Ozdemir
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RESEARCH

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Efficacy of diatomaceous earth, and entomopathogenic fungi, *Beauveria bassiana*, and *Trichoderma asperellum* in combination and separately, against *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae)

Ismail Oguz Ozdemir[1*]

Abstract

Background *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) is one of the most significant pests infesting leguminous crops since it is found in tropical and subtropical climates, as well as in Turkey. The most often utilized methods of managing these insects are fumigants and synthetic insecticides. However, chemical pesticides lead to increased risks for human health, chemical residues, insect resistance, and environmental contamination. Therefore, the present study aimed to determine the effectiveness of entomopathogenic fungi [*Beauveria bassiana* (Bb) and *Trichoderma asperellum* (Ta)] individually or in combination with diatomaceous earth (DE) against *C. maculatus*. The fungi Bb and Ta were applied at 1×10^4, 1×10^6 and 1×10^8 spores/kg of chickpea seeds and mixed with 200, 400, 800 mg/kg of DE. Additionally, the progeny production of the insect on chickpea in the different treatments was evaluated after 40 days of exposure.

Results In all individual treatments, total adult mortality of the insect was accomplished solely by using the highest DE treatment rate (800 mg/kg) after 7 days. The most effective combination that was a mixture at highest application rate of DE/Bb (800 mg/kg of DE + 1×10^8 spores/kg of Bb) caused 100% mortality after 6 days of exposure and had the lowest LT_{50} (2.97) and LT_{90} (5.46) values (days). Although other DE/Bb binary combinations caused 100% mortality of *C. maculatus* 6 days after treatment, their LT_{50} and LT_{90} values were lower. Insect mortalities were 100% in all DE/Ta binary combinations on days 7 and 8, and the highest application rate (800 mg/kg of DE + 1×10^8 spores/kg of Ta) of this combination had the lowest LT_{50} (4.14) and LT_{90} (6.17) values (days). Individual treatments of DE, Bb, Ta and their binary combinations caused significant reduction in progeny production after 40 days of treatment compared with progeny production in the control of *C. maculatus*. The highest progeny production (88.9%) was observed at the highest treatment rate of DE/Bb combinations (800 mg/kg of DE + 1×10^8 spores/kg of BB).

Conclusions The treatments used in combination of Bb or Ta with DE resulted in increased insecticidal effectiveness against *C. maculatus*. These natural agents caused considerable decreasing of progeny production of the pest. Even

*Correspondence:
Ismail Oguz Ozdemir
oguzozdemir@subu.edu.tr
Full list of author information is available at the end of the article

with reduced application rates, the agents with a promising potential against the pest showed acceptable results in binary combinations.

Keywords *Callosobruchus maculatus*, Legumes, Chickpea, Storage pest, Biocontrol, Progeny production

Background

Legumes have a significant role in human nutrition due to their high protein, lipid, and carbohydrate content, as well as in soil fertility. Some seed beetles cause significant damage in the places where these legumes are cultivated and stored. The *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) is one of the most significant pests infesting leguminous crops since it is found in tropical and subtropical climates all over the world, as well as many areas of Turkey (Gad et al. 2021). This pest attacks legume in the field and in storage, causing physical damage and quality loss through post-harvest feeding and reproductive activities. Furthermore, it causes significant economic losses in stored legume seeds due to decreased weight and germination (Musa and Adeboye 2017). The most often utilized methods of managing these pest are fumigants and synthetic insecticides (Wolfson et al. 1991). However, chemical pesticides lead to increased risks for human health, chemical residues, insect resistance, and environmental contamination (Rizwan et al. 2019). For these reasons, the application of sustainable alternative management tools such as eco-friendly botanical insecticides, physical treatments, inert dusts, and microbial/biocontrol agents has been assessed (Abdelgaleil et al. 2021).

