossypii, Kim and Roberts (2012) observed 70% fewer conidia on the dorsal surfaces of first instar nymphs and slower germination of these conidia than observed on third instars. Ramegowda et al. (2007) have reported very high mortality in C. lanigera, at the tenth day after spraying B. bassiana. A progressive reduction in the mortality of aphids was observed with decreasing concentrations. In the sublethal concentrations, the mortality of aphids was no higher for all entomopathogens tested.

The EPF mycelium virulence depends not only on the target aphid species, but also on the environmental temperature and relative humidity (RH) (Yeo et al., 2003; Vu et al., 2007); thus, it is important to select an entomopathogenic fungus appropriate for specific climatic conditions (Vu et al., 2007; Yeo et al., 2003).

Given the aphids high reproductive capacity, is important to assess the EPF potential as biocontrol agents on adult aphids and to reduce their reproduction. Our results concur with Hesketh et al. (2008), Shan and Feng (2010) and Tes-faye and Seyoum, 2010, where after applying EPF, high M. persicae adult mortality (>75%) was recorded. As aphids have long reproductive periods, death due to mycosis, even if relatively slow compared to other control agents, can reduce their reproduction rate. This study results revealed M. persicae reproduction reduction ranging from 36 to 74% after EPF applications at high doses, as reported by He and Li (2008) and Gurulingappa et al. (2011). However, reproduction reductions have little long-lasting effect on the aphid population. This behaviour is largely determined by the first few days of reproduction, and fungi normally exhibit an initially slow, terminally abrupt mode of action (Baverstock et al., 2006).

Results showed in Tables 1 and 2 indicate same impact on fungal infection in M. persicae reproduction cycle. Same results in both survival times and reproductive period could be due to infection time and reproductive cycle, in order to prevent population growth. M. persicae population growth among the untreated plants passed the population in the sprayed plants on day 6, and reproduction continued increased on day 9. Thus, by day 6 of the experiment, the population of M. persicae in sprayed plants was slightly decreased compared with the untreated plants, but on the day 9, the M. persicae population was significantly lower to that observed in the untreated plants. This significantly decreased pattern of the population was found in all treatments where EPF were applied, regardless of conidia concentration.

Treatment with M. brunneum strain Meta Br1 had the highest effect on aphid reproduction reduction only in treatment where 10^8 conidia/mL were applied. In contrast to Jandricic et al. 2014’s report, who found M. brunneum strain F52 had the highest reduction on aphid reproduction, in our study two fungus, B. bassiana 7R and T. gamsii, were effective regardless the tested conidia concentration. Our observations agree with several previous studies, showing that fungal infected aphids continue to produce normal numbers of healthy offspring until near death (Liu et al., 2003; Baverstock et al., 2006, Mantzoukas and Lagogiannis 2019). We also found fungal infection among aphid females, reflected on her offspring viability, as reported by Mantzoukas and Lagogiannis (2019).

Our results indicated that applied entomopathogens had no high effects on population of M. persicae when the conidial concentration was less than 1×10^6 conidia/mL. The use of misting systems to increase RH temporarily (i.e., 24 h) have been explored, but it is unclear currently what level of manipulation of conditions is needed to support activity of insect-EPF relationships without promoting plant pathogens. Further research into this area or the use of more progressive approaches are needed. Therefore, a Greece-Mexico collaboration led us to test a newly reported approach (Lopez et al. 2014) to control the yellow sorghum aphid, an economically important pest in America, based on the previously discussed findings using B. bassiana, but applying both a commercially available strain and a Mexican native strain.

The yellow sorghum aphid M. sacchari was first recorded only in sugarcane crops in the states of Florida in 1977, and Louisiana in 1999. However, in 2013 the United States Department of Agriculture reported the presence of these aphids damaging sorghum crops in several counties of Texas, which months later spread to Louisiana, Oklahoma and a county in Mississippi (Bowling et al. 2016). Later, the plague reached three states of Mexico. By the end of 2015, the yellow aphid already inhabited crops in 40 counties spread over 17 states in the United States and was present in all sorghum-producing regions in Mexico (Bowling et al. 2016). The states of Mexico where this pest is currently present are Coahuila, Guanajuato, Morelos, Nayarit, Nuevo Leon, Oaxaca, Puebla, Queretaro, San Luis Potosi, Sinaloa, Veracruz and Tamaulipas (SENASICA, 2020). Aphid control is predominantly achieved with chemical insecticides. However, this practice has caused environmental problems and resistance problems. The current managing strategies include biological control agents such as EPF, among others (SENASICA, 2020).

The use of B. bassiana in seed treatments in cotton under field conditions has resulted in lower insect pest damage and higher plant growth and yield (Lopez et al. 2014). In this
study, results of a field experiment, were a native *B. bassiana* (PTG4) strain and a commercially available (GHA) strain were applied on sorghum seeds, demonstrated that were able to control this economically important aphid. We used a relatively new approach proved to be efficient for *A. gossypii* control in cotton fields in Texas (Lopez et al. 2014). After seed treatments, plant germination and emergence were not affected. Even we did not test for endophyte establishment of both strains, our most significative finding was that the aphid population decreased in the *B. bassiana* PTG4 treated plants and that this treatment had positive effects on the crop flowering index. On addition, the fresh stem weight and juice volume was significatively increased mainly in the *B. bassiana* GHA treated plants. In general, the yield obtained measured as g of sugar/plant was higher in the treated plants than in the controls. Overall, results indicate that this EPF application protocol to sorghum seeds can be used in IPM strategies to control aphid’s population and may redound in higher crop yield.

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Authors’ contributions All authors contributed to this study’s conception and design. Material preparation, data collection and analysis were performed by MJER, SM and IL. The first draft of the manuscript was written by SM and MJER and all authors commented on previous versions of the manuscript and improved them until reaching the final version. All authors read and approved the final manuscript.

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Availability of data and material The data that support the findings of this study are shown within the text, tables and figures. Any further inquiry would be sent to the first and the corresponding author.

Declarations

Conflicts of interest/Competing interests No conflict of interest or competing interests have been declared by all authors.

Ethical approval was waived by the local Ethics Committee of the Autonomous University of Nuevo Leon and the University of Ioannina, in view of the nature of the study in the field on natural aphids’ populations, whereas all the procedures being performed were part of the routine care followed in the rearing and bioassays using the aphids’ laboratory colonies.

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