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# Pathogenicity of some entomopathogenic fungal strains to green peach aphid, *Myzus persicae* Sulzer (Homoptera: Aphididae)

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## Abstract

Pathogenicity of four fungal strains, two of *Beauveria bassiana* (designated as BB-72 and BB-252), and two of *Lecanicillium lecanii* (designated as V-4 and CS-625) to green peach aphid, *Myzus persicae* Sulzer (Homoptera: Aphididae) was assessed. All treatments showed highly significant effects on the mortality of the aphid when applied as filtrate or conidia. The application methods did not have a significant effect on the mortality rate. The overall mortalities caused by both applications were statistically similar. The maximum overall mortality was caused by BB-72, followed by CS-625 in both application methods. On the other hand, (V-4) caused the minimum mortality rate, followed by (BB-252), which was statistically similar to those caused by V-4. The time also had significant effects on aphid mortality rates. The mortality was the minimum after 3 days reaching its maximum after 10 days. As the number of days increased, there was a corresponding increase in the mortality showing a direct relationship between mortality and time. The maximum mean individual mortality of 95% was caused by CS-625, followed by BB-72 (92%) after 10 days with the conidial application. Similarly, the combined effects of the three most virulent fungal strains showed highly significant differences on the mortality of peach aphid. The combination BB-72 + BB-252 showed the highest percent mortality, followed by BB-72 + BB-252 + CS-625. The combinations BB-72 + CS-625 and BB-252 + CS-625 had statistically similar effects of causing aphid mortality.

**Keywords:** Green peach aphid, Entomopathogenic fungal strains, Pathogenicity, biocontrol, Mortality

## Background

Aphids are important pests of many vegetables and fruits as they suck the sap of plants that is followed by the development of sooty mold on damaged plants (Tang et al. 2017). Among the most damaging and common species of aphids are the green peach aphid, *Myzus persicae* Sulzer; the cabbage aphid, *Brevicoryne brassicae* L.; the potato aphid, *Macrosiphum euphorbiae* Thomas; and the melon (or cotton) aphid, *Aphis gossypii* Glover (Homoptera: Aphididae). Aphids are serious pests as they are known to disseminate the viruses among healthy plants. Peach and apple orchards are intensively treated by insecticides for managing the insect pests. Aphids have developed resistance against commonly used pesticides

(Rousselin et al. 2017). There are more than 700 fungus and nematode species which attack insect pests (Ak 2019; El Hussein 2019a; Javed et al. 2019; Tuncer et al. 2019). The commonly used strains having potential for pest management have been categorized into two groups. One group includes Hyphomycetes in Deuteromycotina and the other belongs to Entomophthorales in Zygomycotina (Feng 1998). Very few entomopathogenic fungi (EPFs), viz. *Nomuraea rileyi*, *Beauveria bassiana*, *Paecilomyces fumosoroseus*, *Metarhizium anisopliae*, *B. brongniartii*, and *Lecanicillium lecanii*, have been registered as products to control insect pests (Shah and Goettel 1999). *B. bassiana* and *L. lecanii*, the two well-known EPFs, have many strains that cause white muscadine disease to a number of insect families and species including aphids. The spores of these EPFs simply need to be in contact with target hosts and rapidly control the insect ultimately. Upon controlling of insects, the produced white molds have more infective spores spreading

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to the healthy pest populations. Aphids in greenhouses and hydroponic systems cannot be treated with insecticides because of released pollinators and other biocontrol agents (Dunne and Donovan 1977) and there are numbers of EPFs which have great potentials for controlling aphids' populations and can be included successfully in biological control programs (Nielsen and Wraight 2009).

*B. bassiana* and *L. lecanii* are the most studied EPFs and well known as virulent biocontrol agents of a wide range of forest, field crops, and greenhouse pests. These EPFs are equally effective in desert, agricultural, and forest habitats (Dhar et al. 2019; El Hussein 2019b; Sayed et al. 2019). The strains of both EPFs can be easily isolated from the infected soil-dwelling pests or from vegetation pests (Freed et al. 2011).

