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RESEARCH

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Efficacy of the entomopathogenic fungus, *Beauveria bassiana* and *Lecanicillium muscarium* against two main pests, *Bemisia tabaci* (Genn.) and *Tetranychus urticae* (Koch), under geothermal greenhouses of Southern Tunisia

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Abstract

Background: The geothermal greenhouses in Southern Tunisia are an important axis of agricultural development. This sector faces many abiotic and biotic constraints that could threat its sustainability. Thus, the heated greenhouses encounter destructive pests such as the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and the two-spotted spider mite *Tetranychus urticae* (Koch) (Acari: Tetranychidae).

Results: This study aimed to assess the effect of the entomopathogenic fungi (EPF) *Beauveria bassiana* (strain ATCC and strain R444) and *Lecanicillium muscarium* strain Ve6 on simultaneous existence of *T. urticae* and *B. tabaci* in the host plants. The EPF had a significant effect on eggs and larvae of *B. tabaci* and on eggs and mobile forms of *T. urticae* in particular. The use of *B. bassiana* ATCC, *B. bassiana* R444 and *L. muscarium* strains Ve6 showed significant efficacies against *B. tabaci* larvae and eggs compared to untreated control. Indeed, the reduction percent of *B. tabaci* eggs varied between 42.65 and 58.52%. Thus, the efficacy against the number of *B. tabaci* larvae was in order to 65.04, 60.26 and 55.52% of *B. bassiana* strain ATCC, *B. bassiana* strain R444 and *L. muscarium* strain Ve6, respectively. In addition, these EPF were very effective on *T. urticae* eggs with a percentage reduction greater than 92.86%, whereas the percentage reduction in the *T. urticae* mobile forms varied between 95.11 and 98.52%.

Conclusions: Use of EPF will be an imperative to develop directed interventions at the integrated management of these two pests in protected and geothermal crops.

Keywords: *Bemisia tabaci*, *Tetranychus urticae*, *Beauveria bassiana*, *Lecanicillium muscarium*, Biological control

Background

The whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is an important agricultural and economic pest distributed worldwide. It can feed on more than 600 species of plants (De Barro et al. 2011). *B. tabaci* is a biting-sucking insect that causes the formation of small yellow spots on leaves or fruits and secretes honeydew which provides a medium for the growth of

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sooty mold, reducing photosynthesis activities (Solanki and Jha 2018). This pest is a vector for large categories of viruses (Jones 2003) most of which belong to the genus Begomovirus, in particular the virus TYLCV «Tomato Yellow Leaf Curl Virus» (Navas-Castillo et al. 2011). In Southern Tunisia, this whitefly species causes harmful damage to crops heated by geothermal waters (Bel Kadhi 2004).

The spider mite *Tetranychus urticae* (Koch) (Acari: Tetranychidae) is one of the most destructive pests, feeding on over 1100 plant species; including vegetable, fruit and ornamental crops (Bensoussan et al. 2018). *T. urticae* is a native to the temperate zone; it is frequently introduced into intensively cultivated regions in the tropics. Due to its fast growth rate and high reproductive potential, this mite can colonize crops quickly (Farazmand and Amir-Maafi 2018). *T. urticae* damages plants by feeding on the contents of leaf mesophyll cells using a stylet (Park and Lee 2002).

The excessive use of chemical pesticides has led to many problems for human health and the environment. In addition, the development of pest resistance and the destruction of non-target organisms was studied (Wang et al. 2018). *B. tabaci* has developed resistance to most of the insecticides used (Basit 2019). According to Wu et al. (2016), the widespread use of pesticides has reduced the number of natural enemies of the predatory mites of *T. urticae* (Nouran et al. 2021). In recent years, research has been developed to use biological control agents, particularly entomopathogenic fungi (EPF) as an ecological alternative to chemical control (Vega 2018). Different isolates of *Lecanicillium muscarium* and *Beauveria bassiana* were effective against sap-sucking insects (Hesketh et al. 2008), especially *B. tabaci* (Abdel-Raheem and Al-Keridis 2017). These EPF were developed as an alternative to synthetic miticides and can manage mite populations naturally (Maniania et al. 2008).

