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In vitro pathogenicity of the fungi *Beauveria bassiana* and *Lecanicillium lecanii* at different temperatures against the whitefly, *Bemisia tabaci* (Genn.) (*Hemiptera: Aleyrodidae*)

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Abstract

The whitefly, *Bemisia tabaci* (Genn.) (*Hemiptera*: *Aleyrodidae*), is one of the most damaging pests in field crops as well as in greenhouses. The present study aimed to evaluate the in vitro pathogenicity of 2 fungus strains of *Beauveria bassiana* (BB-72 and BB-252) and one strain of *Lecanicillium lecanii* (V-2) against the whitefly (*B. tabaci*) at 4 different temperature degrees 20, 24, 28, and 32 °C, using a spray method. Three different bioassays were carried out comprised of conidial concentrations and filtrate of fungal strains, BB-72, BB-252 and V-2, and their binary combinations. The 1.5 ml of fungal filtrate was used in filtrate bioassay for each strain of fungus. Three different concentrations (1×10^6, 1×10^7, and 1×10^8 conidia ml^{-1}) were used in conidial bioassay for each strain of fungus, whereas in bioassay for binary combination (1 ml conidia + 1 ml filtrate) of BB-72 × BB-72, BB-252 × BB-252, and V2 × V2 were used for these strains of fungus. According to the outcomes, the maximum mortality against whiteflies was observed on 12th day post-treatment. In conidial bioassay, the maximum mortality of *B. tabaci* was observed in BB-72 isolate (84%), BB-252 isolate (77%), and V-2 isolate (67%) at the highest concentration (1×10^8 conidia ml^{-1}) at 24 °C, and minimum mortality was recorded in BB-72 isolate (33%), BB-252 isolate (29%), and V-2 isolate (19%) at the lowest concentration 1×10^6 conidia ml^{-1} at 32 °C on 12th day post-treatment. Infiltrate bioassay, BB-72 isolate exhibited maximum mortality (92%) at 24 °C, and V-2 isolate showed minimum mortality (34%) at 32 °C on 12th day post-treatment. Furthermore, in binary combination bioassay, the highest whitefly mortality was recorded in BB-72 × BB-72 isolate (87%), BB-252 × BB-252 isolate (73%), and V2 × V2 isolate (65%) at 24 °C and the lowest mortality in BB-72 × BB-72 isolate (57%), BB-252 × BB-252 isolate (50%), and V2 × V2 isolate (39%) at 32 °C on 12th day post-treatment. In all bioassays, the BB-72 isolate was the utmost virulent, and application of its filtrate was found to be the most impressive against *B. tabaci*.

Keywords: Entomopathogenic fungi, *Beauveria bassiana*, *Lecanicillium lecanii*, Virulence, *Bemisia tabaci*, Temperature degrees

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Background

The whitefly, *Bemisia tabaci* (Genn.) (*Hemiptera*: *Aleyrodidae*), is one of the most destructive pest in the field crops as well as in greenhouses. It directly damages the crops causing heavy losses of the crops (Oliveira et al. 2001). Entomopathogenic fungi (EPF) have the ability to individually affect their host directly through the inward penetration in an insect (Lacey et al. 1996). *Beauveria bassiana, V. lecanii*, and *Metarhizium anisopliae* proved to be the most promising fungi in this respect (Wraight et al. 2007). For the control of whitefly, the entomopathogenic fungi have been used almost all around the world (Jackson et al. 2010). The potential strains best for utilization as pest management tools can be categorized into two groups: one group includes *Hyphomycetes* in *Deuteromycotina* and the other belongs to *Entomophthorales* (EPF) in Zygomycota (Feng 1998).

Very few of these EPF, viz., *Nomuraea rileyi*, *Beauveria bassiana*, *Paecilomyces fumosoroseus*, *Metarhizium anisopliae*, *B. brongniartii*, and *Verticillium lecanii* have been registered as products to control insect pests (Shah and Goettel 1999). *B. tabaci* showed resistance against the chemical insecticides such as nitenpyram, imidacloprid, acetamiprid, and pyrethroid (Horowitz et al. 2008). In china, many recent studies have revealed that most of the commonly available pesticides have lost their effectiveness in the control of whitefly (Wang et al. 2009). Therefore, the development of alternative sources of control is urgently needed. Biological control has now become key area of research in the management of *B. tabaci* over the past 2 decades (Cuthbertson et al. 2010). A number of microbial bio-pesticides based on entomopathogenic organisms like bacteria, nematodes, viruses, and fungi have been playing key roles in plant protection and were used against a broad range of insect pests (Majeed MZ et al. 2017). Entomopathogenic fungi like *M. anisopliae, Isaria fumosorosea*, *B. bassiana,* and *L. lecanii* proved to be impressive, target-specific, and environment-friendly biocontrol agents against several species of sucking insect pests (Cabanillas and Jones 2009). After germination of spores connected to the target insect's cuticle, fungal hyphae enter the body of the insect and cause death of host within a day (Mora et al. 2017). In addition, EPF have less or no residual activity or mammalian toxicity and are specific to the target pest, as well as there is less risk for developing resistance (Zimmermann 2007).

