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 × 10^7 or 1 × 10^8 conidia/mL on pepper plants. Significant differences of aphids' populations were detected between fungus concentration and control (F = 68.743, df = 6.980, P <0.001), where *M. brunneum* at 1 × 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. Mexican PTG4 and commercial GHA *B. bassiana* strains were tested in field (treating seeds with 1 × 10^6 conidia/mL) 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.

Introduction

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 2019). 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 spores 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' cuticle, and the absence of host recognition factors (Sierotzki et al. 2000; Shaw et al. 2002). The infection depends on the EPF genetic ability to infect, the insect ability 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 efforts 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.-Criv.) Vuill., *Isaria fumosorosea* Wize and *Lecanicillium attenuatum* Zare & W. Gams against the cotton aphid, *Aphis gossypii* Glover. Various isolates of EPF such as *Lecanicillium lecanii* Zare & W. Gams, *Isaria farinosa* (formerly *Paecilomyces* ***farinosus***) Wize, *B. bassiana*, *Metarhizium anisopliae* formerly known as *Entomophthora anisopliae* (Metchnikoff) Sorokin, *Cordyceps scarabaeicola* Kobayasi and Shimizu, and ***Metarhizium rileyi*** previously known as *Nomuraea rileyi* (Farlow) 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) (Dorschner 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 after treating seeds, and their effect on several sorghum plants physiology parameters.

Materials And Methods

Entomopathogenic fungal strains source

B. bassiana (strain 7R), *Trichoderma gamsii* Samuels & Druzhin (Hypocreales: Hypocreaceae) (strain Z) and *M. brunneum* (strain Meta Br1) isolated from the Achaia region, Greece, were used for the *in plant* bioassays on pepper plants. 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.

Fungi production for *in plant* bioassays

To achieve the fungi growth, strains from Greece were cultured on Petri dishes with Sabouraud dextrose agar (SDA) culture medium (OXOID LTD), incubated at 25 ± 2°C temperature in dark conditions. After each fungus growth (covering the dish surface), it was isolated once more time, in order to avoid contamination and to achieve pure cultures of each one. The conidia were retrieved from cultures maintained on SDA. Isolates were maintained in Petri dishes on a nutrient SDA incubated at 25 ± 1°C and were renewed every month. 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 Stauros Peloponnesus (*Capsicum annuum* L., Solanales: Solanaceae). Pepper plants were pre-germinated in 2 × 2 cm pots (one seed per pot at a depth of about 1cm) 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 L: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% RH, and 16:8 h L:D photoperiod. Experimental pepper leaves were sprayed with 5 mL of the desired conidial concentration suspension (at 1×10^7 or 1×10^8 conidia/mL) of either *B. bassiana* strain 7R, *T. gamsii* strain Z, or *M. brunneum* strain Meta Br1, using a Badger 100 artist's airbrush (Badger Air-Brush Company, Franklin Park, IL). 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 these 24 h, 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 16L/8D photoperiod at three different temperatures: 20 °C, 25 °C, and 30 °C, under 90% RHs.

Fungi production for in field experiments

The Mexican GHA and PTG4 *B. bassiana* strains were growth using different culture conditions than those used for fungi production in Greece. In Mexico, the strains were activated by plating stock cultures onto potato dextrose agar (PDA, BD Difco, CDMX, Mexico) and incubated in darkness at 25 ± 2 °C for a week. To obtain a monosporic culture, a single selected colony was inoculated onto a PDA plate. This was considered the monosporic culture stock plate. Fresh conidia, collected from PDA plates that started from the monosporic stock, were incubated for 7 d in darkness at 25 ± 2 °C. A conidial suspension was 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* 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 León, 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. Cornstarch (CS) (Unilever Manufacturera, S. de R.L. de C.V., CDMX, 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 a 150 mL volume of conidia + CS, incubated 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. One irrigation and no fertilizers nor herbicides were applied.

