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RESEARCH

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# Antifungal activity of local isolates of *Beauveria bassiana* (Balsamo) Vuillemin against *Verticillium dahliae* Kleb. causing wilt disease of cotton

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## Abstract

**Background** *Verticillium dahliae* Kleb. is a soil-borne pathogen with a broad host spectrum and causes yield losses in cotton cultivation worldwide. *Beauveria bassiana* is an environmentally friendly entomopathogenic fungus (EPF) that has been recognized, used and studied for many years.

**Results** As a control, all local of *B. bassiana* isolates inhibited mycelial growth of the pathogen at different rates. The highest percentage of inhibition against non-defoliating (PHCVd3 isolate) and defoliating (PHCVd47 isolate) pathotypes was determined in Bb18 (85.3; 81.7%) and Bb1 (82.3; 77.5%) isolates, respectively, when applied 2 days ago. Isolates Bb1 (80.6%) and Bb18 (77.8%) had the highest percentages of inhibition against the PHCVd3 isolate, and Bb18 (75.8%) against isolate PHCVd47, when EPF and pathogen were applied at the same time.

**Conclusions** Bb18 and Bb1 isolates of *B. bassiana* showed a hope against both pathotypes of wilt disease caused by *V. dahliae* under in vitro conditions. Especially, *B. bassiana* was observed to induce higher inhibition rates, when EPF isolates developed in Petri dish 2 days before the pathogen.

**Keywords** *Verticillium dahliae*, *Beauveria bassiana*, Local isolates, Antifungal activity, Biological control

## Background

Cotton (*Gossypium* spp.) is an industrial crop that, with its widespread and mandatory fields of use, has great economic importance for humanity and creates added value and jobs for producing countries (Majumdar et al. 2019). Turkey ranks sixth in world cotton production after India, China, the USA, Brazil and Pakistan (USDA 2021).

One biotic factor affecting cotton production and quality is wilt disease caused by *Verticillium dahliae* Kleb. (Fradin and Thomma 2006). *V. dahliae* is a soil-borne fungal plant pathogen responsible for *Verticillium* wilt, which affects a variety of plants, including crops, flowers and vegetables (Jimenez-Diaz et al. 2012). This pathogen can persist in the soil through microsclerotia for up to 14 years (Xiao and Subbarao 1998). Globally, the pathogen has two pathotypes, defoliating and non-defoliating. While the defoliating pathotype causes complete shedding and leaf death, the non-defoliating pathotype causes wilting and less leaf destruction (Bejarano-Alcazar et al. 1995). Both pathotypes have been reported to occur in Turkey, with 93% of the defoliating isolates found in the Aegean region and 77% of the non-defoliating isolates

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found in the Çukurova and Southeast Anatolia (Göre 2007). Cotton yield losses caused by *Verticillium* wilt average around 10–35% (Song et al. 2020). The pathogen enters by infecting plants through capillary roots and settling in vascular bundles. Then, starting from the lower leaves, it causes wilting, drying, reduced photosynthesis, collapse of small pods, yield loss and changes in fiber quality characteristics (Agrios 2005). Controlling the disease is complicated by the fact that the pathogen is a soil fungus and there is no economical chemical control.

*Beauveria bassiana* (EPF) (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae) has been observed in more than 700 species (Wraight et al. 2000). *B. bassiana* is an entomopathogenic fungus (EPF) used for biological control against both plant pathogens and insect pests and the cause of white muscardine (Feng et al. 2004). Today, *B. bassiana*, *Metarhizium anisopliae*, *Isaria fumosorosea* (=*Paecilomyces fumosoroseus*) and *Lecanicillium l*ecanii are commercially produced among the 700 identified species of EPF (Meyling et al. 2018). EPFs have very important advantages such as being non-toxic to human and environmental health, having a wide host range, not developing host resistance, being used in combination with pesticides, being cheap and easy to use (Sinha et al. 2016). Studies in vivo or in vitro, *Beauveria* spp. showed antagonistic activity against *Botrytis cinerea* Pers., *Fusarium oxysporum* Bad. correct. Snyder & Hansen, *Gaeumannomyces graminis* (Sacc.) Arx & D.L. Olivier, *Pythium* spp., *Rhizoctonia solani* Kühn and *Septoria* spp. (Dara 2019). Meanwhile, plant pathogen control studies of *B. bassiana* are, generally, limited to in vitro studies of plant pathogen growth inhibition and cell wall structure degradation. There is a little research on the efficacy of *B. bassiana* against pathogens under field conditions. *B. bassiana* has been reported to inhibit the mycelial growth of *Rhizoctonia solani* and *Pythium myriotylum* in cotton (Ownley et al. 2008), *Botrytis cinerea* (Barra-Bucarei et al. 2020).