B. bassiana and *L. Lecanii* are the most studied EPF and known as a virulent biocontrol agent of a wide range of forest, field crops, and green house pests. These entomopathogens were equally effective in desert, agricultural, and forest habitats (Annamalai et al. 2016 and Dogan et al. 2017). Lately, (Nazir et al. 2019) demonstrated the toxicity of filtrates and conidia derived from different isolates of *L. lecanii* and *B. bassiana* against the aphid species, *Myzus persicae* (*Sulz.*).

The strains of both the entomopathogens can be easily isolated from the infected soil dwelling pests or from vegetation pests (Freed et al. 2011). As with other organisms, the growth rate of EPF varies by temperature. They are usually cultured nearby 25 °C, even depending on the strain in a single species (Sato 1993). The aim of the present study was to assess the pathogenicity and virulence of EPF, i.e., *L. lecanii* and *B. bassiana* against *B. tabaci* under controlled environmental conditions.

Materials and methods

Whitefly stock culture

Bemisia tabaci individuals were collected from the seedlings of tomato (*Solanum lycopersicum*) grown in the greenhouse at Chinese Academy of Agricultural Sciences (CAAS) Beijing, China. The population of whitefly was reared on the seedlings of same tomato grown in pots kept in cages at 25 ± 2 °C and 50 60% RH with photoperiod of 16:8 h light:dark (L:D). In the entire study period, the plants were replaced weekly with new ones.

Effect of temperature on fungus virulence against *Bemisia tabaci*

Evaluation of the role of temperature, the pathogenicity bioassays of *B. bassiana* and *L. lecanii* against the nymphs of whitely, was carried out at 20, 24, 28, and 32 °C.

Fungal isolates

Two isolates of *B. bassiana,* namely, BB-72 and BB-252, and one isolate of *L. lecanii*, V-2 (Table 1), were cultured on potato dextrose agar (PDA) medium (agar 20 g/l., dextrose 20 g/l, and potatoes 200 g/l) plates and incubated at 24 ± 2 °C in dark for 20–25 days.

Conidial suspensions

The conidia were harvested from 25 days of fungal culture plates using ddH_2O with 0.01% Tween 80® (Sigma-Aldrich, Saint Louis, MO, USA). The suspensions of conidia were collected and then filtered by Miracloth (EMD Milipore Corp., Billerica, MA, USA). Neubauer hemocytometer (Brand GmbH, Wertheim, Germany) was used to adjust the conidial concentrations at 1×10^6, 1×10^7, and 1×10^8 conidia ml^{-1}. Before doing bioassays against whitefly, the conidial viability test was carried out, according to the methods described by (Hywel-Jones and Gillespie 1990).

Fungal filtrate

Adamek's liquid medium (40 g yeast extract (Difco, Detroit, MI, USA), 40 g dextrose and 30 g corn steep liquor (Sigma-Aldrich, Saint Louis, MO, USA), was used for primary

Table 1 Studied fungal isolates, code of strain, source, and geographical area of *Beauveria bassiana* and *Lecanicillium lecanii* strains

Fungal isolates	Code of strains	Source	Geographical area
B. bassiana 72	BB-72	Green peach aphid	Vladivostok (Russia)
B. bassiana 252	BB-252	Green peach aphid	Vladivostok (Russia)
L. lecanni 2	V-2	Whitefly	Moscow (Russia)

culture of fungal filtrate by adding 4 ml of conidial suspension into 100 ml of medium for all the strains of fungi. Cultures were incubated for 72 h at 150 rpm, and then for secondary culture, 5 ml from primary culture was added into 500 ml of Adamek's liquid medium and incubated at 26 °C and 150 rpm for 6 days for each strain. The samples were then centrifuged at 12000 rpm and 4 °C for 30 min. After that, the supernatant was filtered through 0.45 μm pore size filter (Millipore Corp., Billerica, MA, USA) to get filtrate, and pH was sustained at 6.

