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Efficacy of the entomopathogenic fungi; *Beauveria bassiana* and *Metarhizium anisopliae* against the cowpea weevil, *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae: Bruchinae)

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Abstract

The efficacy of the two entomopathogenic fungi, *Metarhizium anisopliae* (TR 106) and *Beauveria bassiana* (TR 217), was tested against the adults of the cowpea weevil, *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae: Bruchinae) in laboratory. Two concentrations of conidial suspensions (1×10^6 and 1×10^8 conidia/ml) of *M. anisopliae* and *B. bassiana* isolates were directly applied on four pairs of adult beetles in Petri dishes (2 ml/dish) and then incubated at two different climatic regimes; 22 ± 1 °C and 26 ± 1 °C with $70 \pm 5\%$ RH, 16:8 h light-to-dark. Lethal time values (LT₅₀ and LT₉₀) were calculated using probit analysis. As a result, the LT₅₀ and LT₉₀ values of *M. anisopliae* (1×10^8 conidia/ml) were 4.45 and 5.34 days at 26 °C and 5.17 and 6.15 days at 22 °C, respectively. LT₅₀ and LT₉₀ values of *B. bassiana* (1×10^8 conidia/ml) were 4.07 and 5.11 days at 26 °C and 4.07 and 5.41 days at 22 °C, respectively. LT₅₀ and LT₉₀ values of *M. anisopliae* (1×10^6 conidia/ml) were 5.42 and 6.43 days at 26 °C and 6.08 and 7.54 days at 22 °C, respectively. The LT₅₀ and LT₉₀ values of *B. bassiana* (1×10^6 conidia/ml) were 5.67 and 7.15 days at 26 °C and 5.47 and 7.50 days at 22 °C, respectively. Approximately 100% of mycoses were obtained in all treatments. In general, the effectiveness of these two entomopathogens increased by increasing suspension concentrations and temperature. These results suggest that the two isolates may be very successful in biological control of the *C. maculatus* and may be alternatives for chemical pest management.

Keywords: *Callosobruchus maculatus*, Cowpea weevil, Entomopathogenic fungi, Virulence, Temperature

Background

Chickpeas, beans, lentils, peas, broad beans, and cowpea, which are important legumes for human nutrition, are low in fat and high in carbohydrate and have a nutritious feature. In the field and storage stages of legumes, some insect pests usually cause significant damages. Among them, the cowpea weevil, *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae: Bruchinae), is found in all regions of Turkey.

The most important damages caused by *C. maculatus* include weight loss, loss in market value (Elhag 2000),

decrease in germination ability of seeds (Baier and Webster 1992), and decrease in nutritional value, particularly proteins. The absence of adult diapause of the pest, infestation in the field and storages, and high reproductive potential increase the importance of management against this pest. Nonetheless, it has been reported that 1–2% field infestation may raise to 80% after 6 months of storage period (Youdeowei 1989).

Numerous synthetic insecticides and fumigants are used against stored product pests (Arthur 1996). However, potential risks posed by synthetic insecticides for mammals, concerns of consumers on pesticide residues in processed legume products, insecticidal-resistant insect populations, ecological implications, increased application costs, and the necessity precautionary measures

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when using these chemicals have urged the researchers to develop alternative control strategies against stored product pests (Mahdi and Rahman 2008).

Entomopathogenic fungi (EPF) are effective biological control agents against various pests (Roy et al. 2006). Recently, there is an increasing interest in the use of EPF for the development of biological control strategies against stored product pests. Among EPF, the biological control potential of *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Metch) Sorok, and *Isaria fumosorosea* Wize has been studied against various pests, in particular, *C. maculatus* (Vilas Boas et al. 1996; Rice and Cogburn 1999; Padin et al. 2002; Batta 2005; Cherry et al. 2005, 2007; Sewify et al. 2014; Athanassiou et al. 2017; Iqbal et al. 2018).

Temperature is among the most important factors limiting the efficacy of EPF (Fernandes et al. 2008). Generally, optimum temperature for germination, development, sporulation, and virulence of EPF ranges from 20 to 30 °C (Tefera and Pringle 2003; Dimbi et al. 2004; Kiewnick 2006). Therefore, different temperatures used in different studies have led to varying results. Lawrence and Khan (2002) reported that two different *B. bassiana* isolates were applied to cowpea seed beetle at different temperatures (20, 25, and 30 °C) and the lowest LC₅₀ and LT₅₀ values were determined at 30 °C.

This study was conducted to assess the efficacy of two conidial concentrations of *M. anisopliae* (TR-106) and *B. bassiana* (TR-217) against *C. maculatus* adults at two different temperatures under laboratory conditions.