The aim of the study is to investigate the antifungal activity of 5 local EPF isolates of *B. bassiana* (ET 10, ET 101, BMAUM-M6-4, Bb1, and Bb18) isolated from different hosts in Turkey against 2 fungal isolates of *V. dahliae* (PHCVd3-non-defoliating pathotype and PHCVd47-defoliating pathotype) under laboratory conditions.

## Methods

### Fungal isolates

The EPF isolates of *B. bassiana* (EPF) and fungal isolates of *V. dahliae* (pathogen) used in this study are shown in Table 1. Five local isolates of *B. bassiana* were obtained from different hosts and locations in Turkey. All *B. bassiana* isolates were developed within the dark at 25 ± 1 °C for 7–15 days and after that subcultured on potato dextrose agar (PDA-Difco). Pure cultures of *V. dahliae* (PHCVd3 isolate-nondefoliating pathotype; PHCVd47 isolate-defoliating pathotype) were obtained from the collection of fungal cultures at Mustafa Kemal University of Agriculture, Department of Plant Protection, and subcultured in PDA at 25 ± 1 °C, 12 h dark/12 h light with an incubation period of 7–15 days.

### Antagonistic potential of *B. bassiana* against *V. dahliae* under laboratory conditions

Effect of local EPF isolates of *B. bassiana* on *V. dahliae* was investigated in the General Microbiology Laboratory of the Department of Organic Farming Business Management, Faculty of Applied Sciences, Pamukkale University. Antifungal interactions between EPF and test pathogen were determined using an in vitro dual culture technique on PDA medium in 90 mm Petri dishes. In the first experiment, actively growing *B. bassiana* mycelial disks (5 mm in diameter, 7–15 days old) were placed 3 cm from the corners of Petri dishes poured with PDA (25 mL). *B. bassiana* was plated, 2 days before *V. dahliae* due to the high growth rate of it. Two days after inoculation, mycelial disks of the test pathogen (5 mm in diameter, 7–15 days old) were placed next to those of *B. bassiana* at a distance of 3 cm from the Petri dish. In a second

**Table 1** Sources and origins of the EPF fungus and tested pathogen fungal isolates used in the experiment

| Entomopathogenic fungus isolate | Isolated from | Origin | References |
|---|---|---|---|
| *Beauveria bassiana*/ET 10 | *Sphenoptera antiqua* | Erzurum, Turkey | Tozlu et al. (2017) |
| *Beauveria bassiana*/ET 101 | Coleoptera larvae | Erzurum, Turkey | In the study |
| *Beauveria bassiana*/BMAUM-M6-4 | Field soil | Isparta, Turkey | Baydar et al. (2016) |
| *Beauveria bassiana*/Bb1 | Forest soil | Düzce, Turkey | In the study |
| *Beauveria bassiana*/Bb18 | Field soil | Düzce, Turkey | In the study |
| **Test pathogen fungus isolate** | **Isolated from** | **Origin** | **Reference** |
| *Verticillium dahliae*/PHCVd3 | *Gossypium hirsutum* | Hatay, Turkey | Erdogan (2020) |
| *Verticillium dahliae*/PHCVd47 | *Gossypium hirsutum* | Hatay, Turkey | Erdogan (2020) |

**Table 2** Scale modified from Bell et al. (1982) used in experiments

| Scale value | Definition |
|---|---|
| 1 | The EPF completely overgrew test pathogen |
| 2 | The EPF overgrew at least 2/3rd growth of test pathogen |
| 3 | The EPF colonized half of the growth of test pathogen and no one seems to dominate each other |
| 4 | Test pathogen overgrew 2/3rd of the growth of EPF and resist invasion |
| 5 | Test pathogen completely overgrew the EPF |

experiment, mycelial disks of *B. bassiana* and *V. dahliae* (5 mm in diameter, 7–15 days old) were placed concurrently at a distance of 3 cm from the corner of a Petri dish with PDA. As a control, only a single mycelial disk (5 mm in diameter, 7–15 days old) containing the test pathogen was placed in the center of the Petri dish. Plates were then, sealed with parafilm and incubated at a temperature of 25 ± 1 °C in the dark for the first and second experiments. Experiments were carried out with five replicates in a completely randomized parcels design. When the test pathogen colonized the whole Petri dish, the percentage of inhibition of mycelial growth of the test pathogen was determined according to the method described by Sundaramoorthy et al. (2012) formula (Eq. 1).