Pathogenicity bioassays

To evaluate the efficacy of filtrate, conidia, and binary combinations, different bioassays were carried out. For binary combination bioassays (conidia + filtrate), 1 ml of the highest concentration of conidia 1×10^8 conidia ml^{-1} of each fungal isolate was combined with 1 ml filtrate of the same fungal isolate. The combinations were BB-72 × BB-72, BB-252 × BB- 252, and V2 × V2. For all treatments ,the 90-mm Petri dishes were used with a skinny layer of 1.5% agar, and 50 mm diameter of leaf discs of tomato was placed in Petri dishes. In the control treatment, the leaf discs were sprayed only by 0.01% Tween 80®. Ten apterous adult of *B. tabaci* were released on the leaf discs at each Petri dish, and 1.5 ml sample of each of the treatments was sprayed on *B. tabaci* with the help of small sprayer and then these Petri dishes were incubated at 20, 24, 28, and 32 °C and 50–60% RH with photoperiod of 16:8 h light:dark (L:D) for 12 days. Ten replications of each treatment were used. *B. tabaci* mortality was recorded after 3, 6, 9, and 12 days post-treatment.

Data analysis

Virulence of the two strains of *B. bassiana* and one strain of *L. lecanii* as filtrate, conidia, and binary combination (conidia + filtrate) were analyzed, using factorial analysis of the variance (Statistix software (version 8.1) (Tallahassee, FL)). Comparisons of the treatment means were performed using Fischer's least significant difference (LSD) test at $a = 0.05$.

Table 2 Factorial analysis of variance of mortality of *Bemisia tabaci* bioassay with the conidia of two strains of *Beauveria bassiana* and one strain of *Lecanicillium lecanii*

Source	DF	SS	MS	***F*** value	***p*** value
Fungus	2	116.3	58.17	101.3	≤ 0.001
Days	3	2784.8	928.27	1616.3	≤ 0.001
Temperature	3	323.4	107.81	187.7	≤ 0.001
Concentration	3	2819.0	939.69	1636.2	≤ 0.001
Fungus × temperature	6	139.1	23.18	40.3	≤ 0.001
Fungus × days	6	6.4	1.06	1.8	0.0843
Fungus × concentration	6	46.1	7.69	13.4	≤ 0.001
Temperature × days	9	110.7	12.30	21.4	≤ 0.001
Temperature × concentration	9	103.9	11.55	20.1	≤ 0.001
Days × concentration	9	986.9	109.65	190.9	≤ 0.001
Fungus × temperature × days	18	113.5	6.30	10.9	≤ 0.001
Fungus × temperature × concentration	18	50.7	2.81	4.9	≤ 0.001
Fungus × days × concentration	18	12.5	0.69	1.2	0.2386
Temperature × days × concentration	27	51.9	1.92	3.3	≤ 0.001
Fungus × temperature × days × concentration	54	51.6	0.95	1.6	0.0019
Error	1728	992.4	0.5		
Total	1919	8709.9			
CV/GM	35.06/2.16				

*$p < 0.001$ (highly significant) four-way factorial analysis of variance (ANOVA) at $a = 0.05$. *DF* degree of freedom, *SS* sum of squares, *MS* mean sum of squares, *F* F-statistic, *CV* coefficient of variation, *GM* grand mean