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) 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, Ciudad de México, Mexico). Juice from those stems was extracted using a juice extractor (QJH-L100A, Kuala Lumpur, Malaysia) and the total volume was measured by a graduated plastic cylinder. 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 g 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 × 10^7 and 1 × 10^8 conidia/mL; and especially by *M. brunneum* treatment, where aphids' population on a 1 × 10^8 concentration treated leaf was almost zero.

The aphid population average after three days exposure to *B. bassiana* treatment resulted in 38 (10^3 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 NP9 0.05%) 38 aphids were detected (Table 1). After six days, the aphid population mean for each treatment was 32 (10^3 conidia/mL) and 23 aphids (10^8 conidia/mL) after *B. bassiana* treatment, 37 (10^3 conidia/mL) and 8 aphids (10^8 conidia/mL) after *M. brunneum* treatment, 32 (10^3 conidia/mL) and 12 aphids (10^8 conidia/mL) after *T. gamsii* treatment, and 48 aphids for control (H2O + Tergitol NP9 0.05%) treatments. Finally, the average population of aphids after nine days application of each treatment was 25 (10^3 conidia/mL) and 5 aphids (10^8 conidia/mL) in *B. bassiana* treatment; 34 (10^3 conidia/ml) and 1 aphid (10^8 conidia/mL) in *M. brunneum* treatment; 28 (10^3 conidia/mL) and 1 aphid (10^8 conidia/mL) in *T. gamsii* treatment, while 54 aphids in the untreated control (H2O + Tergitol NP9 0.05%) were detected (Table 1).

The Kaplan-Meier of aphids' survival analysis (Breslow test) after either EPF application indicated that the average total survival time after applying 1 × 10^3 conidia/mL was 8.5 d, whereas after applying 1 × 10^8 conidia/mL was 5.8 d. Specifically, the overall survival time after *B. bassiana* application was 7.5 d; where applying 1 × 10^3 was 8.6 d, and after applying 1 × 10^8 was 5.7 d. The overall survival time after *M. brunneum* application was 7.4 d, where applying 1 × 10^3 was 8.5 d and after applying 1 × 10^8 was 5.9 d. The overall survival time after *T. gamsii* application was 7.3 d, whereas by the untreated control was 8.9 d (Table 2).

Aerial mycelium *M. persicae* on cadavers after EPF expose and incubated under three different temperatures

Following treatment with *B. bassiana* strain 7R, *T. gamsii* 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) (Figure 1). At 20 °C, the aerial mycelium development on dead aphids was between 78 (*B. bassiana*) and 84% (*T. gamsii*); at 25 °C was between 83 (*B. bassiana*) and 88% (*T. gamsii*); whereas at 30 °C the aerial mycelium development on dead aphids was 75% (*B. bassiana*) and 79% (*T. gamsii*).

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 in the field. 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 solution prepared using an inert sticker. Results showed no differences in seed germination and

plant emergence. However, with both isolates, flowering index was increased compared to the values observed in the control and chemical treatments (Figure 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 (Figure 3).

Yield recorded data was analyzed by PCA means (Figure 4). The Bartlett's sphericity test *p* value (two-tailed) was 0.007, thus indicating significant PCA correlations. The three variables analyzed showed correlations ≥ 0.6 therefore were considered relevant: stem fresh weight (g/plant) = 0.826; juice volume (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 was closely related to the time interval 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 1×10^6 conidia/mL, *B. bassiana*, *M. brunneum* and *T. gamsii* gave appreciable mortality. Ekesi et al. (2000) reported similar results, detecting 91 and 93% mortality of *Aphis craccivora* C.L.Koch, in the seventh day after the spraying. Loureiro and Moino (2006) reported that 1×10^6, 1×10^7 and 1×10^8 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* 1×10^7 conidia/mL applications on 4 days old *A. gossypii* aphids. The control values were calculated upon treated insect's population decrease, compared with the untreated ones.

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) had reported very high mortality in *C. lanigera*, at the tenth day after spraying. A progressive reduction in the mortality of aphids was observed with decreasing concentrations. In the sublethal concentrations, the mortality of aphids ranged as no high for all tested entomopathogens. Filho et al. (2011) demonstrated that the numbers of adults and nymphs of *M. persicae* per leaf were significantly reduced in plots treated with two *B. bassiana* isolates ranging from 57 to 60%.