$$\mathrm{PI}: [(\mathrm{C} - \mathrm{T}) / \mathrm{C}] \times 100 \quad (1)$$

where PI is the percentage inhibition of mycelial growth (%), C is the radial growth of the pathogen in control (mm) and T is the radial growth of the pathogen in the presence of EPF (mm). *B. bassiana* isolates were scored using the modified Bell scale (Bell et al. 1982) based on their ability to suppress mycelial growth of *V. dahliae* and are presented in Table 2.

### Data analysis

Data for this study were performed with the packet statistics program JMP IN (SAS Institute, Cary, NC, 13.0 PC version). Data were examined using ANOVA (one-way analysis of variance). Duncan's multiple range test was used to determine whether there was a significant difference ($P \leq 0.01$) among the mean diameters of zones of the EPF inhibition in vitro.

## Results

Experiments were performed under laboratory conditions according to the dual culture technique, and the effects of local isolates of *B. bassiana* on mycelial growth and the mean inhibition (%) of *V. dahliae* isolates are shown in Table 3. This study had statistically significant ($P \leq 0.01$) mycelial growth and inhibition rates of EPF against test pathogens (non-defoliating and defoliating pathotypes) compared to the control Petri plate. Mycelial growths of PHCVd3 (non-defoliating pathotype) and PHCVd47 isolates (defoliating pathotype) of the pathogen were measured as 89.5 and 71 mm in the control Petri dishes (first and second experiment), respectively.

The lowest mycelial growth against PHCVd3 isolate (non-defoliating pathotype) was in Bb18 (13.2 mm) and Bb1 (15.8 mm) isolates, when *B. bassiana* isolates were applied, 2 days before *V. dahliae*, and these isolates were included in the same statistical group. The highest mycelial growth was found with 43.3 mm in BMAUM-M6-4 isolate. The highest percentage of inhibition against the PHCVd3 isolate was determined in isolates Bb18 (85.3%) and Bb1 (82.3%), with a scale value of 1 for these isolates (Fig. 1). The lowest percentage inhibition was detected in the BMAUM-M6-4 isolate at 51.6%. The Bb18 (13.0 mm) and Bb1 (16.0 mm) isolates also showed the least mycelial development relative to the PHCVd47 isolate (defoliating pathotype), and these isolates were included in the same statistical group. The highest mycelial growth was found at 41.7 mm in the BMAUM-M6-4 isolate. The highest percentage of inhibition against the PHCVd47 isolate was determined in isolates Bb18 (81.7%) and Bb1 (77.5%), which received a scale value of 1 (Fig. 1). The lowest inhibition rate was also recorded with the BMAUM-M6-4 isolate (41.3%). When both *B. bassiana* and *V. dahliae* were applied simultaneously, the lowest mycelial growth was detected in isolates, Bb1 (17.4 mm) and Bb18 (19.9 mm) and the PHCVd3 isolate and these isolates were included in the same statistical group. The highest mycelial growth was measured as the 45.9 mm BMAUM-M6-4 isolate. The highest percentages inhibition against PHCVd3 isolates were detected at isolates Bb1 (80.6%) and Bb18 (77.8%), which received a scale value of 1 (Fig. 2). The lowest percentage inhibition was observed with the BMAUM-M6-4 isolate at 48.7%. Compared to the PHCVd 47 isolate, the lowest mycelial growth was detected at 17.2 mm for the Bb18 isolate, the highest mycelial growth was detected at 40.6 mm for BMAUM-M6-4, and 38.4 mm for the ET 101 isolate. The highest inhibition rate was 75.8% for the Bb18 isolate, which received a scale value of 1 (Fig. 2). The lowest inhibition rate was 42.8% for the BMAUM-M6-4 isolate (Table 3).