The use of biological control agents, either alone or integrated with other pest management strategies, is one of the alternatives to chemical insecticides (Singh et al. 2012). In the biological control, many antagonists have shown efficacy against insect pests. Among these, the fungi *B. bassiana* and *L. lecanii* are ubiquitous in distribution and have the potential to control aphids successfully. The objective of the present study was to evaluate the pathogenicity of two antagonists for the management of green peach aphid, *M. persicae*, under laboratory conditions.

## Materials and methods

### Insect culture

*M. persicae* was collected from cabbage fields and shifted to Chinese cabbage already potted under insect net maintained at 24 °C, R.H. of 45–60%, and 16:8 (light to dark) photoperiod in the laboratory (Khan et al. 2012). The plants were replaced weekly to provide enough nutrition for reared insects.

### Fungal isolates

Four fungal isolates, including two strains of *B. bassiana* and two of *L. lecanii* were evaluated for their pathogenicity against the aphid (Table 1). The fungal isolates were maintained on potato dextrose agar (PDA) slants in tubes at 4 °C. The isolates were grown and multiplied on the PDA at 25 °C for 14 days and stored at 4 °C (Javed et al. 2019).

### Preparation of filtrates and conidial suspensions of fungal isolates

Conidial suspensions of the tested fungal isolates were prepared by harvesting conidia from culture plates of each fungal isolate on the 20th day of culturing in 0.02% Tween solution. The suspensions were vigorously stirred and filtered through a sterile cheese cloth. The conidia in suspensions were counted with the help of a hemocytometer under a microscope and finally adjusted to 10<sup>7</sup> conidia per milliliter. The viability of conidia was confirmed, following the method described by Hywell-Jones and Gillespie (1990).

The culture filtrates of isolates of the tested EPFs were prepared according to the method of Khan et al. (2012). Four milliliters of the conidial suspensions from each isolate were poured separately into 100 ml of Adamek's liquid medium and incubated for 3 days in a shaking incubator at 150 rpm. For the preparation of 1% secondary culture filtrate, 2.5 ml of primary culture filtrate was mixed by 250 ml of Adamek's liquid medium and incubated for 6 days at 26 °C in a shaking incubator at 150 rpm. The solution was filtered, centrifuged at 10,000 rpm at 4 °C for 30 min, and again filtered through 0.45 µm pore size Millipore membrane filter.

### Pathogenicity bioassays

In the first experiment, the virulence of tested fungal isolates was assessed individually against the green peach aphid, using their filtrates and conidial suspensions. Bioassays were performed, using a leaf dip method, following the procedures described by Xu et al. (2002). Leaves from cabbage plants were collected and cut into leaf disks of 50 mm diameter. Leaf disks were separately soaked into the filtrates and conidial suspensions of each fungal isolate. After treatment, the leaf disks were placed on a sterilized filter paper for 30 min to absorb the suspension. The cabbage leaves were transferred to 6 cm Petri dishes containing a piece of damp filter paper. For control, leaf disks were only dipped in 0.05% Tween 80 solution for the same time duration. Ten green peach aphids were released in each Petri dish with the help of a camel hairbrush. All the Petri dishes were kept at 26 ± 2 °C with a light-to-dark ratio of 16:8 h, respectively. The mortality rate was recorded daily up to the 10th day.