The aim of this study was to test the efficacy of commercial EPF *B. bassiana* (ATCC 74,040, R444) and *L. muscarium* Ve6 against two major pests of cucurbit crops, *B. tabaci* and *T. urticae*, which co-exist on the same host plants.

Methods

Cultures of Bemisia tabaci and Tetranychus urticae

Bemisia tabaci and *T. urticae* were collected from a greenhouse at the Technical Center for Protected Crops and Geothermal TCPCG in Gabes, Tunisia. Rearing was placed on *Cucumis melon* plants grown in 0.5 L/ pots. Plants were inoculated with these two pests from the stage of the 5–6 leaves.

Entomopathogenic fungi

Three commercial EPF-based biopesticides were tested: Naturalis® (*B. bassiana* strain ATCC 74,040 (2.3×10^9spores/ml)), Bb-Protec® WP (*B. bassiana* strain R444 ($\geq 2 \times 10^9$ spores/gram), and Mycotal® WG (*Lecanicillium muscarium* strain Ve6 (19–79) (10^{10} spores/gram)). These EPF were cultured on Potato Dextrose Agar (PDA) medium and incubated at 25 ± 1 °C for 7 days to confirm their viability before being used (Nouran et al. 2021).

Experimental protocol

Efficacy study of EPF against *B. tabaci* and *T. urticae* was carried out on melon plants by comparing it with the chemical insecticide, Pegasus® containing the active substance Diafenthiuron (500 g/l) (approved against whitefly and dust mites with a dose of 60 ml/hl) as well as an untreated control. This experiment was conducted in a tunnel-type greenhouse, at an area of 500 m^2, 45 days after pest infestation. Temperature and relative humidity inside the greenhouse were recorded using a portable data logger (Fig. 1).

The Randomized Complete Blocks Design (RCBD) was used for this experiment with five treatments and three replicates. Each experimental unit contains 12 melon plants infested with *B. tabaci* and *T. urticae*. The experimental units were treated with *B. bassiana* strain R 444 (1 g/1 l), *B. bassiana* strain ATCC (1 ml/1 l), *L. muscarium* strain Ve6 (1 g/1L), Diafenthiuron (60 ml/hl) and an untreated control. The treatments were applied using a portable sprayer with a volume of 1.5 L. Treatment was carried out on June 3, 2021.

Sampling

Fifteen leaves were sampled randomly from each treatment for examination using a Leica® EZ4E microscopy model to follow the development of *B. tabaci* and *T. urticae*. The different stages of development of *B. tabaci* and *T. urticae* were calculated for each sample. In order to evaluate the efficacy of this EPF, the rate of reduction for developmental stages of *B. tabaci* and *T. urticae* in melon leaves was calculated according to the formula (Henderson and Tilton 1955), where

$$\begin{aligned} \text{reduction}(\%) = (1 - (&\text{No. in control before treatment} \\ &\times \text{No. in treatment after treatment}) \\ &/(\text{No. in control after treatment} \\ &\times \text{No. in treatment before treatment})) \times 100 \end{aligned} \quad (1)$$

