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COMPATIBILITY OF *BEAUVERIA BASSIANA* ISOLATES WITH FOUR ESSENTIAL OILS *IN VITRO* CONDITIONS

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Abstract

Beauveria bassiana and essential oils (EOs) are among the most widely studied in insect control and plant pathogenic fungi and bacteria. However, the compatibility of *B. bassiana* and EOs when used together is unknown. The vapor-phase technique was used to assess the compatibility of four EOs (peppermint, black cumin, chamomile, and fennel) with Turkey indigenous isolates of *B. bassiana* including ET10 and Bb18 under *in vitro* conditions. Petri plates inoculated with different concentrations (0.5, 1, 2, and 4 µL/petri) of EOs were added to the filter papers and placed on the inner surface of the Petri plates' lid. A 5 mm mycelial plug of *B. bassiana* was inoculated at the center of the Petri plate and incubated for 7 days at 25 ±1 ℃. Petri plates filled with sterile distilled water were used as controls. The experiment was conducted in a randomized plot design with three replications. Mycelial growth decreased and inhibition percentage increased in both *B. bassiana* isolates due to the increase in EO concentration. A low concentration of chamomile oil showed weak toxicity against both *B. bassiana* isolates and received an inhibition class value of 1. Peppermint oil exhibited a higher fumigation effect than other EOs against *Beauveria* isolates and received an inhibition class value of 4. A low concentration of chamomile oil combined with *B. bassiana* can be used as a potential bioagent against insect pests. Further laboratory and field trials are

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needed to examine the effects of EOs and *B. bassiana* on pests, beneficial insects, the environment, and plants.

INTRODUCTION

By 2050, the world's population will approach nine billion, and the need for food will increase by up to 98% (Godfray et al., 2013). Therefore, different methods must be developed to obtain high yields from agricultural fields and protect crops from pests (Azizoğlu et al., 2011). Agricultural pests cause approximately 35-40% of crop losses worldwide, resulting in annual economic losses of approximately $470 billion (Culliney, 2014). Farmers use synthetic insecticides to control pests because they are easy to apply and highly effective. The intensive and indiscriminate use of synthetic insecticides has negative effects on human health and non-target organisms and causes environmental pollution. In addition, synthetic insecticides harm beneficial insects, leading to an increase in pest populations and the development of resistance to synthetic insecticides (Campos et al., 2015). In contrast, despite the increasing demand for food every year, a portion of the world's agricultural products is lost due to many factors, such as negative environmental conditions, plant genetic potential, pests, diseases, and weeds (FAO, 2017). Therefore, more effective, safer, and environmentally friendly alternative control methods to synthetic insecticides should be researched. In this context, EOs and entomopathogenic fungi (EPF) are one of the prominent approaches in the control against diseases and pests.

Plants containing EOs are rich in bioactive compounds such as alkaloids, flavonoids, and terpenoids and have been reported to be effective in biological control, disease (antiviral, antimicrobial, and antifungal), and pest control (ovicide, insecticide, growth and development inhibitor, repellent, and toxic effect) (Kordali et al., 2007). More than 3000 EOs from different plants have been analyzed, and approximately 10% of them are commercially available as potential insecticides and repellents (Batume et al., 2014). Important families containing EO species are Asteraceae, Apiaceae, Brassicaeae, Compositaceae, Chenopodiaceae, Cupressaceae, Lauraceae, Lamiaceae, Myrtaceae, Ridaceae, Rutaceae, Rosaceae, Pineaceae, Poaceae, and Zingiberaceae (Kesdek et al., 2015). According to some reports, monoterpenoid inhibit acetylcholinesterase enzyme (AChE) activity and cause death in insects by exerting a toxic effect (Houghton et al., 2006). EOs are generally not toxic to mammals, birds, and fish (Koul et al., 2008). The mechanism of effect of EOs against pests is either through direct spraying or inhalation, and fumigation is the preferred method against storage pests (Göktürk et al., 2020).