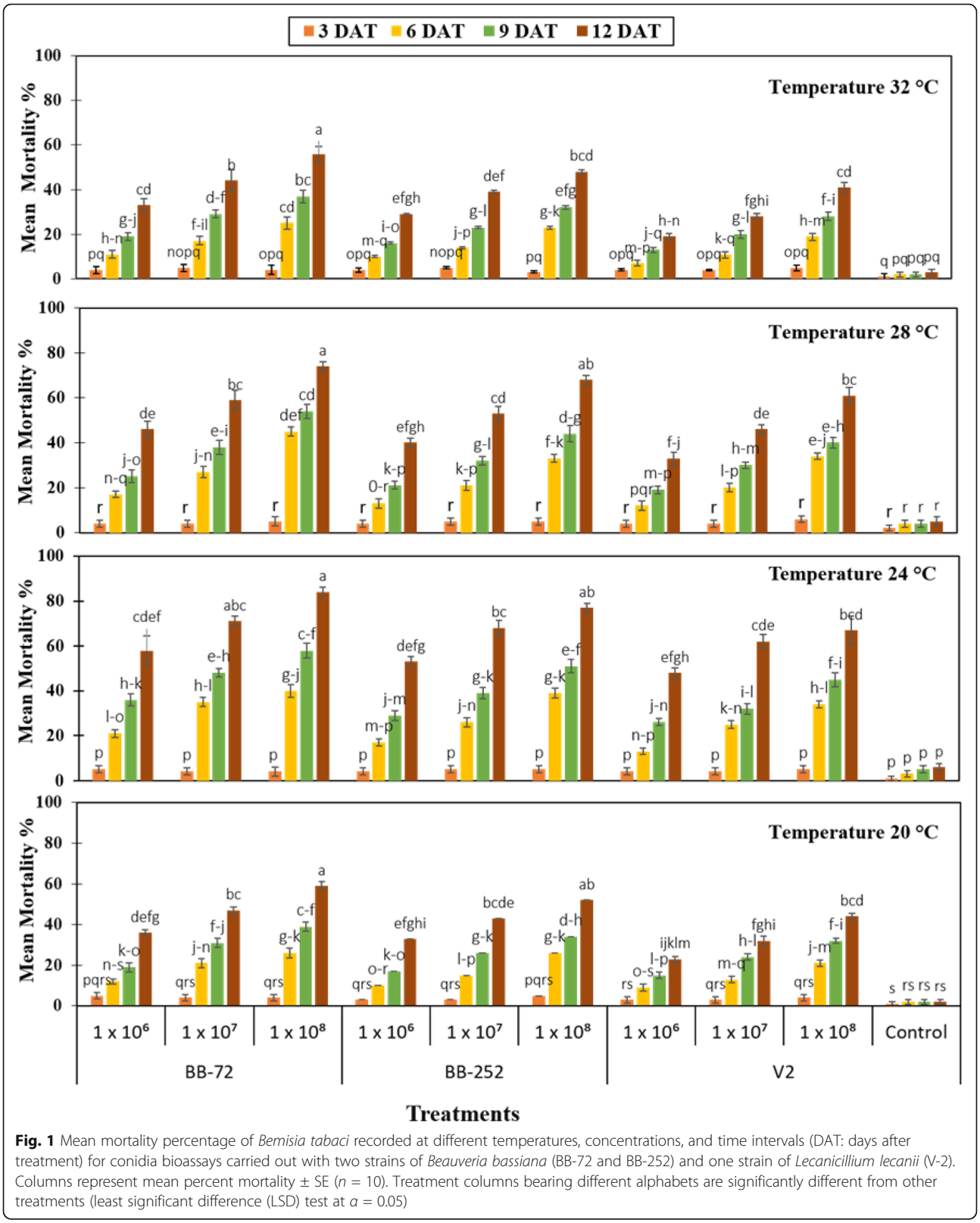

Fig. 1 Mean mortality percentage of *Bemisia tabaci* recorded at different temperatures, concentrations, and time intervals (DAT: days after treatment) for conidia bioassays carried out with two strains of *Beauveria bassiana* (BB-72 and BB-252) and one strain of *Lecanicillium lecanii* (V-2). Columns represent mean percent mortality ± SE (n = 10). Treatment columns bearing different alphabets are significantly different from other treatments (least significant difference (LSD) test at α = 0.05)

Result and discussion

Conidial virulence

In conidial bioassay, the results showed that all the isolates tested revealed significant mortality of whitefly individuals (F = 101.30, p < 0.001; Table 2). Moreover, the factorial analysis of variance exhibited that there was a significant effect of temperature levels, time intervals, concentrations, and their interaction on whitefly mortality rate (Table 2). The maximum mortality of *B. tabacii* was observed in BB-72 isolate (84%), BB-252 isolate