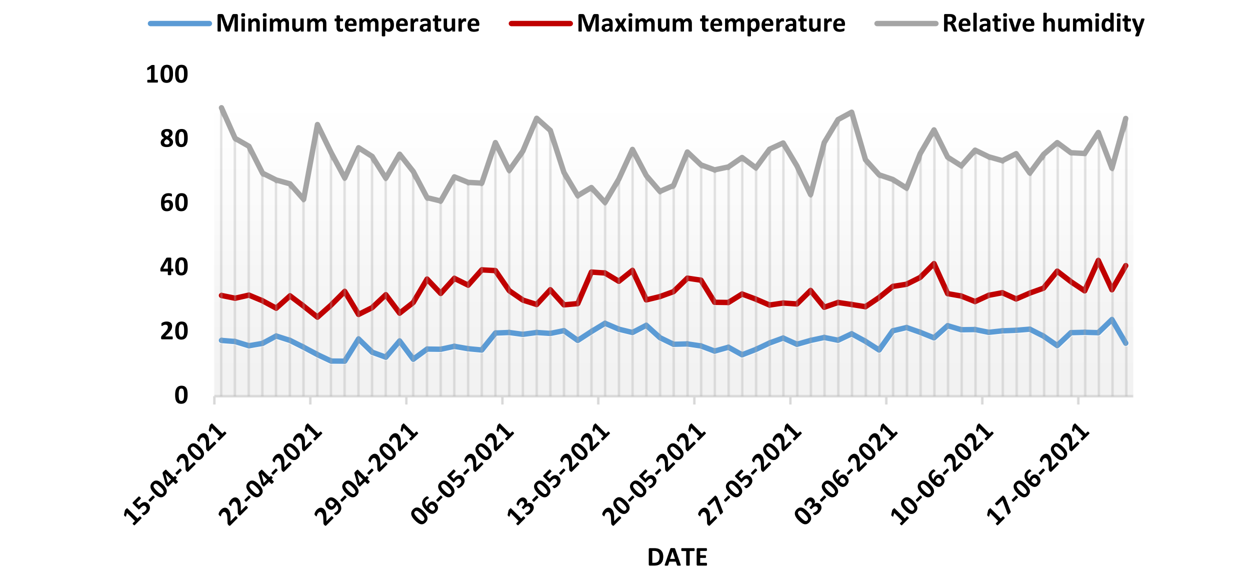

Fig. 1 Minimum temperature, maximum temperature and relative humidity data inside the test greenhouse

Statistical analysis

For the analysis of variance (ANOVA), the number of eggs, larvae of *B. tabaci* and the number of eggs, mobile forms of *T. urticae* as well as the rate of reduction after treatments were subjected to a unidirectional analysis associated with a Duncan test ($P<0.05$) using XLSTAT 2019, Excel® 2013.

Results

Evaluation of the ovicidal effect of EPF against B. tabaci and T. urticae

Number of surviving eggs of *B. tabaci* and *T. urticae* on melon leaves decreased rapidly on plants treated with EPF *B. bassiana* strain ATCC, *B. bassiana* strain R444 and *L. muscarium* Ve6 as well as plants treated with Diafenthiuron. Over the monitoring period, the number of surviving eggs of *B. tabaci* for the untreated plants (control) was relatively high; it exceeded 25.33 eggs/leaf, whereas it decreased to an average of 5.53, 6.86 and 6.6 eggs 3 days after treatment with *B. bassiana* ATCC, *B. bassiana* R444 and *L. muscarium*, respectively. Similar to EPF, Diafenthiuron reduced the number of eggs of *B. tabaci* from 12.26 to only 4.26 eggs/leaf 3 days after treatment (Fig. 2A). After 15 days post-treatment, the number of *B. tabaci* eggs started to increase at all treatments but remained significantly lower compared to those in untreated control ($F=6.412$; $P=<0.0001$).

In addition, the number of surviving eggs of *T. urticae* for the untreated control lots was high, varying between 4.4 and 12.4 eggs/leaf. Indeed, the number of *T. urticae* eggs was 13.93 eggs/leaf before the treatments, which led to a regular decrease of 0.06, 0.06, 0.4 and 0.2 eggs/leaf on the 15th day after treatment for *B. bassiana* ATCC, *B. bassiana* R444 and *L. muscarium* Ve 6, respectively. Similarly, for plants treated with Diafenthiuron, the number of *T. urticae* eggs per leaf decreased from 15.86 before treatment to only 0.2 eggs/leaf on the 15th day after treatment (Fig. 2A). According to the statistical analysis, the three commercial products based on EPF significantly reduced the number of *T. urticae* eggs even as the chemical treatment over time ($F=5.764$; $P<0.0001$).

The percentages of reduction in the total number of live eggs of *B. tabaci* were of 58.52%, 50.45% and 42.65% for the *B. bassiana* strain ATCC, *B. bassiana* strain R444, *L. muscarium* strain Ve6, respectively and 66.03% after treatment with Diafenthiuron (Table 1). The ANOVA analysis of the reduction in *B. tabaci* eggs showed that no significant effect ($F=1.281$; $P=0.325$) between the EPF treatment and the chemical treatment based on Diafenthiuron.