The diatomaceous earth (DE) as an inert dust is a well-studied fossil alternative that occurs naturally and commonly used as a preservative in grains and is safe for natural enemies and mammals (Kalleieratos et al. 2012). This DE has a variety of formulations across the world and used efficiently against many stored product pests (Wakil et al. 2010) by scratching the cuticle of the insects and causing dehydration of its body (Korunic 1998). However, the material, which is effective at high application ratio (1000–3500 mg/kg) (Permual and Le Patourel 1992), has detrimental impacts in the applied products, such as seed bulk density, seed flowability, and visible residues (Golob 1997). For this reason, it is suggested that the dose should be reduced and used in combination with other seed protectants (Ziaee et al. 2021). Several studies have been conducted to assess the efficacy of the binary combination of DE with several entomopathogenic fungi (EPFs), such as *Beauveria bassiana* (Balsamo) (Hypocreales: Clavicipitaceae) (Rizwan et al. 2019) and *Trichoderma harzianum* Rifai (Hypocreales: Hypocreaceae) (Abdelgaleil et al. 2021). Combinations of natural agents with different modes of action, such as DE and EPF, can provide an effective approach to control of storage pests by decreasing toxic residues in products, lowering application doses, and boosting the efficacy of these agents (Gad et al. 2021).

Entomopathogenic fungi are promising alternative agents with their high virulence against stored product insect-pests that can be applied instead of chemicals. They get attention owing to the fact that it does not have any residual activity in stored items and is safe for human health and non-target organisms (Wakil and Ghazanfar 2010). Many biological control agents such as *B. bassiana* and *Trichoderma* strains are commonly used against such devastating stored-grain pests. Although *B. bassiana* has become a well-studied EPF against many storage pests (Karaborklu 2022), but on contrary a few studies have been conducted to assess its virulence against *C. maculatus* (Sewify et al. 2014; El Khoury et al. 2022). As for *Trichoderma* strains, although they are known to be a biocontrol agent against some plant pathogens, but few studies have been reported evaluating the insecticidal efficacy of *Trichoderma* strains against *C. maculatus* as well as some storage pests (Abdelgaleil et al. 2021).

The application of binary combinations of DE and EPFs can assist to minimize the high DE treatment rates which are required to obtain satisfactory results in stored pest control. EPF applications combined with low doses of DE have been shown to increase the efficacy of these materials/agents against *C. maculatus* (Gad et al. 2021). However, a limited study on the application of this strategy for the management of this insect has been conducted. Therefore, the present study aimed to determine the efficacy of EPFs [*B. bassiana* and *Trichoderma asperellum* Samuels (Hypocreales: Hypocreaceae)] alone or in combination with DE against *C. maculatus*. Additionally, the progeny production of the pest on chickpea in the different treatments was evaluated.

Methods

Insect collection and rearing

Callosobruchus maculatus was obtained from natural infested chickpea seeds stored at the Department of Field Crops, Faculty of Agriculture, Ondokuz Mays University, Samsun, Turkey. Before beginning the experiments, chickpeas were kept at 20 °C for two weeks to eliminate of other pests. The chickpea moisture content (10.9%) was measured by the Grain Moisture Meter Wile 55 (Farmcomp Oy, Finland). The adults of *C. maculatus* were placed in glass jars (500 ml) containing 200 g of sterilized chickpea (*Cicer arietinum* L. var. Kocbasi) seeds and covered with a cloth to enable air to enter. The glass jars were kept in conditions of 25 ± 1 °C and 65 ± 5% RH, and 16:8 h light-to-dark periods for laying eggs. Every day, the culture was sieved to obtain the population of male and female adults (Ozdemir et al. 2020).

DE formulation

K14 coded diatomaceous earth was created by combining four native diatom soils from different provinces of Turkey's Central Anatolian Region in certain proportions as described by Bayram (2018) in detail for some of the physical and chemical features of K14.