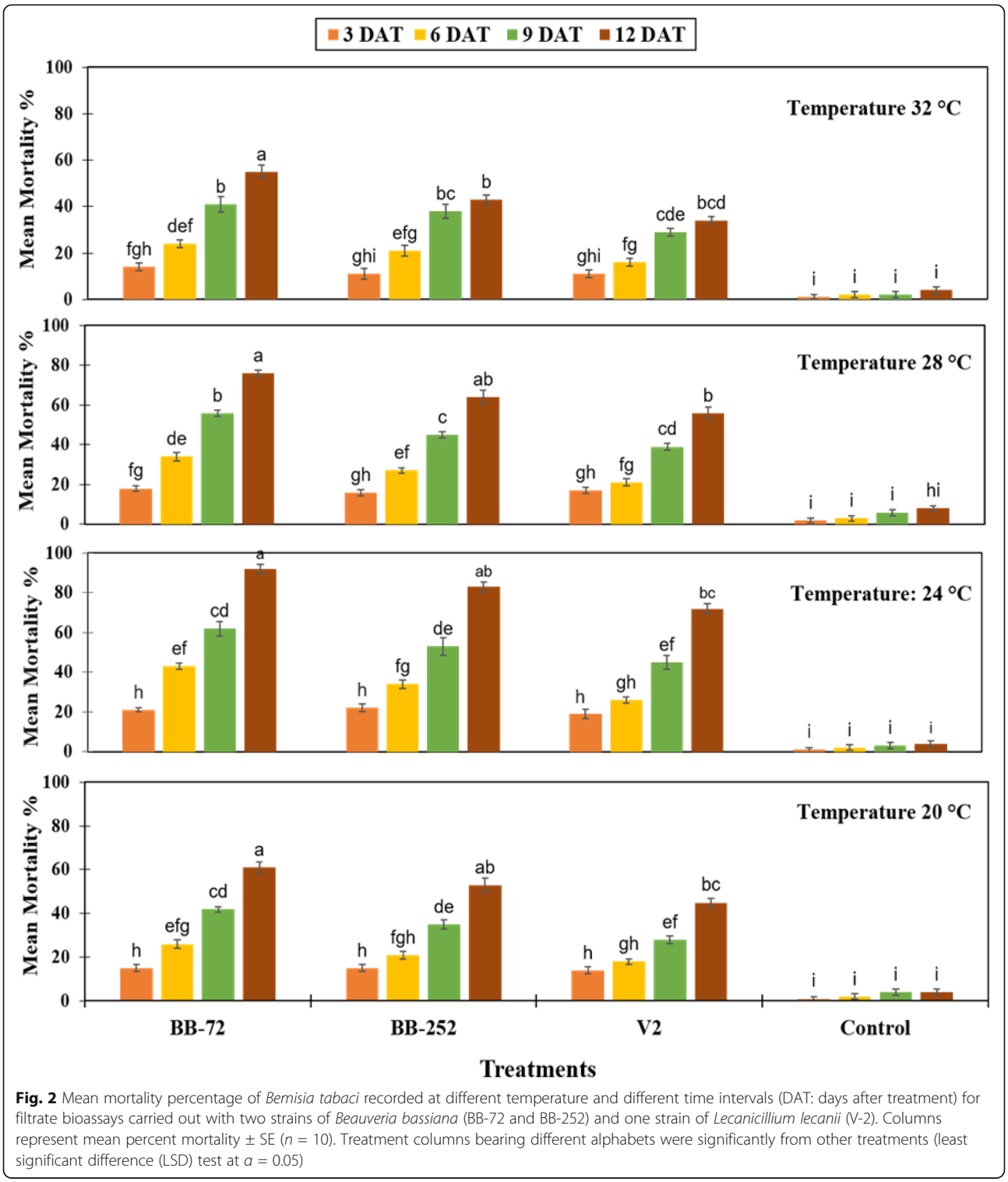

Fig. 2 Mean mortality percentage of *Bemisia tabaci* recorded at different temperature and different time intervals (DAT: days after treatment) for filtrate bioassays carried out with two strains of *Beauveria bassiana* (BB-72 and BB-252) and one strain of *Lecanicillium lecanii* (V-2). Columns represent mean percent mortality ± SE (n = 10). Treatment columns bearing different alphabets were significantly from other treatments (least significant difference (LSD) test at α = 0.05)

(77%), and V-2 isolate (67%) at the highest concentration 1×10^8 conidia ml^{-1}, and low mortalities were recorded at the lowest concentration 1×10^6 conidia ml^{-1} on 12th day of post-treatment at 24 °C (Fig. 1). The minimum mortality of whitefly was recorded by BB-72 isolate (33%), BB-252 isolate (29%), and V-2 isolate (19%) at the lowest concentration 1×10^6 conidia ml^{-1} on 12th day of post-treatment at 32 °C (Fig. 1). The mean mortality at the highest concentration 1×10^8 conidia ml^{-1} in BB-72 isolate (74%), BB-252 isolate (68%), and V-2 isolate (61%,) and at the lowest concentration 1×10^6 conidia ml^{-1} in BB-72 isolate (46%), BB-252 isolate (40%), and V-2 isolate (33%) was recorded on 12th day of post-treatment at 28 °C (Fig. 2). The mean mortality was at the lowest concentration 1×10^6 conidia ml^{-1}in BB-72 isolate (36%), BB-252 isolate (33%), and V-2 isolate (23%), and at the highest concentration 1×10^8 conidia ml^{-1} in BB-72 isolate (59%), BB-252 isolate (52%), and V-2 isolate (44%) was recorded on 12th day of post-treatment at 20 °C (Fig. 1). The mean mortality of *B. tabaci* in the control Petri-plates was noticeably low (6%) after 12th day post-treatment.