The EPF mycelium virulence depends not only on the target aphid species, but also on the temperature and relative humidity (RH) of the environment (Yeo et al., 2003; Vu et al., 2007); thus, it is important to select an entomopathogenic fungus appropriate for 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 aphid adults and their reproduction. Our results concur with Hesketh et al. (2008), Shan and Feng (2010) and Tesfaye 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 reproduction reduction ranging from 36% to 74% after high dose applications of EPF, as reported by *M. persicae* by He and Li (2008) and Gurulingappa et al. (2011). However, reproduction reductions have little long-lasting effect on the aphid population. This statistic 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).

The results presented in Table 1 and Table 2 show the same impact on fungal infection in the reproduction cycle of the *M. persicae*. The same results in both survival times and reproductive period could be due to the alignment of infection time and reproductive cycle in order to prevent population growth. The population growth of *M. persicae* in 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 than in the untreated plants but on the day 9, the population of *M. persicae* in sprayed plants was significantly different to the observed in the untreated plants. This significantly decreased pattern of the population was found in all the concentrations that were used.

Treatment with *M. brunneum* strain Meta Br1 had the highest effect on aphid reproduction only in 10^8 conidia/mL suspensions. In contrast to what was reported by Jandricic et al. 2014, who found *M. brunneum* strain F52 had the highest effect on aphid reproduction, in our study two fungus, *B. bassiana* 7R and *T. gamsii*, were effective at all other used concentrations. Our observations mirror 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 effect of fungal infection of the mother aphid on the viability of her offspring, as reported also 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 1×10^5 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-entomopathogenic fungi relationships without promoting plant pathogens. Further research into this area or the use of more progressive approaches are needed. Therefore, we decided to test a newly reported approach (Lopez et al. 2014) to control an economically important pest, based on the previously discussed findings.

In Mexico, yellow sorghum aphid *M. sacchari* was first recorded in the state of Florida in 1977 and Louisiana in 1999 only in sugarcane crops. However, in 2013 the health authorities of the United States reported the presence of a new plague of aphids that were damaging sorghum crops in several counties of Texas, which months later spread to Louisiana, Oklahoma and a county in Mississippi. Later, the plague reached three states of Mexico. By the end of 2015, the yellow aphid already inhabited crops in 400 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. Aphid control, for the other hand, 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 fungal entomopathogens among others (SENASICA, 2020).

The use of *B bassiana* in seed treatments in cotton has resulted in lower insect pest damage and higher plant growth and yield under field conditions (Lopez et al. 2014). In this work we present a field experiment were a native strain of *B. bassiana* (PTG4) and a commercially available strain (GHA) were used 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 PTG4 treated plants and that this treatment had positive effects on the flowering index. On addition, the fresh stem weight and juice volume was significatively increased mainly in GHA treated plants. In general, the yield obtained measured as g of sugar/plant was higher in the treated plants than in the controls. It indicates that this protocol can be used in IPM strategies to control aphid's population and, in the case of sorghum production, have positive effects on yield.

Declarations

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Conflicts of interest/Competing interests

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

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.

Authors' contributions

All authors contributed to the study 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.

Ethics statement

Ethical approval was waived by the local Ethics Committees of the Autonomous University of Nuevo Leon and the University of Patras, 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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Tables

Table 1. Population (%) (% ± sd) (*F*=8.396, *df*=12.550, *P*<0.001) of the adults of *M. persicae* after treatment with 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_20 plus Tergitol NP9 0.05%). Mean ± sd values with the same letter within a column are not significantly different (P < 0.05)