Fungal cultures

First fungus used in this study, *B. bassiana* (isolate: TR-55-006) (Bb), was isolated from adults of *Anisandrus dispar* Fabricius (Coleoptera: Curculionidae: Scolytinae), which is one of the important hazelnut pests, and this isolate was molecularly characterized (GenBank accession no: MN588120) (Kushiyev et al. 2022). The other fungus, *T. asperellum* (isolate: T-11-25) (Ta), was obtained from soil samples collected from the Black Sea Region of Turkey and was molecularly characterized (GenBank accession no: MT341772) (Kushiyev et al. 2021). Both fungal isolates have been stored in the entomology laboratory collection of Ondokuz Mayıs University, Faculty of Agriculture in Samsun, Turkey. The fungal strains were cultured, and the spore suspension was prepared by following the studies of Abdelgaleil et al. (2021). The fungal spores were adjusted at the rates of 1×10^4, 1×10^6 and 1×10^8 spores/mL, using Neubauer hemocytometer, under Olympus CX31 light microscope (Olympus America Inc., Lake Success, NY), and these rates were also used in bioassays.

Bioassays

Bb and Ta at three rates [1×10^4, 1×10^6 and 1×10^8 spores/kg) and DE at three rates (200, 400 and 800 mg/kg) were applied individually and in combination against *C. maculatus*. These application rates for DE were chosen by taking into account the detrimental impacts of high-rate applications (1000–3500 kg/mg). The three distinct rates of fungal isolates mentioned above were chosen to monitor their overall efficacy and evaluate the difference. A grain mixing technique applied by Abdelgaleil et al. (2021) was used for binary combinations. The binary combinations of each DE application rate with the rate (1×10^8 spores/kg) of Bb and Ta were also evaluated. The 50 g of chickpea seeds (var. Kocbasi) with a 10.9% moisture content that were clean and free of infestation were placed into 200 ml glass jars for this purpose. Each glass jar containing 50 g of chickpea seeds was treated with 50 µl of the spore suspension corresponding to the rate at which Bb and Ta were applied. The treated chickpea seeds were then manually shaken for 2 min in glass jars to achieve an even distribution of fungal spores in the mass of chickpea seeds. The jars were kept for 30 min before releasing adults of *C. maculatus* or, treating with DE in the case of combination. For DE, 50 g of chickpea seeds were placed in glass jars and immediately combined with DE at concentrations of 200, 400, and 800 mg/kg. The jars were then manually shaken for 5 min to ensure that the DE was uniformly distributed throughout the mass of the chickpea seeds before releasing insects. Total 15 treatments (9 individual treatments and 6 combination treatments) were conducted as demonstrated in the (Table 1). Chickpea seeds in control jars were also treated with 50 µl distilled water (Abdelgaleil et al. 2021). After chickpea seed treatment, 10 *C. maculatus* adults (5 ♂ + 5♀) at ≤ 24 h-old were placed into each glass jar separately. All jars were placed into the KBWF 240 incubator for incubation at 25 ± 1 °C and 65 ± 5% RH, and 16:8 h light/dark. Each treatment in the experiments had four replications. Mortalities were recorded for 8 successive days to ensure the independence of each day's observations from each other (Ozdemir et al. 2020). All treatments in the trial were replicated by using the same number of different individuals (n = 40 insects/day/isolate/rate) for each day and after assessing the mortalities of the insects of the relevant day on each counting day, the insects and

Table 1 Concentrations applied in individually and binary combination of diatomaceous earth (DE), *Beauveria bassiana* (Bb), *Trichoderma asperellum* (Ta) used in this study

Diatomaceous earth (mg/kg)	*B. bassiana* (spores/kg)	*T. asperellum* (spores/kg)	Diatomaceous earth (mg/kg) + *T. asperellum* (spores/kg)	Diatomaceous earth (mg/kg) + *B. bassiana* (spores/kg)
200 (DE1)	1×10^4 (Bb1)	1×10^4 (Ta1)	$200 + 1 \times 10^8$ (DE1 + Bb3)	$200 + 1 \times 10^8$ (DE1 + Ta3)
400 (DE2)	1×10^6 (Bb2)	1×10^6 (Ta2)	$400 + 1 \times 10^8$ (DE2 + Bb3)	$400 + 1 \times 10^8$ (DE2 + Ta3)
800 (DE3)	1×10^8 (Bb3)	1×10^8 (Ta3)	$800 + 1 \times 10^8$ (DE3 + Bb3)	$800 + 1 \times 10^8$ (DE3 + Ta3)

jars corresponding to that day were removed from the experiment (Robertson et al. 2007). For the control groups, the same method was followed.