Filtrate pathogenicity

Results of the filtrate bioassay showed that the mortality percentages of whitefly by all isolates of the fungus were more than those of the conidial treatments. The fungal filtrate, observation time, temperature, and their interactions had significant effects on *B. tabaci* mortality (Table 3). Maximum mortality of *B. tabaci* was observed in BB-72 isolate (92%), BB-252 isolate (83%), and V-2 isolate (72%) on 12th day of treatment at 24 °C. Minimum mortality was observed in BB-72 isolate (55%), BB-252 isolate (43%), and V-2 isolate (34%) on 12th day of treatment at 32 °C. On the other hand, the mean mortality of BB-72 isolate (76%), BB-252 isolate (64%), and V-2 isolate (56%) was on 12th day of treatment at 28 °C, while the mean mortality of BB-72 isolate (61%), BB-252 isolate (53%), and V-2 isolate (45%) was on 12th day of treatment at 20 °C (Fig. 2).

Binary combinations

The bioassay was carried out with the binary combinations of (conidia + filtrate) of two strains of *B. bassiana* (BB-72 and BB-252) and one strain of *L. lecanii* (V-2), and they showed significant control of *B. tabaci*. On *B. tabaci* mortality, the binary combinations (conidia + filtrate), observation time, temperature, and their interactions had significant effects (Table 4). Significant and the highest white fly mortality was recorded in BB-12th day of treatment at 24 °C, while the lowest white fly mortality was recorded in BB-72 × BB-72 isolate (57%), BB-252 × BB-252 isolate (50%), and V2 × V2 isolate (39%) on 12th day of treatment at 32 °C. The mean mortality of BB-72 × BB-72 isolate (74%), BB-252 × BB-252 isolate (60%), and V2 × V2 isolate (51%) on 12th day of treatment at 28 °C, whereas the mean mortality of BB-72 × BB-72 isolate (64%), BB-252 × BB-252 isolate (50%), and V2 × V2 isolate (50%) on 12th day of treatment at 20 °C (Fig. 3).

Table 3 Factorial analysis of variance of mortality of *Bemisia tabaci* bioassay with the filtrate of two strains of *Beauveria bassiana* and one strain of *Lecanicillium lecanii*

Source	DF	SS	MS	***F*** value	***p*** value
Fungus	3	1472.2	490.7	1114.2	≤ 0.001
Days	3	097.0	365.6	830.2	≤ 0.001
Temperature	3	276.2	92.0	209.0	≤ 0.001
Fungus × days	9	317.4	35.2	80.0	≤ 0.001
Fungus × temperature	9	90.4	10.0	22.8	≤ 0.001
Days × temperature	9	90.3	10.0	22.7	≤ 0.001
Fungus × days × temperature	27	31.5	1.1	2.6	≤ 0.001
Error	576	253.7	0.4		
Total	639	629.1			
CV/GM	23.43/2.83				

*$p < 0.001$ (highly significant) three-way factorial analysis of variance (ANOVA) at $\alpha = 0.05$. *DF* degree of freedom, *SS* sum of squares, *MS* mean sum of squares, *F* F-statistic, *CV* coefficient of variation, *GM* grand mean

The development of biocontrol research tools based on entomopathogenic fungi with improved efficacy against the target pests is one of the key areas of biological control research. Several insect pests have been controlled by the different entomopathogenic fungal strains (Yun et al. 2017).

Obtained results are in accordance with the previous studies describing the effectiveness of different isolates of *L. lecanii* and *B. bassiana* against sucking insect pests (Hesketh et al. 2008). In all bioassays, the

Table 4 Factorial analysis of variance of mortality of *Bemisia tabaci* bioassayed with the binary combinations (conidia + filtrate) of two strains of *Beauveria bassiana* and one strain of *Lecanicillium lecanii*