T. urticae egg reduction rates were generally higher than *B. tabaci* egg reduction rates exceeding 92.86% (Table 1). The analysis of variance showed that *B. bassiana* ATCC and *B. bassiana* strains R444 had a more effective effect than *L. muscarium* strain Ve6 15 days after treatments.

Evaluation of the treatments effects on the larval stages of B. tabaci and the mobile forms of T. urticae

Density of *B. tabaci* larvae decreased from 10.73, 10.26 and 11.73 larvae per leaf before treatment to 3.26, 3.93 and 4.26 larvae 3 days after treatments with *B. bassiana*

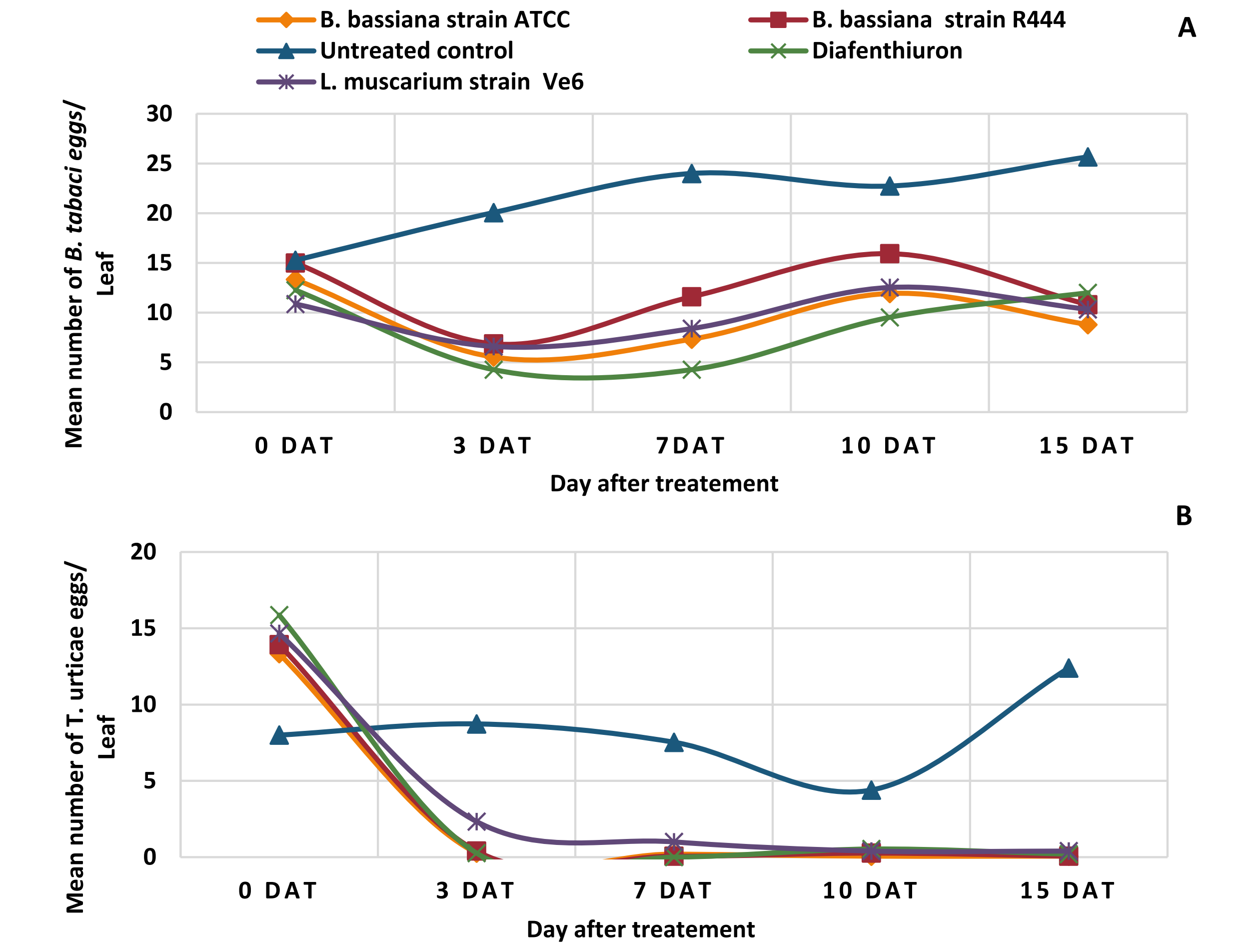

Fig. 2 Mean number of *Bemisia tabaci* eggs and *Tetranychus urticae* as a function of treatment with entomopathogenic fungi for 15 days (**A**: *Bemisia tabaci* eggs, **B**: *Tetranychus urticae* eggs)