Progeny production

All adults (alive and dead) were removed from the jars after 8 days of treatments in the trial. The jars were then placed into the incubator and maintained for an additional 40 days under the same conditions as before. After the completion of 40 days, the jars were checked for the emergence of F1 progeny of *C. maculatus*, and the adults that emerged were counted (Parkin 1956).

Statistical analyses

When the pathogenicity testing mortality rates exceeded 5%, the data were corrected using Abbott's formula (Abbott 1925). The log-probit algorithm with the Probit analysis program (POLO-PLUS ver. 2.0) was used to calculate independent-time mortality data statistics from the bioassays, which were reported as 50% lethal time (LT_{50}) and 90% lethal time (LT_{90}). The slopes of the regression lines were compared using standard errors, and the isolates' and/or DE' LT_{50} and LT_{90} values were compared using confidence intervals (95%). The data on progeny production were analyzed using one-way analysis of variance (ANOVA) in Minitab 17.0 statistical software, and the means were separated using the pair-wise multiple comparison analysis with the Tukey's test, with differences at $P<0.05$ considered significant.

Results

Lethal time and mortality rate of *C. maculatus* on treated chickpea seeds

The LT_{50} and LT_{90} values and mortality rates of *C. maculatus* adult after 8 days of exposure to chickpea seeds, treated with DE, Bb and Ta alone and their binary combination, are shown in (Table 2 and Fig. 1). The lowest LT_{50} and LT_{90} values to DE alone applied to chickpea seeds at three different rates against the adult beetles were 3.19 and 5.02 days at the highest concentration (800 mg/kg), respectively, and it was statistically different from other applications of DE ($P<0.05$) (Table 2). After 4 and 7 days of exposure, this application resulted in 75 and 100% mortality rate in *C. maculatus*, respectively (Fig. 1). During the applications of Bb alone, the lowest LT values were determined for the highest concentration (Table 2) and it caused 95% mortality at the end of 7[th] (Fig. 1). The LT_{50} and LT_{90} values were not determined because of the extreme low mortality rates for the Ta alone applications (200 and 400 mg/kg) that were the result (Table 2 and Fig. 1). These values in the highest concentration were 8.23 and 13.76 days, respectively, and a mortality rate of less than 50% in the insect population after 7 days of exposure was determined. In the binary combinations of DE and Bb, the lowest LT values (days) were 2.97 (LT_{50}) and 5.45 (LT_{90}) to DE3+Bb3 combination, respectively. Starting on the first day of exposure, this concentration resulted in 45% mortality on the third day and 100% death on the sixth day. Similarly,

Table 2 Probit analysis data on mortality time (days) of *Callosobruchus maculatus* after applied on chickpea seeds at different rates of EPFs [*Beauveria bassiana* (Bb) and *Trichoderma asperellum* (Ta)] alone or in combination with diatomaceous earth (DE)