Source	DF	SS	MS	***F*** value	***p*** value
Fungus	3	1333.99	444.663	950.02	≤ 0.001
Days	3	1196.14	398.712	851.85	≤ 0.001
Temperature	3	151.91	50.638	108.19	≤ 0.001
Fungus × days	9	363.33	40.369	86.25	≤ 0.001
Fungus × temperature	9	53.65	5.961	12.74	≤ 0.001
Days × temperature	9	28.70	3.189	6.81	≤ 0.001
Fungus × days × temperature	27	18.69	0.692	1.48	0.0578
Error	576	269.60	0.468		
Total	639	3416.00			
CV/GM	24.88/2.75				

*$p < 0.001$ (highly significant) three-way factorial analysis of variance (ANOVA) at $\alpha = 0.05$. *DF* degree of freedom, *SS* sum of squares, *MS* mean sum of squares, *F* F-statistic, *CV* coefficient of variation, *GM* grand mean

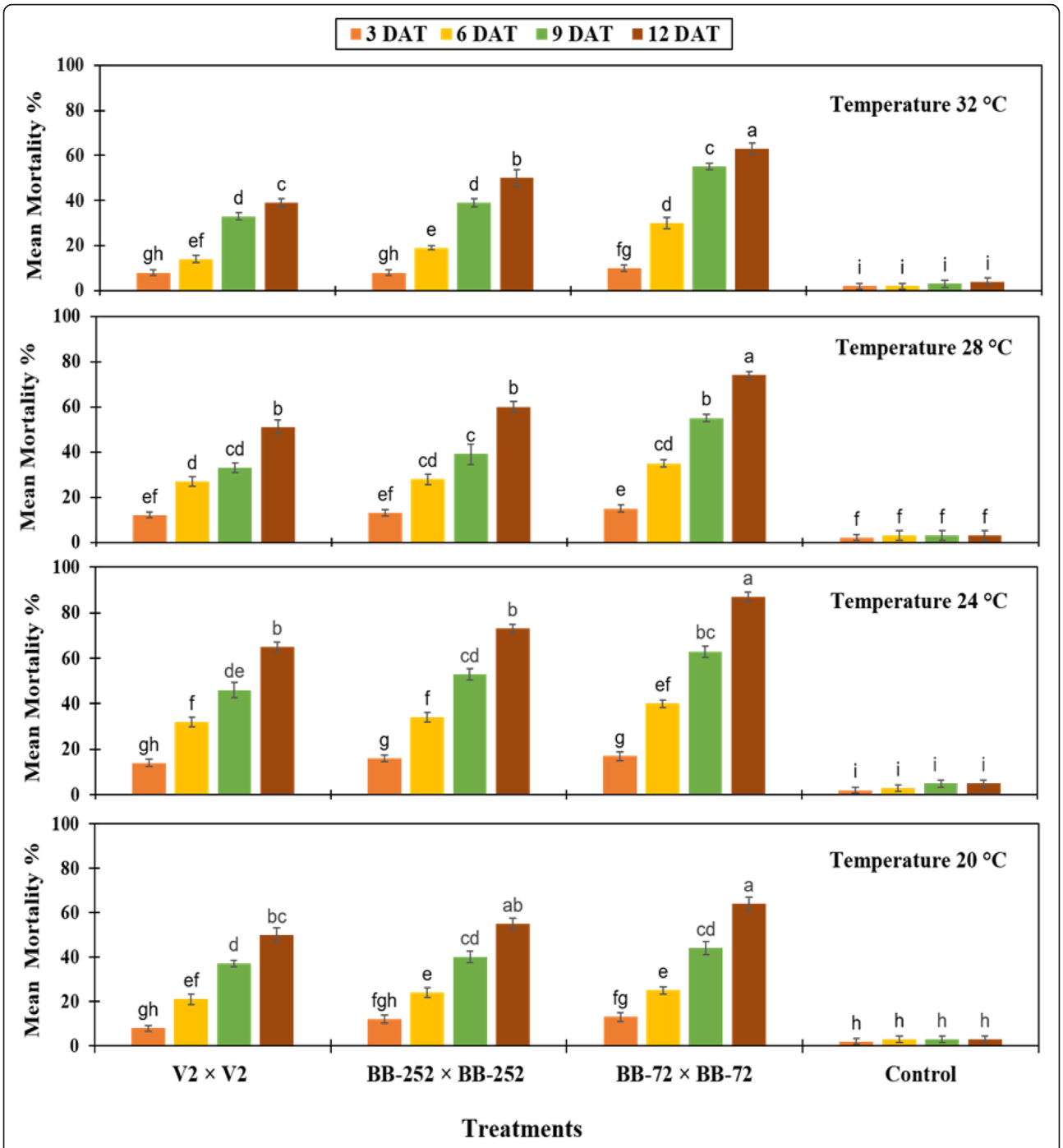

Fig. 3 Mean mortality percentage of *Bemisia tabaci* recorded at different temperature and time intervals (DAT: days after treatment) for binary combinations (conidia + filtrate) and bioassays carried out with two strains of *Beauveria bassiana* (BB-72 × BB-72 and BB-252 × BB-252) and one strain of *Lecanicillium lecanii* (V2 × V2). Columns represent mean percent mortality ± SE (n = 10). Treatment columns bearing different alphabets are significantly different from other treatments (least significant difference (LSD) test at α = 0.05)