EPF has good potential as a microbial pest control agent (Roberts, 1989). The potential capabilities of the EPF *Beauveria bassiana* as a biological pest control agent was first recognized in the early 19th century (Van Driesche and Bellows, 1996). *B. bassiana* has two different insecticidal properties. First, the fungus multiplies in the insect's flora and disrupts the insect's body balance. In the second case, the insect dies because of the toxins it produces (Clarkson and Chamley, 1996; Kershaw et al., 1999). In general, many insect orders (Lepidoptera, Hemiptera, Hymenoptera, Coleoptera, and Diptera) are susceptible to fungal diseases (Alavo et al., 2002). Currently, 700 EPF species belonging to 90 genera have been identified, including *B. bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae), *Lecanicillium* (= *Verticillium*) lecanii (Zimm.) Zare & Gams (Hypocreales: Clavicipitaceae), and *Purpureocillium lilacinum* (Thom.) Luangsaard, Hou-braken, Hywel-Jones, and Samson (Hypocreales: Ophiocordycipitaceae) insecticides are used commercially in many countries to control many pests (Ambethger, 2009). EPFs penetrate the integument, gain access to the hemocoel, and then deplete nutrients from the host (Vega et al., 2012). EPFs have many advantages over synthetic insecticides, including cost-effectiveness, high yield, absence of harmful side-effects for beneficial organisms, fewer chemical residues in the environment, and increased biodiversity (Lacey, 2016). EOs and *B. bassiana* are toxic to insects when applied alone. However,

their compatibility when applied together is not fully known. This study was conducted to investigate the compatibility of Turkey indigenous isolates of *B. bassiana* including ET10, and Bb18 and EOs of peppermint (*Mentha piperita* L.), fennel (*Foeniculum vulgare* Mill.), chamomile (*Matricaria chamomilla* L.), and black cumin (*Nigella sativa* L.) under *in vitro* conditions by the vapor-phase technique.

MATERIALS AND METHODS

Essential oils

EOs from different families supplied by a private company (Arpaş Arifoğlu Company, Istanbul-Turkey) using steam distillation method were used in the study (Table 1). The EOs were stored in dark and tightly closed bottles at +4^{o}C in the refrigerator until further use.

Table 1: List of EOs under study

Scientific name	Family	Common name	Brand name
Mentha piperita L.	Lamiaceae	Peppermint	Peppermint oil
Foeniculum vulgare (Mill.)	Apiaceae	Fennel	Fennel oil
Matricaria chamomilla L.	Asteraceae	Chamomile	Chamomile oil
Nigella sativa L.	Ranunculaceae	Black cumin	Black cumin oil

Indigenous isolates of *B. bassiana*

Two indigenous isolates of *B. bassiana*, including ET10 and Bb18, were obtained from different hosts and locations in Turkey (Table 2). *B. bassiana* isolates were subcultured on potato dextrose agar (PDA-Merck, 39 g) in 9-cm diameter Petri plates and incubated in dark at 25±1°C for 7 days.

Table 2: Indigenous *B. bassiana* isolates used in the study

B. bassiana isolate	Isolated from	Origin	Reference
B. bassiana ET10	*Sphenoptera antiqua*	Erzurum, Turkey	Tozlu et al. (2017)
B. bassiana Bb18	Field soil	Düzce, Turkey	Erdoğan and Sağlan, (2023)

Compatibility of EOs with *B. bassiana*

The vapor-phase technique was used to determine the compatibility of four EOs with *B. bassiana* (Soylu et al., 2010). Plastic Petri plates (90 mm diameter, 25 mL PDA media) were used to determine the vapor-phase technique effect. Petri plates inoculated with different concentrations of EOs were added to sterile filter papers (70 mm diameter, Whatman no.1) and placed on the inner surface of the lid of Petri dishes to obtain final concentrations of 0.5-4 mL/petri. *B. bassiana* was inoculated by plating the center of each Petri plate with a 5 mm diameter EPF disc, cut with a sterile cork borer from the edge of actively growing cultures on PDA plates. The Petri dishes filled with sterile distilled water were used as controls. The Petri plates were immediately sealed with parafilm to prevent the loss of EO vapors and incubated for 7 days at 25 ±1℃. The experiment was conducted with three replicates depending on a completely randomized plot design. Readings were taken at right angles using a ruler, and the percentage inhibition of the *B. bassiana* isolates was calculated using the following formula (Eq. 1):

$$I (\%) = C\ \ T / C \times 100 \qquad \text{(Equation 1)}$$

Where;

I (%)= percent inhibition (%),

C = mycelial growth diameter of the *Beauveria* isolate in the control (mm)

T = mycelial growth diameter of the *Beauveria* isolate in treatment (mm) (Vincent, 1947).

Inhibition levels were used to evaluate the effect of the EOs on B. bassiana (Ambethger, 2009): 1 = harmless (< 25%), 2 = slightly harmful (25-35%), 3 = moderately harmful (36-50%), and 4 = harmful (> 50%).