Treatment/Mortality	Concentration	3 days	6 days	9 days
B. bassiana	10^3	38±6.1b*	32±3*b	25±3.6*c
	10^4	42±6.1b*	27.6±4.6*b	23±2.1*c
	10^5	32±6*ab	24.3±3.1*ab	15.7±3.2*b
	10^6	33±7.5*ab	20±4.2*ab	15±3.1*b
	10^7	25±6*a	15±3.1*a	4.6±5*a
	10^8	24±1.53*ab	13±3.2*a	6±5.3*a
M. brunneum	10^3	44.6±2.4*d	37±3.5*c	29±4.5*d
	10^4	39.3±3.5*c	29.3±1.5*b	25±1*c
	10^5	32.6±1.1*b	25.3±3.8*b	20.7±3.1c
	10^6	28.3±1.7*b	22.6±5*b	16.3±3.8*b
	10^7	29.3±1.1*b	12±10.4*ab	8±7.2*ab
	10^8	21.3±5.8*a	7.6±6.8*a	0.7±1.1*a
T. gamsii	10^3	34.7±3.5*c	29.3±1.5*c	26.6±2.1*d
	10^4	33.3±6.6*c	31.6±5.5*d	27.6±5.5*d
	10^5	29±1.7*b	24.7±3.1*c	21±2.6*c
	10^6	26.3±1.5*b	24±4*c	16.3±2.3*b
	10^7	21.7±5.5*b	12±10.4*bc	5±4.6*a
	10^8	20.3±1.5*a	11.6±1.5*a	1±1.73*a
Control	*H_2O + Tergitol Np9*	38±3.9	48.1±8.8	54±8.3

Table 2. Survival time of adults *M. persicae* from Kaplan-Meier (Breslow test) after 9 days.

Treatment	Concentration	Median Survival Time (days)	Std. Deviation	95% Confidence internal	
				Lower Bound	Upper Bound
B. bassiana	10^3	8.5	.217	8.076	8.924
	10^4	8.3	.241	7.827	8.773
	10^5	8.0	.265	7.481	8.519
	10^6	7.7	.313	7.087	8.313
	10^7	6.5	.325	5.864	7.136
	10^8	5.8	.351	5.113	6.487
	Overall	7.5	.137	7.198	7.735
M. brunneum	10^3	8.6	.148	8.511	8.889
	10^4	8.1	.299	7.514	8.686
	10^5	7.9	.309	7.295	8.505
	10^6	7.6	.315	6.983	8.217
	10^7	6.7	.344	6.026	7.374
	10^8	5.5	.300	5.112	6.288
	Overall	7.4	.139	7.094	7.639
T. gamsii	10^3	8.5	.184	8.340	9.060
	10^4	8.0	.407	7.002	8.598
	10^5	7.9	.345	7.224	8.576
	10^6	7.5	.376	6.762	8.238
	10^7	6.2	.288	5.636	6.764
	10^8	5.9	.337	5.239	6.561
	Overall	7.3	.152	7.002	7.598
Control		8.9	.098	8.707	9.093