mortality of whiteflies was dependent on temperature, time, and concentration, and it increased by increasing the concentrations of conidia and time after application at optimum temperature. Mortality of 92, 83 and 72% was recorded after 12 days of filtrate application of BB-72, BB-252, and V-2 isolate, in that order, while 84, 77, and 67% mortality of whiteflies was found after 12 days at the highest concentration 1×10^8 conidia ml^{-1} of BB-72, BB-252, and V-2 isolate at 24 °C. Another experiment was carried out and reported 87,

73, and 65% mortality of whiteflies after 12 days through binary combination of conidia + filtrate of BB-72 × BB-72, BB-252 × BB-252, and V2 × V2 isolates. These results are in accordance with (Wraight et al. 2000) who reported that *B. bassiana* caused up to 97% mortality, and *V. lecanii* showed 100% mortality of *Chilo partellus*. Obtained results are in accordance with (Halimona and Jankevica 2011), who tested various concentrations of *B. bassiana* against *A. fabae* and *Metopeurum fuscoviride* and found that the highest concentration of conidia $1 \times 108\,ml^{-1}$ showed maximum mortality after 7th day of application.

The percentage of mortality was affected by temperature, conidial concentration, and exposure time (Ansari et al. 2004). *B. bassiana* showed a great effect on the *B. tabaci* and *Aphis craccivora* infesting cucumber (Maniania 1991). The *V. lecanii* showed high mortality in early stages of *B. tabaci* and less mortality in old instars (Zaki 1998). EPF showed a good control of *B. tabaci* (Abdel-Raheem et al. 2016). The filtrate application was more effective to control the insect pests than the conidial application mainly in the case of short life cycled insects. There may be a possible cause that the less attachment of conidia to the insect cuticle as compared to huge infiltration of filtrates (Hanan et al. 2020a b).

Concerning the combined application obtained results is identical to (Yun et al. 2017) who studied the double behavior of EPF *B. bassiana* and *M. anisopliae* against *M. persicae* and *B. cinerea* and witnessed that the usage of filtrate with blastospores provided the maximum mortality of *M. persicae*. Furthermore, the mortality percentage was near to the combination of filtrates with blastospores; however, the combination of conidia and filtrate gave the minimum mortality as compared to filtrate application. So, it is clear that the fungal filtrate application had extreme virulence efficiency. Moreover, EPF are considered safe and environmentally friendly than the chemical pesticides (Goettel and Jaronski 1997); so, they are suggested as a control agent against destructive insect pests, like whitefly *B. tabaci*.

Conclusion

The in vitro study exhibited the efficiency of 2 strains of *B. bassiana* and one strain of *L. lecanii* against *B. tabaci*. *L. lecanii* showed lower mortality than both strains of *B. bassiana* either singly or in binary combinations (conidia + filtrate) of same strain at optimum temperature. The application of filtrate was utmost appropriate material to control *B. tabaci*. The binary combination of filtrate + its conidia had some irreconcilable effect and cannot effectively be used to control *B. tabaci*. Further studies are still needed to assess the role of EPF against *B. tabaci*, especially under field conditions.

Abbreviations

PDA: Potato dextrose agar; ddH_2o: Double-distilled water; L:D: Light:dark; DF: Degree of freedom; μm: Micrometer; ml: Milliliter; pH: Potential of hydrogen; Rpm: Revolutions per minute; DAT: Days after treatment; SS: Sum of squares; MS: Mean sum of squares; F: F-statistic; CV: Coefficient of variation; GM: Grand mean

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Authors' contributions

AUK, TN, and DQ conceived and designed the experiments. MAG and TN collected the data. GHJ and TA analyzed the data. AUK and TA performed the experiment. AUK, TS, and YAA wrote the paper. DQ critically revised of the manuscript for intellectual content. All authors read and approved the final manuscript.

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Availability of data and materials

All data and materials are mentioned in the manuscript.

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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