Treatment (Concentration, mg/kg or spores/kg)	LT_{50} (95% CI)	LT_{90} (95% CI)	Slope ± SE	Regression	X^2	Df	Heterogeneity
DE1 (200)	5.53(4.80–6.22) c[a]	8.10(7.02–11.18) bc[a]	7.74 ± 1.0	y = -5.75 + 7.74x	12.44	30	2.07
DE2 (400)	4.46(3.68–5.07) bc	7.44(6.36–10.27) b	5.77 ± 0.79	y = -3.75 + 5.77x	9.80	30	1.63
DE3 (800)	3.19(2.84–3.48) a	5.02(4.56–5.72) a	6.52 ± 0.85	y = -3.29 + 6.52x	3.32	30	0.66
Bb1 (1×10^4)	6.24 (5.73–6.71)[a]c	8.41(7.62–10.35) b	9.87 ± 1.55	y = -7.85 + 9.87x	6.77	30	1.12
Bb2 (1×10^6)	5.65(5.06–6.17) c	7.98(7.13–9.98) b	8.55 ± 1.17	y = -6.44 + 8.55x	8.57	30	1.42
Bb3 (1×10^8)	5.45(4.81–5.98) c	7.2(6.49–8.86) b	10.6 ± 1.35	y = -5.08 + 6.76x	12.11	30	2.01
Ta1 (1×10^4)	-b	-b	10.50 ± 2.79	y = -9.47 + 10.50x	10.23	30	1.70
Ta2 (1×10^6)	-b	-b	7.58 ± 2.23	y = -6.91 + 7.58x	8.73	30	1.45
Ta3 (1×10^8)	8.23(7.39–10.36) d	13.76(10.74–26.48) c	5.74 ± 1.36	y = -5.26 + 5.74x	5.27	30	0.87
DE1 + Bb3	4.38 (4.09–4.61) b	5.52 (5.21–6.05) a	12.71 ± 2.01	y = -8.15 + 12.71x	11.30	30	0.37
DE2 + Bb3	4.27 (3.96–4.52) b	5.47 (5.14–6.02) a	11.96 ± 1.95	y = -7.55 + 11.96x	16.50	30	0.55
DE3 + Bb3	2.97(2.41–3.39) a	5.45(4.76–6.79) ab	4.87 ± 0.68	y = -2.31 + 4.87x	42.71	30	1.42
DE1 + Ta3	5.68 (5.29–5.98) c	7.36 (6.91–8.21) b	11.39 ± 1.94	y = -8.60 + 11.39x	21.15	30	0.70
DE2 + Ta3	5.01(4.41–5.45) bc	6.69(6.09–8.03) b	10.23 ± 1.47	y = -7.16 + 10.23x	8.98	30	1.49
DE3 + Ta3	4.14(3.34–4.66) ab	6.17(5.38–8.65) ab	7.38 ± 1.16	y = -4.56 + 7.38x	8.19	30	1.63

[a] Within columns, means followed by the same lower-case letters do not differ significantly at $p \leq 0.05$

[b] Because of the extremely low mortality rates, LT_{50} and LT_{90} values were not be calculated