Figures

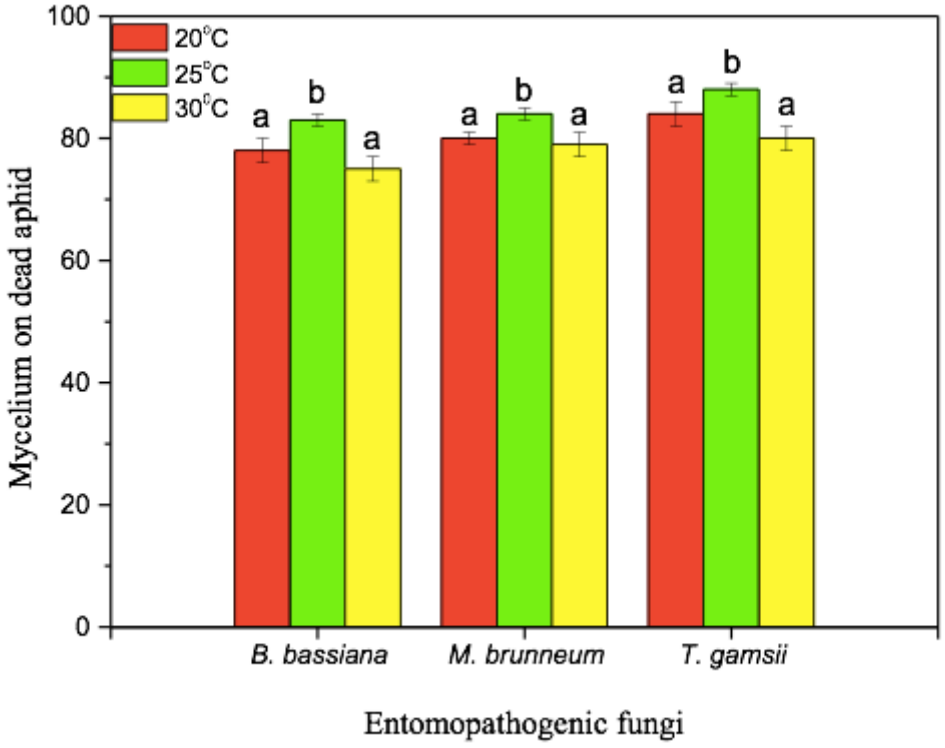

Figure 1

Fungal growth on cadavers of M. persicae after the expose to the entomopathogenic fungi at different temperatures in laboratory conditions. Percentage of presence of 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 $p < 0.05$.