Statistical analysis

The JMP IN statistical program (SAS Institute, Carry, NC, 13.0 PC version) was used for all calculations. One-way analysis of variance (ANOVA) was performed, and means were compared using the student's t-test at a significance level of $P \leq 0.01$.

RESULTS AND DISCUSSION

Two indigenous *B. bassiana* isolates were grown on PDA media amended with four different EO concentrations. Mycelial growth diameter of *B. bassiana* isolates exposed to different EO concentrations was recorded on the 7th day after inoculation. Table 3 shows the compatibility results of EOs with *B. bassiana* isolates. The EO concentrations were found to be significant according to the statistical analysis results ($p \leq 0.01$) of the *in vitro* experiment. The highest mycelial growth diameter was found in the control Petri dishes (40.0 mm and 40.9 mm) of *B. bassiana* isolates (ET10 and Bb18). From the observed records at 7th day after inoculation, The maximum mycelial growth diameter (mm) against isolates of *B. bassiana* (ET10 and Bb18) was determined at low concentration (C1) of chamomile oil (35.0 mm and 37.9 mm), followed by black cumin oil (27.1 mm) against isolate ET10 and fennel (31.9 mm) and black cumin (30.7 mm) oils against Bb18 isolate. The lowest percentage inhibition rate among the EOs was determined as 64.3% and 61.3% at the high concentration (C4) of chamomile oil against ET10 and Bb18 isolates, respectively. The maximum mycelial growth diameter and lowest inhibition percentage were determined for all chamomile oil concentrations compared with other EOs. The low concentration of chamomile oil received an inhibition class value of 1 against both *B. bassiana* isolates. Meanwhile, the low concentration of fennel oil received an inhibition class value of 1 only against the Bb18 isolate. The minimum mycelial growth diameter against both isolates of *B. bassiana* was determined at high concentrations of peppermint oil (3.9 mm and 4.7 mm), followed by fennel oil (9.9 mm and 6.4 mm). Conversely, the highest inhibition percentages in Bb18 and ET10 isolates were determined as 90.4% and 88.3%, respectively, at high concentrations of peppermint oil. This oil received an inhibition class value of 4 against the Bb18 isolate at all concentrations except the low concentration. All EOs received an inhibition class value of 4 against both *B. bassiana* isolates at high concentrations. The second highest percentage of inhibition against ET10 and Bb18 isolates was obtained from black cumin oil (84.0% and 75.7%), respectively (Table 3).

In this study, the compatibility of indigenous isolates of *B. bassiana* with different concentrations of EOs, such as peppermint, black cumin, chamomile, and fennel, was tested *in vitro* using the vapor-phase technique. Mycelial growth diameter of *B. bassiana* isolates decreased and inhibition percentage increased due to the increase in EO concentration. This may be because EOs are from different families and contain different secondary metabolites. Similar to our findings, Oussalah et al. (2007) reported that the variability in the fungicidal activity of EOs was related to differences in their active components, such as phenols, aldehydes, and ketones. Contrary to our findings, in some studies, fungal growth was not altered by EOs. For example, *Ocimum sanctum* leaves, roots, stems, and seeds extracts did not affect the conidial growth of *Metarhizium anisopliae* (Borgio et al., 2008). Another study reported that *B. bassiana* mycelial growth increased with decreasing EO concentration (Liu, 2012). M. piperita oil exhibited higher fungicidal activity than the other three EOs (*F. vulgare*, *M. chamomilla*, and *N. sativa*). The inhibition of the growth of these fungal pathogens may be due to the main components, such as menthone, menthol, and menthofuran (Moghddam et al., 2013). According to Kostik et al. (2015), the two main components of peppermint oil are menthol and menthone. The chemical composition of *M. piperita* oil mostly detected linalool (40.4%) and linalyl acetate (32.6%) (Mejdoub et al., 2019). Güven et al. (2023) tested the effects

of thyme, rosemary, and eucalyptus EOs on *B. bassiana* spore germination and mycelium development *in vitro*, and pure thyme oil concentration inhibited B. bassiana mycelial development and spore germination. The effect of EOs on the mycelial growth of fungi was evaluated; all EOs except peppermint and rosemary oils were found to be compatible with *B. bassiana* isolates, and peppermint and rosemary oils showed toxic effects against the OZ1 isolate (Sohrabi et al., 2024).