Table 1 Percentage reduction in surviving *Bemisia tabaci* eggs and *Tetranychus urticae* eggs as a function of treatment with entomopathogenic fungi

Fungi	Reduction rate of surviving *Bemisia tabaci* eggs	Reduction rate of surviving *Tetranychus urticae* eggs
Beauveria bassiana strain ATCC	58.52[a]	98.83[a]
B. bassiana strain R444	50.45[a]	98.26[a]
Diafenthiuron	66.03[a]	97.88[a]
Lecanicillium muscarium strain *Ve6*	42.65[a]	92.86[b]
F value	1.281	3.039
P value	0.325	0.071

Different letters denote means are significantly different from one another, as determined by Duncan

strain ATCC, *B. bassiana* strain R444 and *L. muscarium* strain Ve6, respectively. Similarly, the substance Diafenthiuron reduced the number of *B. tabaci* larvae from 12.13 to 3.26 larvae per melon leaf 3 days after treatment (Fig. 3A). The ANOVA analysis of the numbers of *B. tabaci* larvae highlighted a significant effect after the EPF treatments by comparing to the untreated control ($F=4.851$; $P=0.001$).

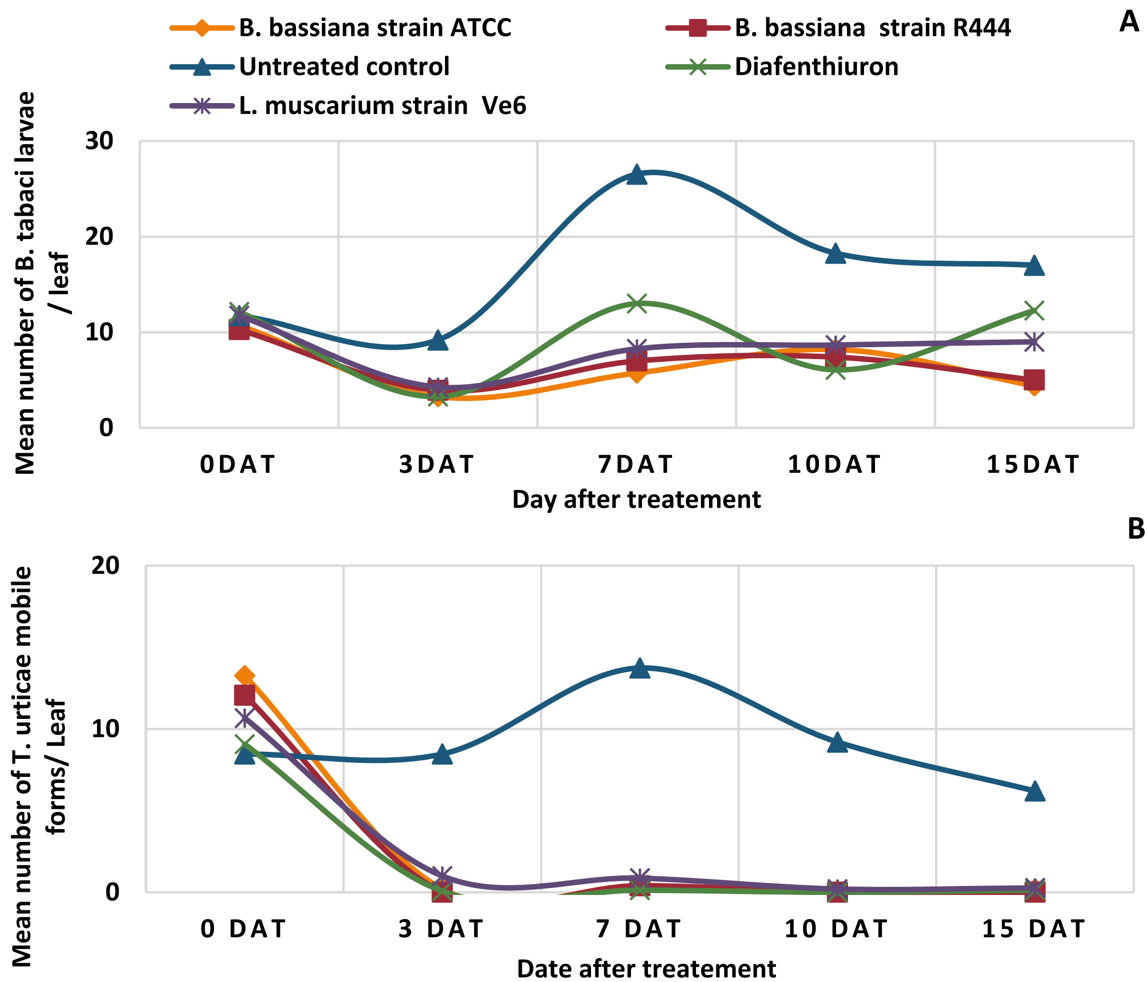

Fig. 3 Mean number of *Bemisia tabaci* larvae and *Tetranychus urticae* mobile forms as a function of treatment with entomopathogenic fungi for 15 days (**A**: *Bemisia tabaci* larvae, **B**: *Tetranychus urticae* mobile forms)

In addition, during the monitoring period, the total number of surviving mobile forms of *T. urticae* for the untreated control plants increased and varied between 6.2 and 13.73 mobile forms/leaf. On the other hand, the number of larvae, protonymphs, deutonymphs and adults of *T. urticae* decreased rapidly for plants treated with *B. bassiana* (strain ATCC and strain R444) and *L. muscarium* strain Ve6 as well as those treated with Diafenthiuron until their abandon (Fig. 3B). The EPF affected significantly ($F=12.845$; $P<0.0001$) the number of mobile forms of *T. urticae*.