In the second experiment, three isolates showing the maximum mortality rates in single bioassays (BB-72, BB-

**Table 1** Detail of entomopathogenic fungi used in the study

Sr. No	Entomopathogenic fungi	Isolate code	Source	Geographical area
1	<i>Beauveria bassiana</i>	BB-72	Green peach aphid	Vladivostok (Russia)
2	<i>B. bassiana</i>	BB-252	Green peach aphid	Vladivostok (Russia)
3	<i>Lecanicillium lecanii</i>	V-4	Whitefly	Moscow (Russia)
4	<i>L. lecanii</i>	CS-625	Green peach aphid	Suwn (Korea)

252, and CS-625) were tested again but in combinations. The treatments were as follows:

- T1 = BB-72 + BB-252
- T2 = BB-72 + CS-625
- T3 = BB-252 + CS-625
- T4 = BB-72 + BB-252 + CS-625
- T5 = Tween 80
- T6 = Water

Dead aphids were counted and placed into a dark room for 10 days to confirm mortality by the pathogens. After confirmation, only those aphids were counted on which pathogen growth appeared. Mortality was observed on a daily basis, and percentages were calculated after the 3rd, 6th, and 10th day of treatment. The percent mortality in each treatment was calculated and corrected by Abbott’s formula (Abbott 1925) as follows:

$$\text{Mortality (\%)} = \frac{t-c}{c} \times 100$$

where *t* is the percent mortality in the treatments by fungal isolates and *c* is the percent mortality in the control.

**Statistical analysis**

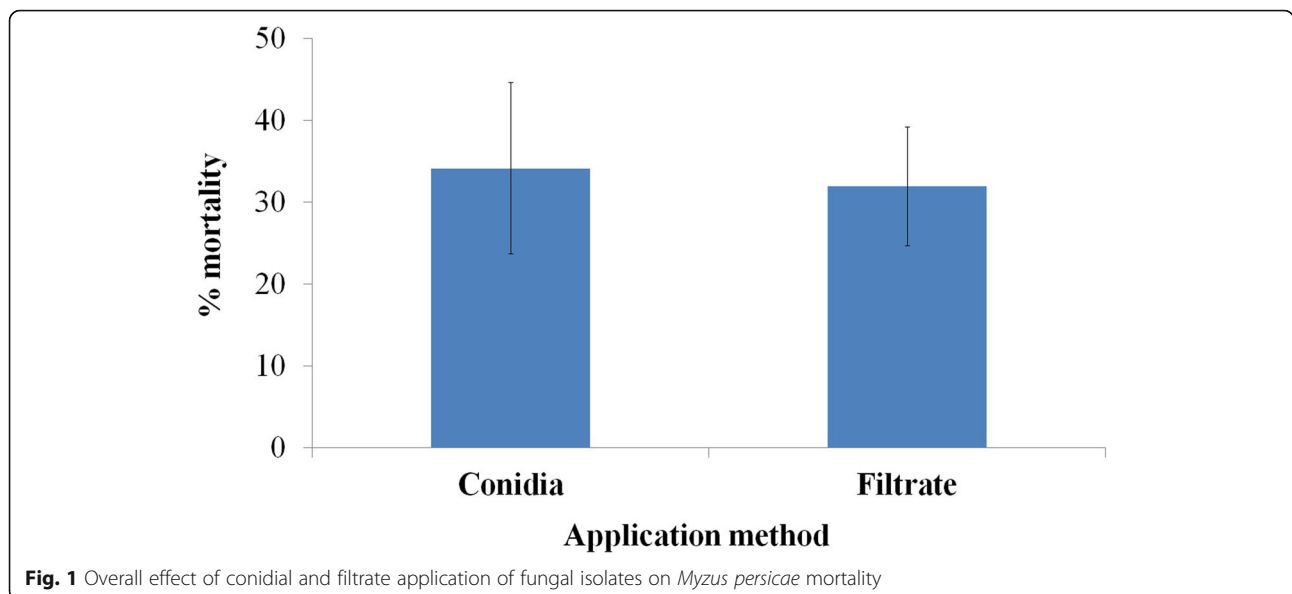
All the treatments were repeated four times. The corrected aphid mortality rate was analyzed by using two-way factorial analysis of variance (ANOVA), using Statistix (8.1) software. Comparisons of treatment means were performed, using Fischer’s protected least significant difference (LSD) test at *P* = 0.05.