Materials and methods

Insect culture

Initially, *C. maculatus* was obtained from chickpea seeds stored in the Department of Field Crops, Faculty of Agriculture, Ondokuz Mayıs University, Samsun, Turkey. Chickpeas were kept at -20 °C for 2 weeks to eliminate all infesting pests before starting the experiments (Cherry et al. 2005). The obtained *C. maculatus* adults were placed in glass jars (500 ml) having 200 g of sterilized chickpea

seeds. The glass jars were placed under conditions of 26 °C, 70% relative humidity, and 16:8 light-to-dark period for obtaining eggs. The culture was sieved daily to obtain male and female adults.

Preparation of entomopathogenic fungi

M. anisopliae TR-106 and *B. bassiana* TR-217 isolates used in this study were isolated from adults of *Xylosandrus germanus* Blandford (Coleoptera: Curculionidae: Scolytinae), which is one of the important hazelnut pests (Tuncer et al. 2018). The isolates were plated on potato dextrose agar (PDA; Merck Ltd., Darmstadt, Germany) in 9-cm-diameter Petri dishes, incubated at 25 °C with a complete dark for 10–12 days. At the end of the growth period, 10 ml sterile distilled water containing 0.02% Tween 20 was added to each dish. The spore suspensions were then filtered through two layers of cheesecloth to remove the micelle structures and were homogenized by vortexing for 3 min. The resulting spore suspensions were adjusted to concentrations of 1×10^6 and 1×10^8 conidia/ml, using Neubauer hemocytometer, under Olympus CX31 light microscope (Olympus America Inc., Lake Success, NY) (Kushiyeve et al. 2018).

Conidial germination assessment

The viability of conidia of the EPF isolates was determined. A conidial suspension was adjusted to 1×10^4 conidia/ml and 0.1 ml was sprayed onto Petri dishes (9 cm diameter, containing PDA), and the dishes were incubated at 25 °C. After 24 h of incubation, percentage of germinated conidia was determined by examining of 200 conidia from each of three replicate dishes, using Olympus CX31 compound microscope (-400). Conidia were regarded as germinated when they produced a germ tube at least half of the conidial length (Erper et al. 2016).

Application of EPF to *C. maculatus* adults

Two layers of sterile filter papers were placed in plastic Petri dishes and moistened with 1 ml of sterile distilled

Table 1 The LT₅₀ and LT₉₀ values of *Metarhizium anisopliae* against *Callosobruchus maculatus* at 2 different concentrations and temperatures

Variables	22 °C		26 °C	
	1×10^6	1×10^8	1×10^6	1×10^8
LT ₅₀ (95% fid. limit)	6.64 (6.08–7.38)a	5.46 (5.17–5.74)b	5.42 (4.72–6.01)bc	4.44 (3.89–5.01)c
LT ₉₀ (95% fid. limit)	8.43 (7.54–11.06)a	6.52 (6.15–7.15)b	6.43 (5.84–8.31)ab	5.34 (4.79–7.27)b
Slope ± SE	12.37 ± 1.98	16.54 ± 2.55	17.31 ± 2.71	16.08 ± 2.71
Regression equation	$y = -10.18 + 12.37$	$y = -12.19 + 16.54$	$y = -12.72 + 17.31$	$y = -10.42 + 16.08$
χ^2	46.1	15.54	75.92	46.14
df	22	22	22	16
Heterogeneity	2.09	0.7	3.45	2.88

*Means followed by same lowercase letter within a row do not differ significantly at $p \leq 0.05$

Table 2 The LT_{50} and LT_{90} values of *Baeuveria bassiana* against *Callebbruchus maculatus* at two different concentrations and temperatures

Variables	22 °C		26 °C	
	1×10^6	1×10^8	1×10^6	1×10^8
LT_{50} (95% fid. limit)	5.87 (5.47–6.32)a	4.61 (4.07–5.15)b	5.67 (5.35–5.99)a	4.07 (3.71–4.39)b
LT_{90} (95% fid.limit)	8.28 (7.5–9.7)a	6.1 (5.41–7.71)ab	7.15 (6.69–7.93)a	5.11 (4.7–5.9)b
Slope \pm SE	8.59 \pm 1.28	10.49 \pm 1.4	12.77 \pm 1.89	12.97 \pm 2.11
Regression equation	$y = -6.6 + 8.59x$	$y = -6.96 + 10.49x$	$y = -9.63 + 9.63x$	$y = -7.91 + 12.97x$
χ^2	11.77	52.63	16.2	22.34
<i>df</i>	22	22	22	18
Heterogeneity	0.53	2.39	0.73	1.24