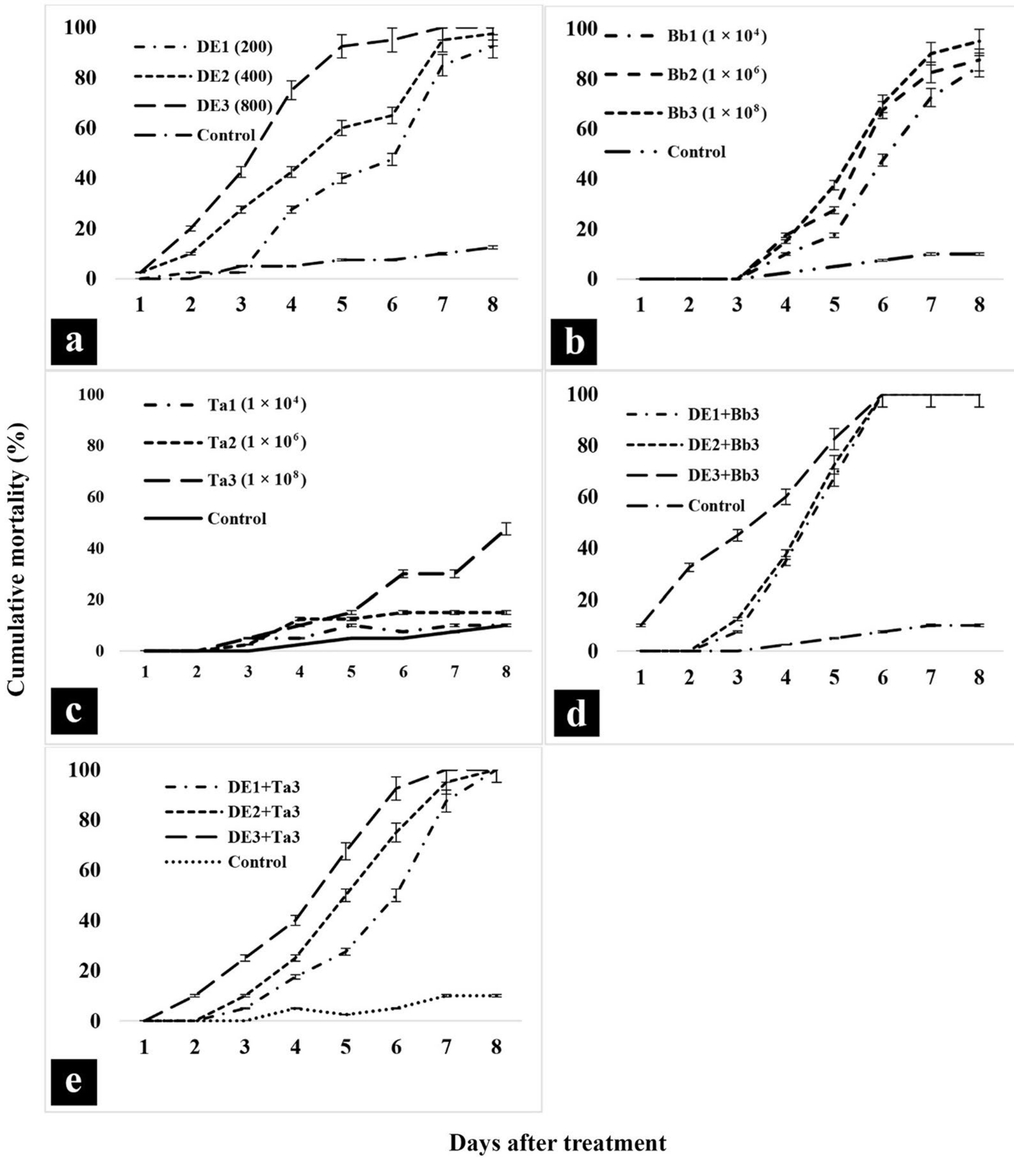

Fig. 1 Mortality rates of *Callosobruchus maculatus* on chickpea seeds treated with **a** DE at 200, 400, 800 mg/kg; **b** *Beauveria bassiana* at 1×10^4, 1×10^6, 1×10^8 spores/kg; **c** *Trichoderma asperellum* at 1×10^4, 1×10^6, 1×10^8 spores/kg; **d** *Beauveria bassiana* at 1×10^8 spores/kg in combination with DE at 200, 400, 800 mg/kg; **e** *Trichoderma asperellum* at 1×10^8 spores/kg in combination with DE at 200, 400, 800 mg/kg

the lowest LT_{50} and LT_{90} values to the highest concentration the binary combinations of DE and Ta were 4.14 and 6.17 days, respectively, and mortalities began on the second day of exposure, reached 40% by the fourth day, and reached 100% by the end of the seventh day (Table 2 and Figs. 1, 2).

The effect of applications on *C. maculatus* progeny production

Applications in individually and binary combinations of Bb, Ta with DE caused significant decrease in progeny production after 40 days of treatment than the progeny production in the control of the insect (263.5 ± 26.3) (Table 3). The lowest progeny production was observed at the highest application concentration of binary (800 mg/kg of DE + 1×10^8 spores/kg of Bb).

Discussion

The present study showed that the mortalities of *C. maculatus* adults in chickpea seeds treated with binary combination of DE with Bb or Ta were significantly superior to alone treatments after 6 and 7 days.