**Results and discussion**

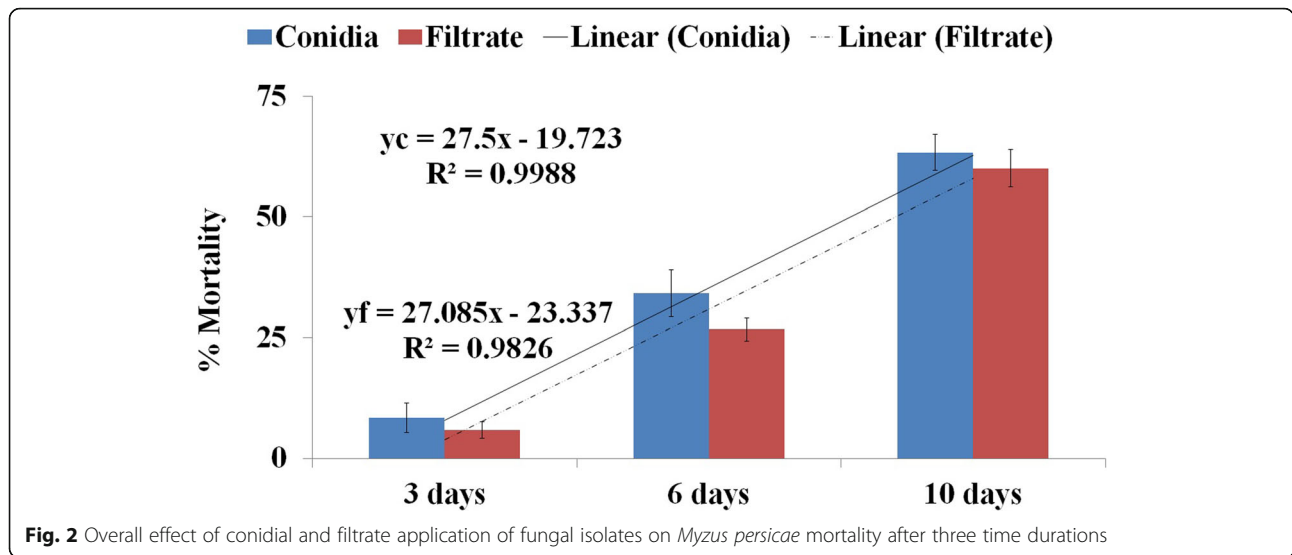
**Pathogenic potential of fungal strains against *M. persicae***

The analysis of variance showed highly significant effects of the biocontrol treatments (*F* = 122.62; *df* = 5,

105; *P* < 0.001) on the mortality of the peach aphid, when applied as filtrate or conidia. However, there was an insignificant effect on the mortality rates between the application methods (*F* = 2.24; *df* = 1, 105; *P* = 0.138). The mortality rates caused by conidial treatment were slightly higher (Fig. 1). The maximum overall mortality rate (54.17 and 52.5%) was caused by *B. bassiana* (BB-72), followed by *L. lecanii* (CS-625) showing 50.83 and 47.55% mortalities for both application methods. On the other hand, *L. lecanii* (V-4) caused the minimum mortality rate (40 and 39.17%), followed by *B. bassiana* (BB-252) which gave 41.67 and 39.17% mortality rates. They were statistically similar to those caused by V-4 as shown in Fig. 2. The duration also had significant effects (*F* = 452.73; *df* = 2, 105; *P* < 0.001) on aphid mortality. The mortalities were found to be the minimum after 3 days and reached the maximum after 10 days. A direct correlation was observed between mortality and time. As the number of days increased, there was a corresponding increase in mortality. These relationships are shown by trend lines and regression equations in Fig. 3. The interaction between duration and treatments was found highly significant (*F* = 39.90; *df* = 10, 105; *P* < 0.001) and that between application methods and duration was also significant (*F* = 4.44; *df* = 2, 105; *P* = 0.014). Contrarily, the interactions between application methods and treatments (*F* = 0.31; *df* = 5, 105; *P* = 0.908) and that between application methods, duration, and treatments were insignificant (*F* = 0.4; *df* = 10, 105; *P* = 0.943). The maximum mean individual mortality of 95% was caused by CS-625, followed by BB-72 (92%) after 10 days at conidial application. The individual mortalities by each biocontrol agent with both application methods at three time intervals are given in Table 2.