Chamomile oil showed lower toxicity against *B. bassiana* isolates than the other three EOs. The antifungal effect of chamomile oil is related to its terpene-type components (Pauli-Magnus and Griensven, 2006). Similar to our results, Sokovi´c and van Griensven (2006) reported that chamomile EO has weak antifungal potential. Sharma et al. (2016) observed the different antimicrobial effects of chamomile and ginger oils against bacteria and fungi. The compatibility of EOs and *B. bassiana* isolates varied with increasing concentration. The reasons for this may be attributed to the differences in the host, region, and virulence levels of the *Beauveria* isolates. Roy et al. (2006) reported that the ecology, physiology, and life cycle of EPF are variable.

Table 3: Mycelial growth, inhibition (%), and inhibition class of *B. bassiana* isolates

Treatment	***B. bassiana* isolate ET10**											
	Mycelial growth diameter (mm)				**Inhibition (%)**				**Inhibition class***			
	C1	**C2**	**C3**	**C4**	**C1**	**C2**	**C3**	**C4**	**C1**	**C2**	**C3**	**C4**
Mint oil	25.7 b[1]	20.7 c	9.7 e	4.7 e	35.3	47.9	75.6	88.3	2	3	4	4
Black cumin oil	27.1 c	20.9 c	14.9 c	9.8 c	31.7	47.3	62.6	75.4	2	3	4	4
Chamomile oil	35.0 d	27.4 b	20.3 b	14.2 b	11.7	31.1	48.9	64.3	1	2	3	4
Fennel oil	25.7 b	20.7 c	12.5 d	6.4 d	35.3	47.9	68.5	84.0	2	3	4	4
Control	40.0 a	40.0 a	40.0 a	40.0 a	0.0	0.0	0.0	0.0	-	-	-	-
$CD_{(P=0.01)}$	1.9	3.9	4.3	3.6								
Treatment	***B. bassiana* isolate Bb18**											
	Mycelial growth diameter (mm)				**Inhibition (%)**				**Inhibition class***			
	C1	**C2**	**C3**	**C4**	**C1**	**C2**	**C3**	**C4**	**C1**	**C2**	**C3**	**C4**
Mint oil	26.6 d[1]	19.3 d	13.8 d	3.9 e	34.9	52.9	66.3	90.4	2	4	4	4
Black cumin oil	30.7 c	24.1 c	16.7 c	12.4 c	24.9	41.0	59.2	69.6	2	3	4	4
Chamomile oil	37.9 b	31.3 b	22.4 b	15.8 b	7.2	23.5	45.1	61.3	1	2	3	4
Fennel oil	31.9 c	23.8 c	16.0 c	9.9 d	22.0	41.8	60.8	75.7	1	3	4	4
Control	40.9 a	40.9 a	40.9 a	40.9 a	0.0	0.0	0.0	0.0	-	-	-	-
$CD_{(P=0.01)}$	2.3	1.9	3.1	5.3								

[1]Mean values followed by different letters within the column are significantly different according to LSD Test (P≤ 0.01). C1: 0.5 µL/Petri, C2: 1 µL/Petri, C3: 2 µL/Petri, C4: 4 µL/Petri. *Inhibition classes according to Ambethgar (2009): 1 = harmless (< 25%), 2 = slightly harmful (25–35%), 3 = moderately harmful (36%–50%), and 4 = harmful (> 50%). CD: Critical difference

CONCLUSION

Compatibility with *B. bassiana* isolates decreased with increasing EO concentrations. Peppermint oil showed more toxic effects than the other three EOs against both *B. bassiana* isolates and received an inhibition class value of 4 against the Bb18 isolate of *B. bassiana* at all concentrations except the low concentration. The high fumigation effect of peppermint oil against *Beauveria* isolates can be attributed to the presence of two main compounds, menthol and menthone. In particular, low-concentration chamomile oil showed weak toxicity against both *B. bassiana* isolates and received an inhibition class value of 1. This oil contains terpene-type compounds and thus showed better compatibility with the two *B. bassiana* isolates. The compatibility of *B. bassiana* isolates varies depending on the EO concentration. This may be associated with the different host, region, and virulence

levels of *B. bassiana* isolates. In conclusion, chamomile oil with a weak toxic effect can be used together with *B. bassiana*. Additionally, field trials should be conducted using these EOs and *B. bassiana* isolates together to determine their effects against pests and beneficial insects.

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