*Means followed by same lowercase letter within a row do not differ significantly at $p \leq 0.05$

water. Afterwards, four pairs of *C. maculatus* adults were released into the dishes containing 15 g chickpeas and sprayed with 2 ml of spore suspensions (1×10^6 or 1×10^8 conidia/ml). The control insects were sprayed by sterile distilled water containing 2 ml of 0.02% Tween 20. All Petri dishes were sealed with a parafilm and incubated for 10 days at two different temperatures (22 and 26 °C), 70% RH, and 16:8 light-to-dark. The mortality rate was recorded daily for 8 days. The experiment was repeated daily at the same time with the same number ($n = 24$ adults/day/isolate/concentration/temperature) and different adults to keep the mortality rates of every day independent from each other (Robertson et al. 2007). The dead *C. maculatus* individuals were surface sterilized by 1% sodium hypochlorite and 70% ethyl alcohol. Then, these insects were washed with sterile distilled water, placed in Petri dishes having filter paper, and kept at 25 °C and 90% RH for 10 days. The dead adults were examined under a microscope to determine whether the cause of death was fungus, and mycosis rates were determined.

Statistical analyses

The daily mortality rates at different doses were corrected according to the Abbott formula when the mortality rate in the control exceeded 5% (Abbott 1925). The LT_{50} and LT_{90} values were determined by probit analysis, using the log-probit method (POLO-PLUS ver.2.0). The slopes of the regression lines were compared with each other using standard errors, and the LT_{50} and LT_{90} values of the isolates were compared using confidence intervals (95%).

Results and discussion

Conidia viability of the two isolates (TR-106 and TR-217) was assessed before bioassays, and approximately (100%) germination was obtained. The LT_{50} and LT_{90} values for *M. anisopliae* (1×10^8 conidia ml^{-1}) against *C. maculatus* were 4.44 and 5.34 days, respectively, at 26 °C, whereas at 22 °C, they were 5.46 and 6.52 days, respectively (Table 1). The respective LT_{50} and LT_{90} for *B.*

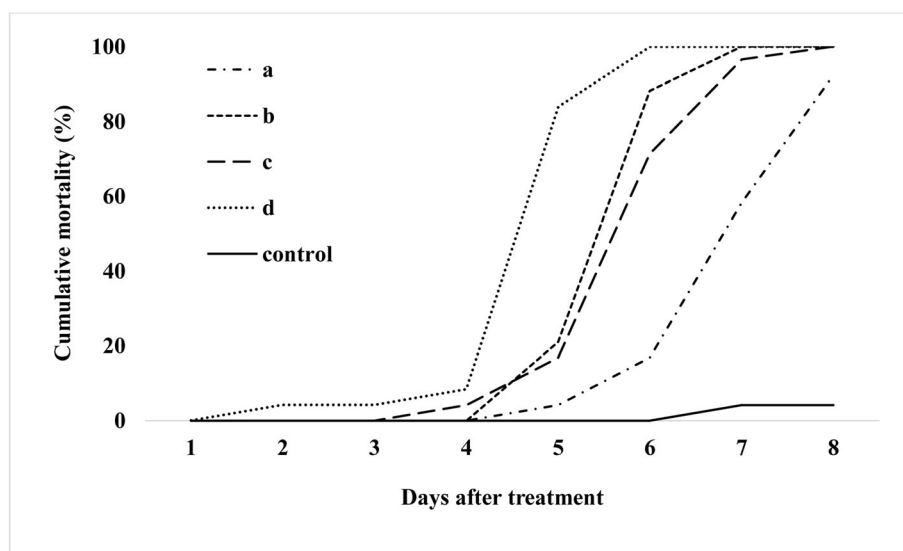


Fig. 1 The efficacy of *Metarhizium anisopliae* against *Callosobruchus maculatus* adults. **a** 22 ± 1 °C, 1×10^6 conidia ml^{-1} ; **b** 26 ± 1 °C, 1×10^6 conidia ml^{-1} ; **c** 22 ± 1 °C, 1×10^8 conidia ml^{-1} ; **d** 26 ± 1 °C 1×10^8 conidia ml^{-1}