**Fig. 1** Overall effect of conidial and filtrate application of fungal isolates on *Myzus persicae* mortality

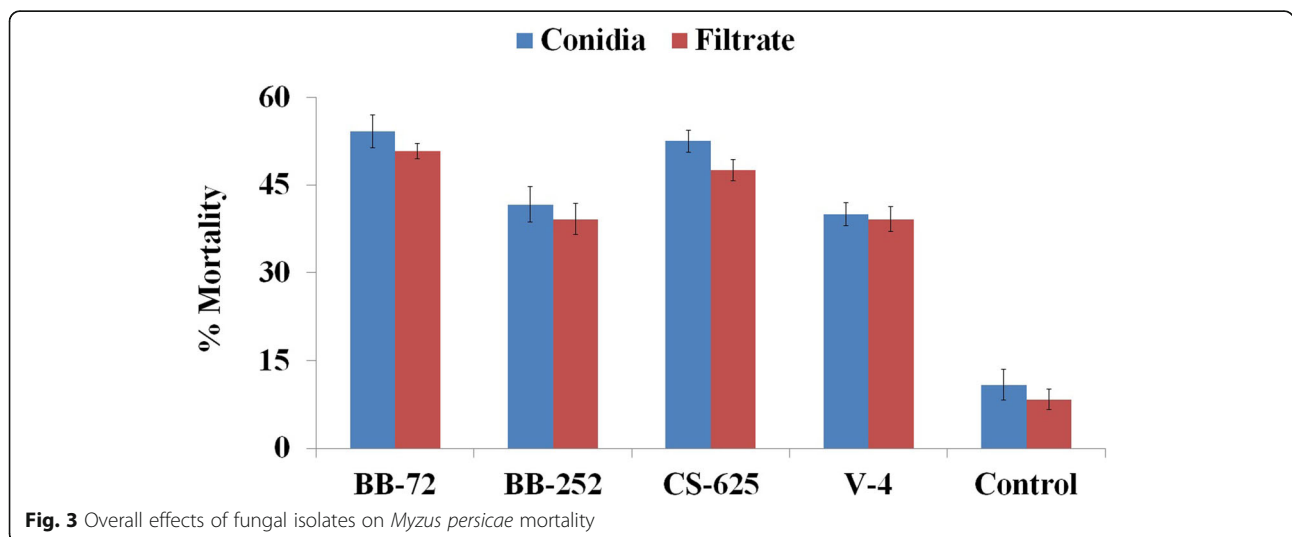


**Pathogenicity of combined conidial suspensions of fungal strains against *M. persicae***

The analysis of variance regarding combined effects of three virulent fungal strains ( $F = 73.66$ ;  $df = 4, 42$ ;  $P < 0.001$ ), duration ( $F = 279.83$ ;  $df = 2, 42$ ;  $P < 0.001$ ), and their interactions ( $F = 13.69$ ;  $df = 8, 42$ ;  $P < 0.001$ ) showed highly significant effects on the mortality rates of peach aphid. The combination of BB-72 + BB-252 showed the highest percent mortality of 89.04%, followed by BB-72 + BB-252 + CS-625 combination which caused 86.66% mortality. The combinations of BB-72 + CS-625 and BB-252 + CS-625 were statistically at par with each other’s in causing aphid mortality as shown in Fig. 4. The individual mortality rate by each combination of fungal strains at three time intervals is given in Table 3.