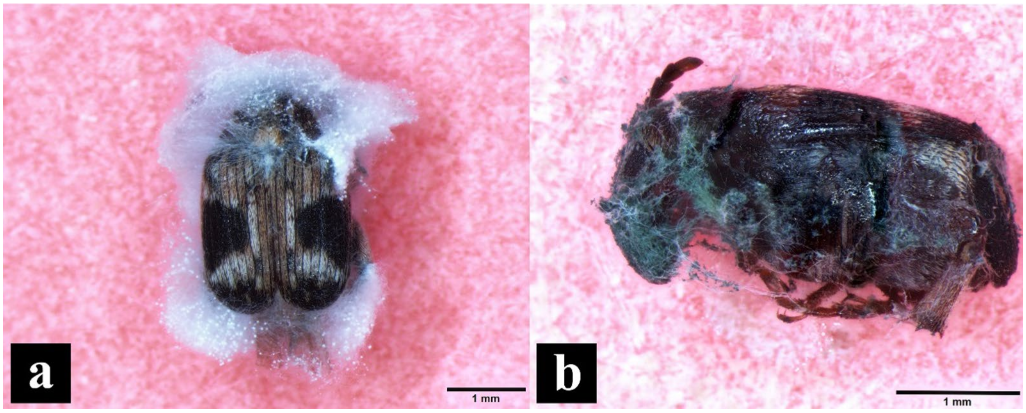

Fig. 2 Fungal colonization of *Beauveria bassiana* (**a**) and *Trichoderma asperellum* (**b**) isolates on the body surface of *Callosobruchus maculatus* adults 8 days after inoculation

Table 3 Mean progeny production (±SE) and mean reduction (%) of *Callosobruchus maculatus* on chickpea seeds treated with diatomaceous earth (DE), *Beauveria bassiana* (Bb) and *Trichoderma asperellum* (Ta), applied individually or in combination at different application rates after 40 days of treatment

Treatment (Concentration, mg/kg or spores/kg)	Progeny production (±SE) after 40 days	
	No of progeny/50 g chickpea	Progeny reduction (%)
Control (0.0)	263.5 ± 26.3 a[a]	0.0
DE (200)	132.0 ± 23,6 bc	49.9
DE (400)	90.0 ± 12,9 def	65.8
DE (800)	55.8 ± 23.6 fg	78.8
Bb1 (1×10^4)	59.0 ± 12.9 efg	77.6
Bb2 (1×10^6)	48.7 ± 13.0 g	81.5
Bb3 (1×10^8)	32.7 ± 8.6 g	87.5
Ta1 (1×10^4)	140.7 ± 17.9 b	46.6
Ta2 (1×10^6)	114.0 ± 18.5 bcd	56.7
Ta3 (1×10^8)	95.5 ± 16.8 cde	63.7
DE (200) + Bb3	49.5 ± 13.3 g	81.2
DE (400) + Bb3	38.0 ± 10.0 g	85.5
DE (800) + Bb3	29.2 ± 3.5 g	88.9
DE (200) + Ta3	51.0 ± 4.0 fg	80.6
DE (400) + Ta3	45.5 ± 12.9 g	82.7
DE (800) + Ta3	42.5 ± 11.8 g	83.8
F	58.6	
P	< 0.000	

[a] Values within each column followed by the same letter are not significantly different ($P < 0.05$)

The insecticidal efficiency of DE in combination with several EPFs was evaluated against some stored grain insect pests (Gad et al. 2020) as well as *C. maculatus* (Gad et al. 2021). The findings of the study are consistent with previous studies on the efficacy of binary (DE + EPF) combinations against stored grain insects. For example, Vassilakos et al. (2006) combined the DE with *B. bassiana* and determined that the mortalities of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) were increased in binary combination applied on stored wheat. Also, Batta (2004) reported that the DE improved the insecticidal activity of *Metarhizium anisopliae* (Metschnikoff) Sorokin (Hypocreales: Clavicipitaceae) against *S. oryzae* adults on treated wheat. Riasat et al. (2011) found that highest mortality (79.8%) of *R. dominica* was observed when applying to wheat upon binary combination of *B. bassiana* (2.23×10^9 conidia/kg) with DE (400 mg/kg). Gad et al. (2020a) concluded that application of a binary combination of *T. harzianum* (Th) (2.1×10^7 spore/kg) with DE (800 mg/kg) was highly effective against *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae), with 93.9% mortality after 7-days exposure. Our findings also showed that combining the binary mixture (Bb and Ta with DE) resulted in higher mortality than individual treatments, as well as lower LT values. Similar findings were reported by Khoobdel et al. (2019) who noticed that the $LC_{50}^{Bb} + LC_{50}^{DE}$ combination, which is the highest concentration, resulted in mortality rates of 90–95% for *C. maculatus*, following a 10-day exposure. In a study conducted by Abdelgaleil et al. (2021), it was shown that binary combinations of just DE (1000) + Th (1×10^7) ve kaolin (KA) (1000) + Th (1×10^7) caused 100% mortality, demonstrating the highest efficiency, 7 days after application against *C. maculatus* and *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae) in individual and combination of Th at three application doses (1.0×10^5, 1.0×10^6 and 1.0×10^7 spores/kg) and inert dusts such as DE and KA (100, 500 and 1000 mg/kg) at three application doses. A parallel study of Gad et al. (2021) stated that the using of DE, spinosad (SP), Th alone showed lower effectiveness against *C. maculatus and C. chinensis* than their binary and ternary combinations treatments. Binary combination of DE (500 mg/kg) + Th (1×10^6 spores/kg) caused 83.1 and 85.7% mortality of both insects, while the highest application rate of ternary combination (DE (500 mg/kg) + SP (0.5 mg/kg) + Th (1×10^6 spores/kg)) resulted in 100% mortality of both insects. Furthermore, it was emphasized that binary and ternary combinations including DE were more successful than others, and that DE was the most effective substance in these mixtures (Gad et al. 2021). Similarly, when the DE combinations used in the present investigation were compared to the individual treatments of Bb and Ta, all applications resulted a considerable decrease in LT values, resulting in 100% mortality between the sixth and eighth days after the applications.