## Discussion

In this study, the highest inhibition rate was recorded in Petri dishes of *B. bassiana* isolates applied, 2 days before *V. dahliae*. This may be because *B. bassiana* possesses

**Table 3** Percentage of inhibition of *Verticillium dahliae* by *Beauveria bassiana* under in vitro conditions

| Isolate code | Isolate PHCVd3 | | | Isolate PHCVd47 | | |
|---|---|---|---|---|---|---|
| | Mycelial growth (mm)[a] | MGI (%) | Bell's scale** | Mycelial growth (mm)[a] | MGI (%) | Bell's scale** |
| Application of *B. bassiana* to the medium, 2 days before *V. dahliae* | | | | | | |
| ET 10 | 33.3 c* | 62.8 | S2 | 31.4 c | 55.8 | S3 |
| ET 101 | 29.0 c | 67.6 | S2 | 27.2 c | 61.7 | S2 |
| BMAUM-M6-4 | 43.3 b | 51.6 | S3 | 41.7 b | 41.3 | S4 |
| Bb1 | 15.8 d | 82.3 | S1 | 16.0 d | 77.5 | S1 |
| Bb18 | 13.2 d | 85.3 | S1 | 13.0 d | 81.7 | S1 |
| Control | 89.5 a | 0.0 | – | 71.0 a | 0.0 | – |
| CD $_{(p= 0.01)}$ | 13.8 | | | 14.5 | | |
| Application of *B. bassiana* and *V. dahliae* to the medium at 2 days | | | | | | |
| ET 10 | 32.3 c* | 63.9 | S2 | 33.2 c | 53.2 | S3 |
| ET 101 | 38.3 c | 57.2 | S3 | 38.4 b | 45.9 | S4 |
| BMAUM-M6-4 | 45.9 b | 48.7 | S4 | 40.6 b | 42.8 | S4 |
| Bb1 | 17.4 d | 80.6 | S1 | 25.6 d | 63.9 | S2 |
| Bb18 | 19.9 d | 77.8 | S1 | 17.2 e | 75.8 | S1 |
| Control | 89.5 a | 0.0 | | 71.0 a | 0.0 | – |
| CD $_{(p= 0.01)}$ | 13.5 | | | 9.7 | | |

[a] Data are means of five replicates, *Means followed by different letters within a column are significantly different according to Duncan's test ($P \leq 0.01$), MGI: Mean growth inhibition (%), CD: Critical difference, **Bell's Scale: S1: Antagonist completely overgrewtest pathogen, S2: Antagonist overgrew 2/3rd growth of test pathogen, S3: Antagonist colonized half of test pathogen, S4: Test pathogen overgrew 2/3rd growth of antagonist and S5: Test pathogen completely overgrew antagonist

mechanisms such as mycoparasites, competition and antibiotics. Researchers have reported that *B. bassiana* suppresses plant diseases through direct mechanisms such as mycoparasitism, competition and antibiotics and exhibits multiple mechanisms of antagonistic interactions (Ownley et al. 2010).

*B. bassiana* isolates showed varying degrees of mycelial growth and percent inhibition of both pathotypes. In agreement to our results, Gothandapani et al. (2015) reported that three different entomopatogenic fungi (*B. bassiana*, *M. anisopliae* and *V. lecanii*) were tested against *Alternaria porri*. All the three EPF showed inhibitory effect against *A. porri* subjected to in vitro experiments under dual culture technique, conidial germination, mycelia germination and seed germination. The percentage inhibition of mycelial growth of *A. porri* was 69.24, 56.17 and 45.81%, after *B. bassiana*, *M. anisopliae* and *V. lecanii* treatments, respectively, and the percentage inhibition of conidial germination was detected 97.81, 42.11 and 67.69%, after the same treatments, respectively. *B. bassiana* exhibited effective antagonism *against A. porri*, showing the highest percentage inhibition of mycelial growth and conidial growth. As a result, EPF significantly inhibited *A. porri*. Culebro-Ricaldi et al. (2017) reported a 72% inhibition of pathogen development, when applied to the EPF, *B. bassiana* 1215 strain, 2 days before *F. oxysporum* f. sp. *lycopersici* strain 3. Culture filtrates of *B. bassiana* SD1, SD7, SD8, SD12, SD14, SD15, and *M. anisopliae* SD3 at 100% concentration showed varying levels of antifungal activity against *B. cinerea*, whereas *B. bassiana* SD8, SD12, SD14, SD15 and *M. anisopliae* SD3 isolate have been shown to be important in suppressing *B. cinerea* mycelial growth at low concentrations (Yun et al. 2017). Jaber and Alananbeh (2018) reported that two entomopathogens as endophyte (*B. bassiana* NATURALIS) and *M. brunneum* BIPESCO5) found to be antagonistic against three *Fusarium* species (*F. oxysporum*, *F. culmorum* and *F. moniliforme*). Fuentes et al. (2020) observed that both the EPF, *M. brunneum* and *B. bassiana* inhibited mycelial growth of the sunflower wilt pathogens *V. dahliae* and *Cadophora helianthi* under in vitro conditions. Deb and Dutta (2021) reported that all 22 native *B. bassiana* isolates inhibited mycelial growth of the tomato root rot pathogen *Pythium myriotylum* by 68–82% under in vitro conditions.