In the present study, all the biocontrol agents caused mortality of green peach aphid with various degrees singly and in combinations and can be used effectively for the management of *M. persicae*. Fungal isolates are effective biological tools against many target pests of controlled and semi-controlled glass-houses without causing remarkable loss to other biocontrol agents and pollinators. Various studies have found entomopathogens as a strong tool of biological control of sucking insect pests (Amnuaykanjanasin et al. 2013; Yun et al. 2017; El Hussein 2019c; Mohamed 2019; Sain et al. 2019).

In the present study, the selected strains of EPFs were found effective to control the green peach aphid under laboratory conditions. The results are strengthened by the findings of previous studies that fungal strains of *B. bassiana* and *L. lecanii* effectively controlled *M. persicae*



**Table 2** Mortality of green peach aphid in conidial and filtrate applications

Treatments	% Mortality of green peach aphid in					
	Conidial application after			Filtrate application after		
	3 days	6 days	10 days	3 days	6 days	10 days
<i>Beauveria bassiana</i> , (BB-72)	10.00 ab	50.00 fg	92.50 ij	15.00 abc	60.00 g	87.50 hij
<i>B. bassiana</i> , (BB-252)	7.50 ab	25.00 cd	85.00 hij	10.00 ab	35.00 de	80.00 hi
<i>Lecanicillium lecanii</i> , (CS-625)	7.50 ab	40.00 ef	95.00 j	12.50 abc	55.00 g	90.00 ij
<i>L. lecanii</i> , (V-4)	5.00 ab	32.50 de	80.00 hi	5.00 ab	40.00 ef	75.00 h
Tween	2.50 a	7.50 ab	15.00 abc	5.00 ab	10.00 ab	17.50 bc

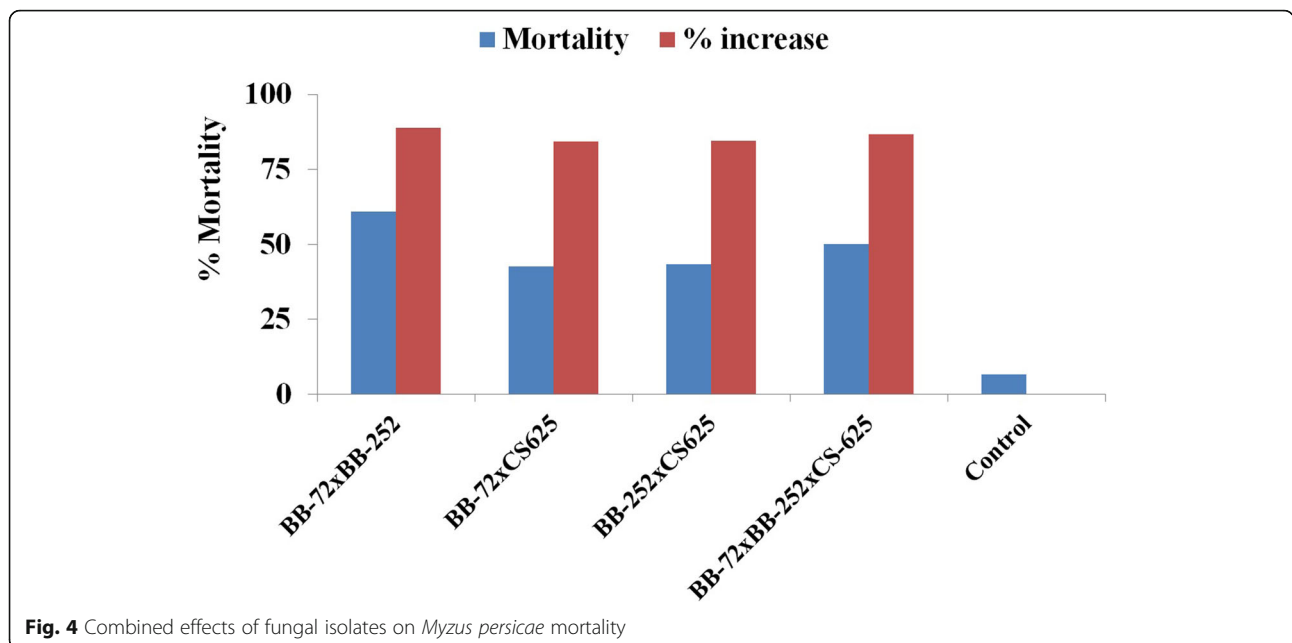
Values are means of four replications  
 Means sharing common letters do not differ significantly ( $P = 0.05$ )