The evaluation of the efficacy of the EPF tested is based on the calculation of reduction rates against *B. tabaci* larvae and mobile forms of *T. urticae*. This test consisted of studying the effectiveness of EPFs and comparing them to that of a Pegasus® insecticide (Diafenthiuron) approved against *B. tabaci* and *T. urticae* and an untreated control.

The calculation of the reduction percentages against the total number of *B. tabaci* larvae showed a reduction exceeding 54.09% in the plants treated with EPF as well as those treated with Diafenthiuron. In addition, the percentages of efficacy against mobile forms of *T. urticae* were of 98.52, 99.48 and 95.11%, following treatment with *B. bassiana* strain ATCC, *B. bassiana* strain R444 and *L. muscarium* strain Ve6, respectively (Table 2).

Discussion

The treatments with *B. bassiana* (strain ATCC and strain R444) and *L. muscarium* strain Ve6 showed a very significant efficacy against different stages of both pests *B. tabaci* and *T. urticae* than the untreated control. In fact, they can reduce the number of *B. tabaci* eggs by up to 65% and the number of *B. tabaci* larvae by up to 58% compared to untreated plants. In this context, Assadi

Table 2 Percentage reduction in *B. tabaci* larvae and mobile forms (larvae, protonymphs, deutonymphs and adults) of *Tetranychus urticae* as a function of treatment with entomopathogenic fungi

Fungi	Reduction rate of surviving *Bemisia tabaci* larvae	Reduction rate of surviving *Tetranychus urticae* mobile forms
Beauveria bassiana strain ATCC	65.04[a]	98.52[a]
B. bassiana strain R444	60.26[a]	99.48[a]
Diafenthiuron	54.09[a]	99.08[a]
Lecanicillium\muscarium strain *Ve*6	55.52[a]	95.11[b]
F value	0.658	4.905
P value	0.593	0.019