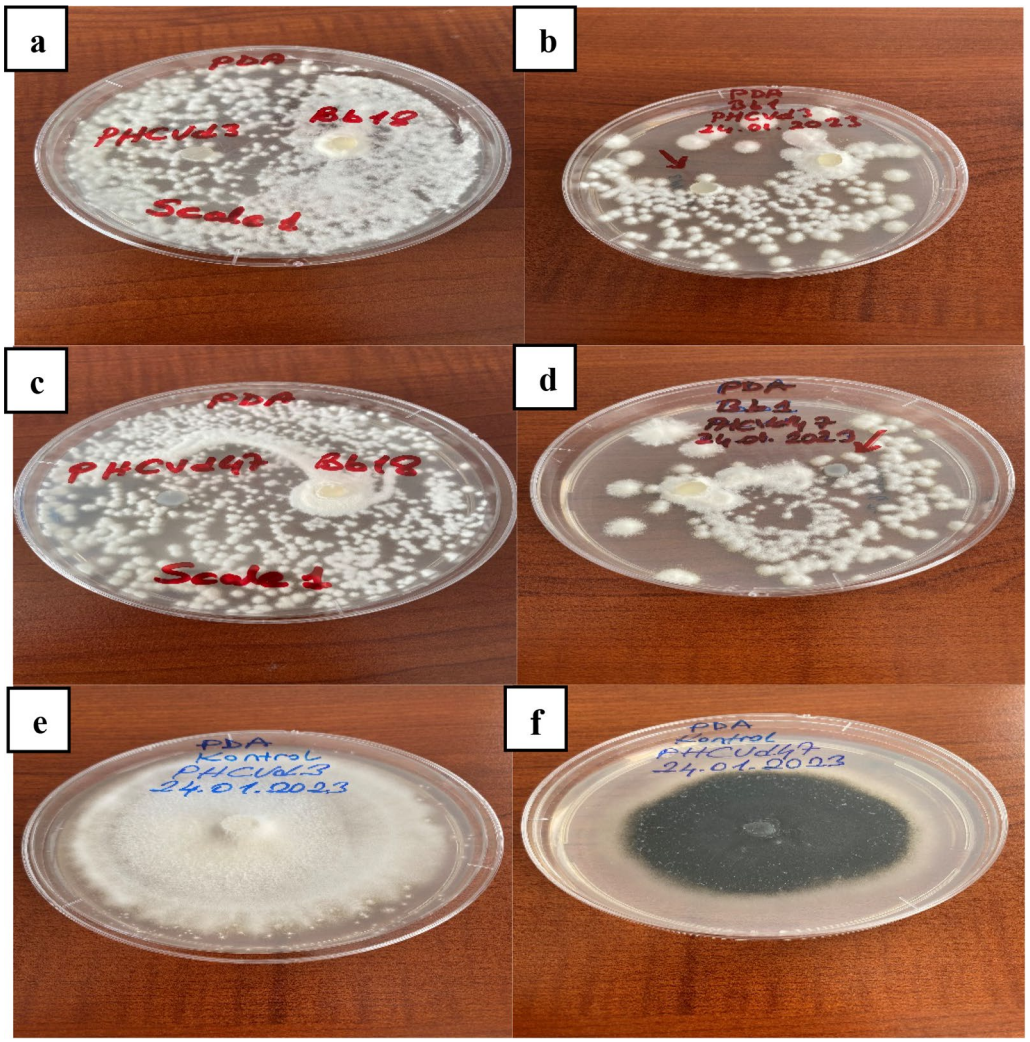


**Fig. 1** Antifungal effect of *Beauveria bassiana* applied to potato dextrose agar plates 2 days before *Verticillium dahliae*: (**a**, **b**) High activity from *B. bassiana* Bb18 and Bb1 to PHCVd3 (scale value 1) (**c**, **d**) High activity from *B. bassiana* Bb18 and Bb1 to PHCVd47 (scale value 1) (**e**, **f**) Control Petri dishes (PHCVd3 and PHCVd47 isolates)