and many other aphid species (Khan et al. 2012; Nazir et al. 2019). *B. bassiana* and *L. lecanii* were found to be the most virulent EPFs strains for controlling *M. persicae* with more than 80% mortality on the 10th day of treatment. A previous study by Hayden et al. (1992) reported that *B. bassiana* and *V. lecanii* caused maximum mortality of *S. avenae* after the ninth and eight day of treatment, respectively.

In previous studies, 12 strains of EPFs, viz. *L. lecanii*, *P. farinosus*, *B. bassiana*, *M. anisopliae*, *C. scarabaeicola*, and *N. rileyi* were screened for their effectiveness against *M. persicae* in which *B. bassiana* and *L. lecanii* were found to grow and control aphids in a broad range of temperature (15–30 °C) and humidity. The effective concentration of conidial suspension was also reported to be  $1 \times 10^7$  conidia/ml, which corroborated the present findings (Yeo et al. 2003; Hong and Kim 2007). The results of the present study also showed that *L. lecanii* effectively controlled aphid when applied singly but had

antagonistic effect when combined with *B. bassiana*. These findings are in conformity with those reported by Hall and Burges (1979) who claimed that *V. lecanii* can be used for the control of aphid populations but was not found effective when used in combination with other antagonists under the same laboratory conditions.

In all treatments, the mortality rate of *M. persicae* was found to be time dependent in cases of filtrate, conidial suspension, and even when used in combinations. The highest percent mortality was observed after 10 days, while after 3 days, the mortality was the minimum. Almost similar results were reported by Loureiro et al. (2004) who found an increase in mortality with the increase in time and concentration. Contrarily, Araujo et al. (2009) reported (90%) mortality at the high concentration ( $10^7$  spore/ml) of *B. bassiana* after 4.4 days. However, there might be differences in mortality rates due to difference in strains being evaluated, concentration, laboratory conditions, and material used.



**Fig. 4** Combined effects of fungal isolates on *Myzus persicae* mortality



**Table 3** Effect of combined treatments on the mortality of green peach aphid

Combined treatment of fungal isolates	% mortality after		
	3 days	6 days	10 days
BB-72 + BB-252	15.0 b	67.5 d	100.0 e
BB-72 + CS625	10.0 ab	42.5 c	75.0 d
BB-252 + CS625	7.5 ab	45.0 c	77.5 d
BB-72 + BB-252 + CS-625	12.5 ab	47.5 c	90.0 e
Control (Tween)	2.5 a	5.0 ab	12.5 ab

Values are means of four replications

Means sharing common letters do not differ significantly ( $P = 0.05$ )

## Conclusion

The fungal strains, *Beauveria bassiana*, and *Lecanicillium lecanii*, used as filtrate and conidial suspensions, showed high mortality rates and can be effectively used for the control of green peach aphid. The effectiveness can be enhanced by combining different fungal strains.

## Abbreviations

PDA: Potato dextrose agar; rpm: Revolutions per minute

## Authors' contributions

All authors read and approved the final manuscript.

## Availability of data and materials

Not applicable

## Ethics approval and consent to participate

Not applicable

## Consent for publication

Not applicable

## Competing interests

The authors declare that they have no conflict of interest.

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