Different letters denote means are significantly different from one another, as determined by Duncan

et al. (2021) demonstrated that *B. bassiana* strain R444 and *L. muscarium* strain Ve6 were significantly effective on all developmental stages of *B. tabaci* under controlled conditions. In addition, several studies have shown that EPF are very effective against the whitefly. According to Keerio et al. (2020), two strains of *B. bassiana* (BB-72 and BB-252) and one strain of *L. lecanii* (V-2) caused a maximum mortality of *B. tabaci* after 12 days of treatment at different temperatures. Wari et al. (2020) showed that *B. bassiana* strain GHA caused a significant reduction in different life stages of *B. tabaci* under greenhouse conditions. Application of several *B. bassiana* isolates against *B. tabaci* development stages on different host plants showed significant efficacy that affected the reproductive rates (Zafar et al. 2016). Others, *B. bassiana* and *M. anisopliae* caused the highest larval mortality of *B. tabaci* under greenhouse conditions (Sain et al. 2021).

In addition, the tested EPF showed very high efficacy against eggs and motile forms of *T. urticae* with a reduction percentage greater than 92%. Chandler et al. (2005) found that *B. bassiana* (Naturalis-L) reduced the number of *T. urticae* adults, larvae and eggs by up to 97%. Additionally, fungal isolates of *M. anisopliae* and *B. bassiana* were pathogenic against the mite females, which were more virulent at 25, 30, and 35 °C than at 20 °C (Bugeme et al. 2009). Indeed, these EPF are highly capable of causing mortality for all stages of *T. urticae.* Several studies have shown that spider mites are susceptible to and affected by EPF. *B. bassiana* strains showed a high virulence for *T. urticae* eggs and adults (Hassan et al. 2017). *M. anisopliae* isolates with potential for the control of *T. urticae* (Elhakim et al. 2020), especially for adult females (Castro et al. 2018). Al Khoury et al. (2020) showed that following the application of *B. bassiana* against all life stages of *T. urticae*, where the mortality rate was 52, 67.9 and 95.3% in eggs, mobile forms and adults, respectively. Moreover, the two isolates of *B. bassiana* and 17 isolates of *M. anisopliae* caused a mortality rate of 80% against *Tetranychus evansi* Baker & Pritchard (Wekesa et al. 2005). Thus, the use of *B. bassiana* on bean plants led to a reduction in adult populations of *T. urticae* by significantly affecting the fecundity of females (Wu et al. 2020) and adverse effects on certain other biological parameters of this mite (Kheradmand et al. 2021).

Bean seed treatment with *B. bassiana* and *M. robertsii* isolates resulted in endophyte colonization and reduced *T. urticae* populations under greenhouse conditions (Canassa et al. 2019). However, EPF had no obvious pathogenicity for the phytoseiid predatory mites *Neoseiulus californicus* and *Phytoseiulus persimilis* (Dogan et al. 2017). In addition, mortality of *T. urticae* was obtained when predatory mites carried conidia *B. bassiana* or *M. anisopliae*. Predatory mites that were able to control *T. urticae* could be associated with EPF (Castillo-Ramírez et al. 2020).

Conclusion

In conclusion, it appears that EPF treatment can be used as a mean of controlling *B. tabaci* and *T. urticae* in protected and geothermal crops. They are very effective, especially on the eggs and mobile forms of *T. urticae*. This finding inspires us to apply this fungus on the first clutches. It can be considered as an innovative biological control tool for managing these two pests of cucurbits in heated greenhouses in Southern Tunisia. Since these pests are found on the same host plants, the use of this EPF can reduce the number of chemical applications.

Abbreviations

EPF: Entomopathogenic fungi; TCPG: The technical center for protected and geothermal crops in Chenchou; PDA: Potato dextrose agar; RCBD: Randomized complete blocks design; ANOVA: Analysis of variance; DAT: Day after treatment; P: *P* Value.

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Author contributions
SC and MSB conceived and designed the experiments. SC and BHA performed the experiments. SC analyzed the data. SC, KLG, and MSB wrote the paper. All authors read and approved the final manuscript.

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Availability of data and materials
All data of the study have been presented in the manuscript, and high-quality and grade materials were used in this study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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