## Conclusions

It was concluded that Bb18 and Bb1 isolated from *B. bassiana* isolates included in the study showed a high antagonistic activity against both pathotypes of *V. dahliae*. Notably, inhibition rates for Bb18 and Bb1 isolates were high in experiments performed, 2 days before the pathogen. The reason for this can be traced back to place competition in *B. bassiana* and the mechanism of effect of antibiotics. However, plant pathogen control depended not only on *B. bassiana* isolate characteristics, but also on biotic and abiotic factors. Therefore, this situation should be taken into account in the biological control strategies. In addition, more detailed studies under field conditions are needed on the efficacy of promising isolates, their role in disease management, plant growth promotion and increased yield.

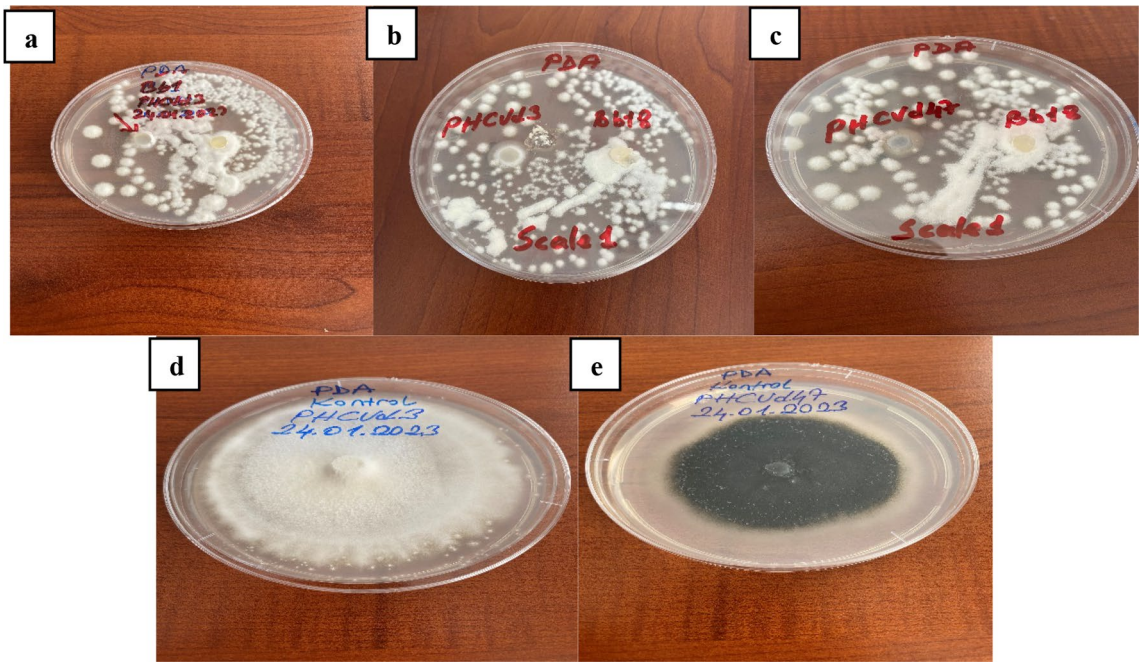


**Fig. 2** Antifungal effects of *Beauveria bassiana* applied concurrently with *Verticillium dahliae* in potato dextrose agar plates: (**a**, **b**) High activity of *B. bassiana* Bb1 and Bb18 against PHCVd3 (scale value 1) (**c**) High activity from *B. bassiana* Bb18 to PHCVd47 (scale value 1) (**d**, **e**) Control Petri dishes (PHCVd3 and PHCVd47 isolates)

**Abbreviations**

| | |
|---|---|
| ANOVA | Analysis of variance |
| *B. bassiana* | *Beauveria bassiana* |
| *V. dahliae* | *Verticillium dahliae* |
| EPF | Entomopathogenic fungi |
| PDA | Potato dextrose agar |

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**Author contributions**
OE and ZS collaborated in the creation of the manuscript. ZS conducted the experiments and recorded data. OE wrote the manuscript with statistical analysis, editing and review. Both authors read and approved the final manuscript.

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**Availability of data and materials**
All presented data are original.

## Declarations

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**Consent for publication**
We agree to publish this paper in the EJBC.

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

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