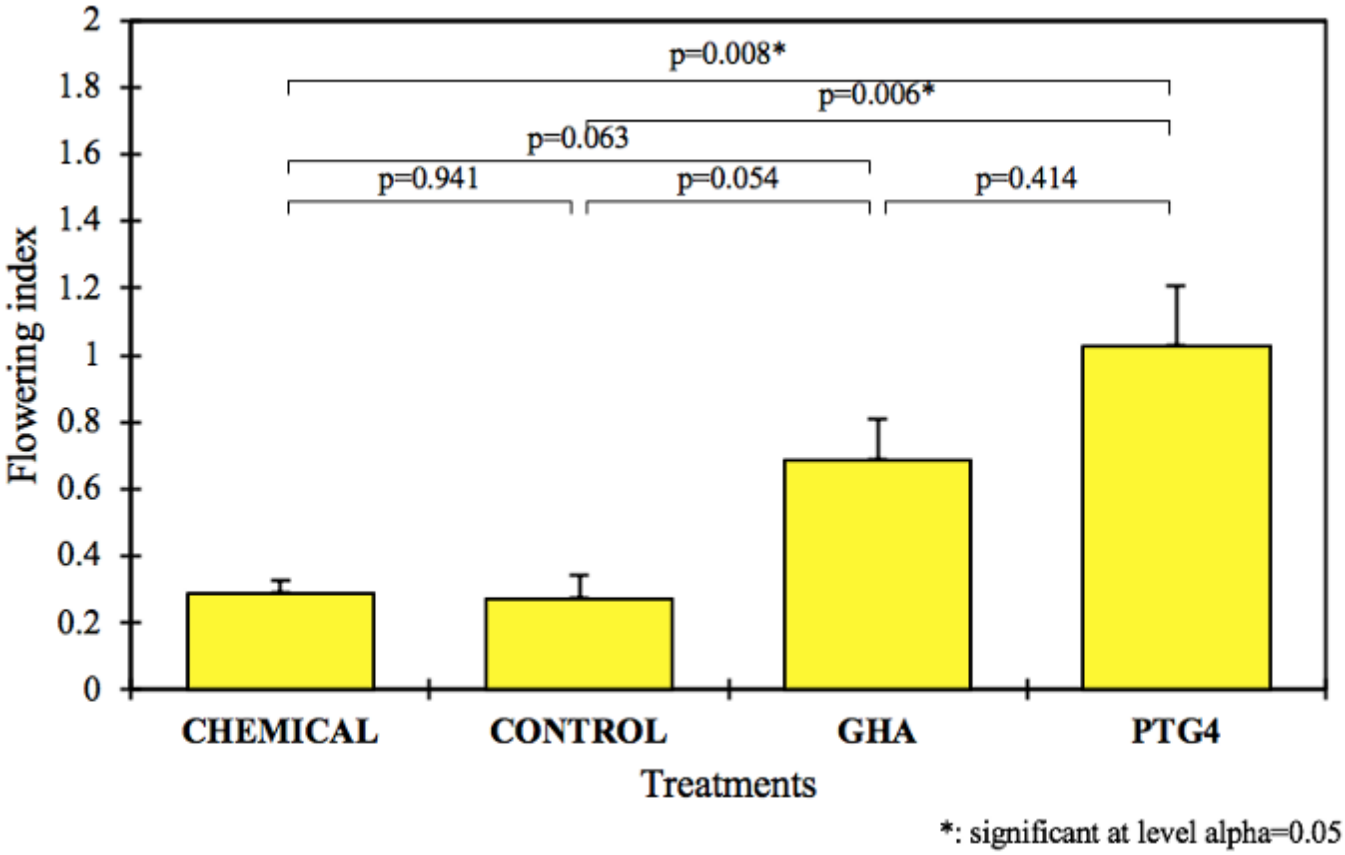

Figure 2

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. PTG4 treatment was significantly different (P=0.006) as well as chemical treatment vs. PTG4 treatment (P=0.008).

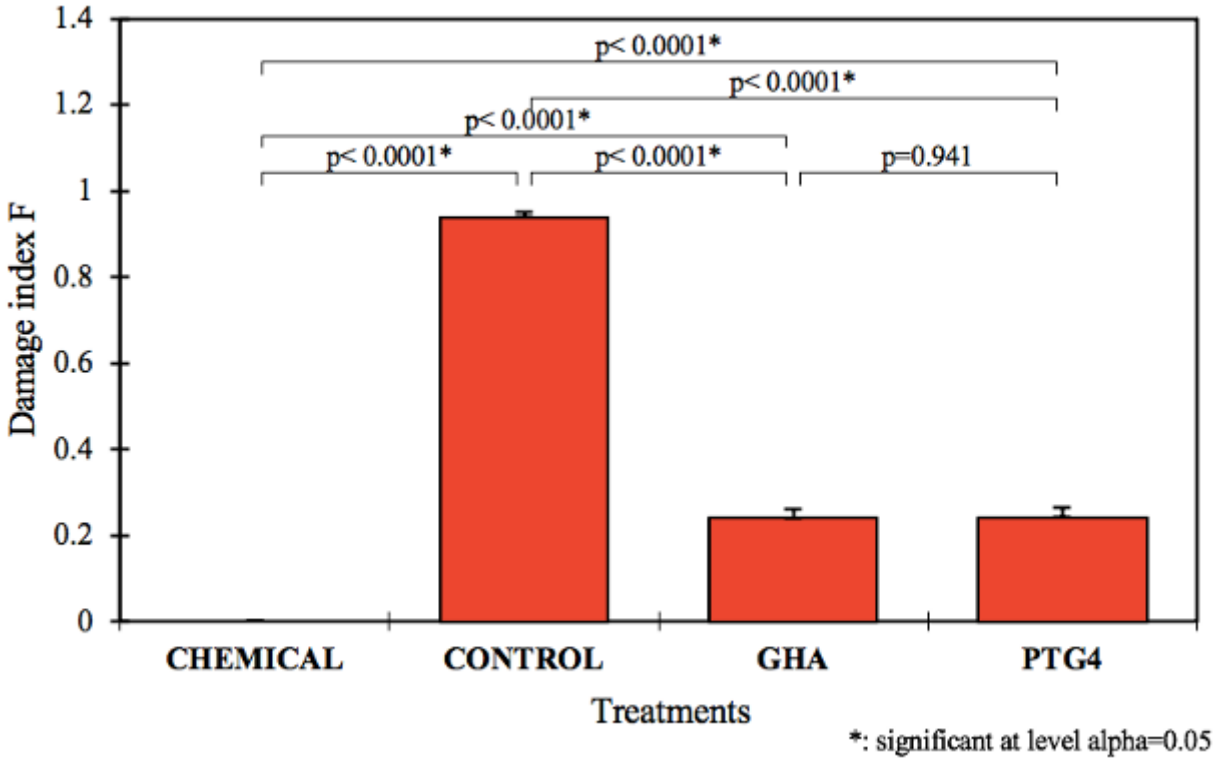

Figure 3

Aphids damage in the field experiment. Damage index F (arcsine 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 GHA and 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.