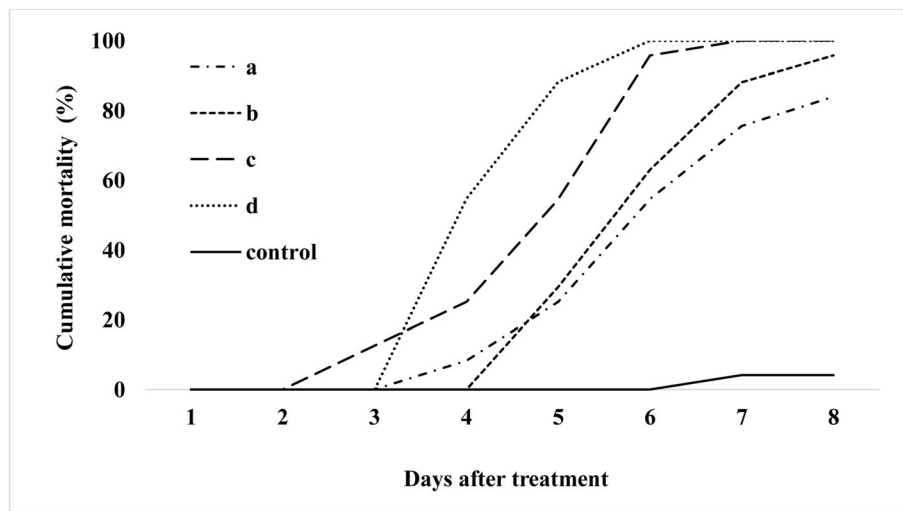


Fig. 2 The efficacy of *Beauveria bassiana* against *Callosobruchus maculatus* adults. **a** 22 ± 1 °C, 1 × 10⁶ conidia ml⁻¹; **b** 26 ± 1 °C, 1 × 10⁶ conidia ml⁻¹; **c** 22 ± 1 °C, 1 × 10⁸ conidia ml⁻¹; **d** 26 ± 1 °C, 1 × 10⁸ conidia ml⁻¹

bassiana were 4.07 and 5.11 days at 26 °C and 4.61 and 6.10 days at 22 °C.

The LT₅₀ and LT₉₀ values for *M. anisopliae* (1 × 10⁶ conidia ml⁻¹) were 5.42 and 6.43 days, respectively, at 26 °C, whereas at 22 °C, they were 6.64 and 8.43 days, respectively (Table 1). The respective values for *B. bassiana* were 5.67 and 7.15 days at 26 °C and 5.87 and 8.28 days at 22 °C (Table 2). In addition, approximately 100% mycosis rate was obtained in all treatments.

It was observed that the two tested concentrations of the two isolates at both temperatures started to cause mortality in 4 days after application and mortality increased by the time (Figs. 1 and 2). The 1 × 10⁸ concentration of the two species caused 100% mortality, 8 days after application. Similarly, the 1 × 10⁶ concentration of *M. anisopliae* caused 100% mortality, 8 days after application at 26 °C. Nonetheless, 84 to 96% mortality rate was noted in the other treatments (Figs. 1 and 2).

B. bassiana and *M. anisopliae* have been found to be the most effective EPF against various pests (Vilas Boas et al. 1996; Lawrence and Khan 2002). Cherry et al. (2005) reported that *B. bassiana* (0306 isolate) obtained from the insect belonging to the Scolytidae family did not have high virulence against *C. maculatus*.

Temperature is one of the leading abiotic factors affecting the development of fungi, which in turn significantly affects the virulence of EPF against harmful insects. For instance, Ak (2019) reported that *B. bassiana* caused the highest mortality (93.66%) at 25 °C and the lowest (40.74%) against *Sitophilus oryzae* (Coleoptera: Curculionidae) at 20 °C. Similarly, Vassilakos et al. (2006) reported that *B. bassiana* was more effective against *S. oryzae* and *Rhizopertha dominica* (Coleoptera: Bostrichidae) at 26 °C than at 30 °C. Similar results were obtained by Athanassiou and Steenberg (2007).

Comparing the LT₅₀ values obtained in the present study to the others, Vilas Boas et al. (1996) reported that *M. anisopliae* and *B. bassiana* at a concentration of 1 × 10⁸ conidia/ml against the adults of *C. maculatus* ranged between 3.46 for *B. bassiana* and 6.78 for *M. anisopliae*. Similarly, Cherry et al. (2005) reported that the LT₅₀ values for *C. maculatus* by *M. anisopliae* 0351 isolate and *B. bassiana* 0362 isolate at 1 × 10⁸ spores/ml were found to be 3.27 and 3.11 days, respectively.

Conclusion

The two evaluated EPF in this study (*M. anisopliae* TR-106 and *B. bassiana* TR-217 isolates) showed a virulence against *C. maculatus* (causing up to 100 % mortality). Thus, they seem to be promising biocontrol agents against this pest. However, further studies are needed to determine their effectiveness under storage conditions.

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Authors' contributions

IOO, CT, IE, and RK designed the study, supervised the work, and wrote the manuscript with input from all the authors. IOO, CT, IE, and RK carried out the experiments. CT analyzed the data. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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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