Obtained findings demonstrated that all individual and combination applications of DE, Bb, and Ta against *C. maculatus* considerably decreased the pest's progeny production after 40 days of treatments. Progeny production was dramatically reduced in binary combination treatments and at their higher doses. It was concluded from several studies that using inert powders and EPF to various storage pest beetles have reduced the progeny production, which consistents with our findings. For example, Gad et al. (2020) demonstrated that individual and binary mixture treatments of DE, Th reduced *A. obtectus* progeny production after 60 days, particularly at higher mixture rates. Abdelgaleil et al. (2021) found that binary treatments at the highest doses of DE (1000 mg/kg) + Th (1×10^7 spores/kg) and KA (1000 mg/kg) + Th (1×10^7 spores/kg) dramatically reduced/prevented progeny production of *C. maculatus* and *C. chinensis* at rates of 90.4–95.6 and 85.8–100%, respectively, after 45 days of treatments. Similar results were reported in a research using binary and ternary combinations of DE, SP, and Th against the same insects, and it was noted that the decrease in progeny production might be attributable to the quick mortality of insects, following the treatments (Gad et al. 2021). These results are consistent with our study, which indicated that progeny poaching reduced with increasing dosage and fall by nearly 90% in the combined treatment of DE (800 mg/kg) + BB (1×10^8 spores/kg). Furthermore, it was emphasized by Athanassiou et al. (2005) that the decrease in progeny was more noteworthy than the death rate of adults exposed to the treated stored items, because grain protectants needed to safeguard these products during long storage periods.

Conclusion

In the present study, individual and binary combinations of Bb, Ta, and DE were tested. The treatments used Bb or Ta with DE resulted in increased insecticidal effectiveness against *C. maculatus*. These natural agents caused considerable decreasing of progeny production of the pest. Even with reduced application rates, the agents with a promising potential against the pest showed acceptable results in binary combinations. This promising approach, may help to reduce the application rates of the DE used in this study, could promote more investigations to use practically against *C. maculatus* under long-term storage conditions.

Abbreviations

DE	Diatomaceous earth
EPF	Entomopathogenic fungi
Bb	*Beauveria bassiana*
Ta	*Trichoderma asperellum*
Th	*Trichoderma harzianum*
KA	Kaolin
SP	Spinosad
LT	Lethal time
LT_{50}	50% lethal time
LT_{90}	90% lethal time

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Author contributions

IOO designed the study, and wrote the manuscript. IOO carried out the experiments and analyzed the data. The author 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.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The author declares that they have no competing interests.

Author details

[1]Department of Plant Protection, Faculty of Agriculture, Sakarya University of Applied Sciences, 54580 Arifiye, Sakarya, Turkey.

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