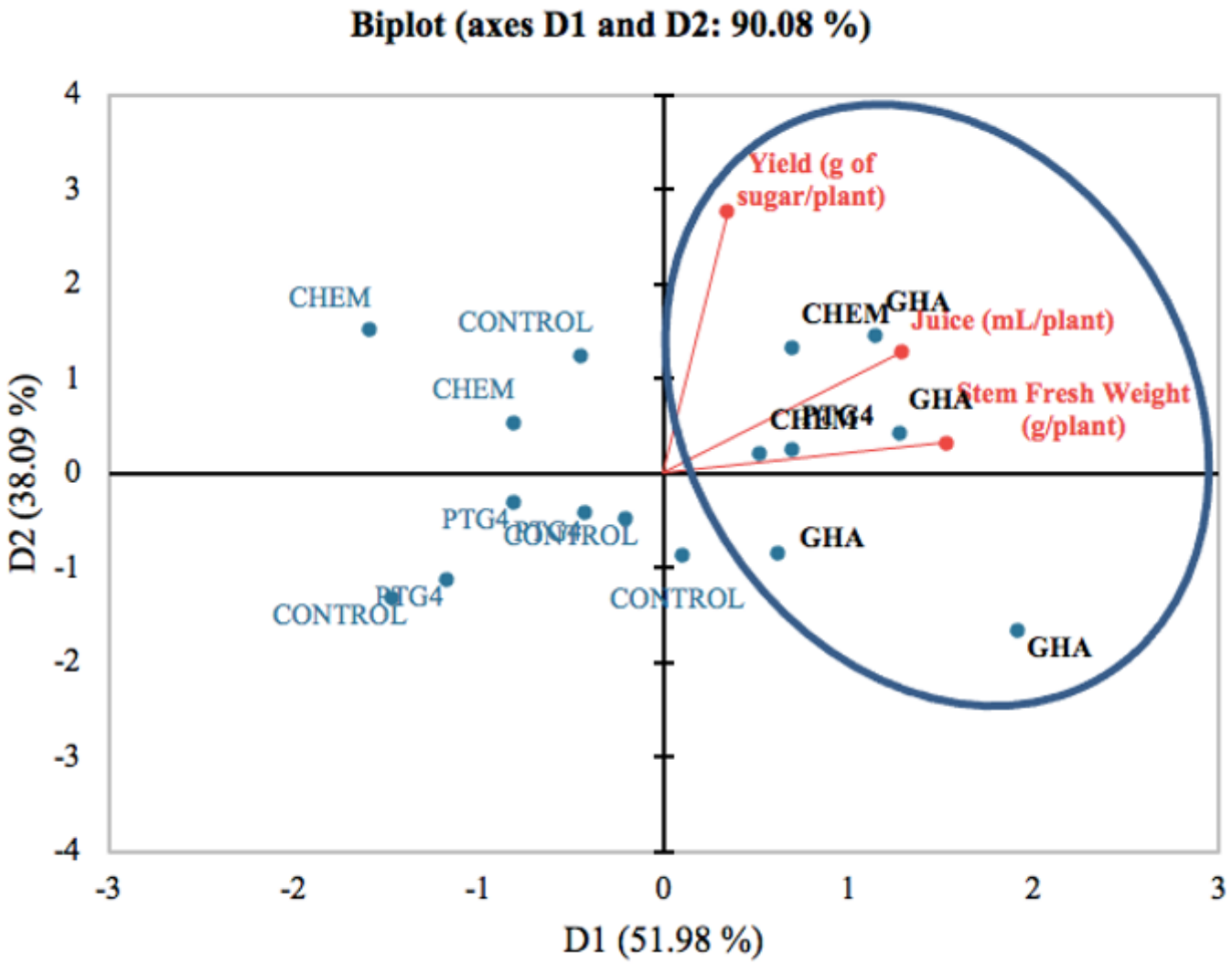

Figure 4

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.