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Effect of the entomopathogenic fungus, *Beauveria bassiana*, combined with diatomaceous earth on the red flour beetle, *Tribolium castaneum* (Herbst) (Tenebrionidae: Coleoptera)

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Abstract

An assay was designed to evaluate the efficacy of each entomopathogenic fungus, *Beauveria bassiana*, and diatomaceous (DE) and their combinations at concentrations of 1×10^6 and 1×10^8 conidia kg^{-1} of wheat and 200 and 400 ppm, respectively, on the red flour beetle, *Tribolium castaneum* (Herbst) (Tenebrionidae: Coleoptera), infesting wheat. Percent mortality of *T. castaneum* was recorded after 7, 14, and 21 days. Percent mycosis on dead cadavers was assessed after 21 days. Alive adults were removed from plastic jars after 21 days and kept for the next 60 days to assess the progeny production. The results indicated that the highest concentrations of *B. bassiana* and DE in their combinations were more effective for the virulence and progeny suppression of *T. castaneum*. Maximum mean percent mortality (88.13%) was recorded by *B. bassiana* (1×10^8 conidia kg^{-1} of wheat) and DE (400 ppm) in their combination after a 21-day exposure interval, while a minimum percent mean mortality (10.00%) was recorded by *B. bassiana* (1×10^6 conidia kg^{-1} of wheat) alone. The maximum percent mycosis (78.89%) on dead cadavers was recorded at a low concentration rate (1×10^6 conidia/kg) of *B. bassiana*. Mean progeny adult emergence was the highest (62.67 adults) at the low concentration of *B. bassiana* alone. Present studies showed that *B. bassiana* and DE are more effective in combination against *T. castaneum* on wheat as both substances are advantageous.

Keywords: *Beauveria bassiana*, Diatomaceous earth, *Tribolium castaneum*, Wheat grains, Virulence, Progeny

Background

The red flour beetle, *Tribolium castaneum* (Herbst) (Tenebrionidae: Coleoptera), is among the most destructive insect pests of stored products all around the world (Mahroof and Hagstrum 2012). Both larvae and adults cause damage to the grains. Studies showed that insects cause about 5–10% of world's grain production losses. These losses extend to 50% in sultry countries where heat and moisture run high during summer season (Ahmad and Ahmad 2002).

The insecticides are a common and effective tool acting as grain protectants against stored products' insect pests in Pakistan and worldwide. However, with the presence of insecticide resistance in insects, increasing demands for residue-free food products and environmentally safe practice, attempts have been made to find non-toxic and environmentally safe alternative protectants.

The possible use of entomopathogenic fungi (EPF), as a non-chemical alternative to traditional chemical products for cereal grains, has acknowledged increased attention during the recent years (Moore et al. 2000 and Lord 2001). EPF have a reuse capacity as they persist on the product and could recycle in the cadavers under certain environmental conditions, reestablishing further inoculum

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in the grains. Hence, while persistence is considered as a disadvantage in the case of customary grain protectants, it is an advantageous property in the case of fungi (Stathers 2002).

Diatomaceous earth (DE) is one of the known non-chemical substances and the most promising alternative to systematic use of insecticides (Vayias and Stephou 2009). DE is a naturally occurring non-toxic, non-chemical, and safe substance for controlling the stored grain insect pests with a unique mode of action for bruising and desiccation (Subramanyam and Roesli 2000). There are commercially available formulations of DE that have been proved effective for a number of insect species with different concentrations on various stored grains. However, the efficacy of DE often varies with the formulations, treatment methodology, treated commodity, and other factors (Athanassiou et al. 2007 and 2008).

Both DE and EPF as grain protectants have different mechanisms of action and affect the insect cuticle, so their use in combination has been proposed by scientists as means of minimizing the applied doses (Akbar et al. 2004). Akbar et al. (2004) testified that the blend of *Beauveria bassiana* with the diatomaceous earth had a synergistic effect and better results against the larvae of *T. castaneum*. Furthermore, since both substances EPF and DE persist on the grains, it appears that such a mixture may have a certain gain as a durable and long-term protectant. Recent studies indicated that *Sitophilus oryzae* (L.), *T. castaneum*, and *Rhyzopertha dominica* (Fab.) are susceptible to *Metarhizium anisopliae* (Metsch.) Sorokin, *B. bassiana*, and DE (Michalaki et al. 2006).

In the present study, the possibility of a long-term safety of wheat grains against *T. castaneum*, through the collective use of *B. bassiana* and DE, was assessed. In addition to parental adult mortality, the rate of mycosis and progeny production of *T. castaneum* on the treated kernels was evaluated.

Materials and methods

Insect culture

Adults of *T. castaneum* were obtained from the culture that reared in the laboratory on wheat flour at 28 ± 2 °C and 65–75% R.H. The cultures were kept at the stored grain management cell, Department of Entomology, University of Agriculture, Faisalabad, Pakistan. All adult insects used in these tests were 6–7 days old.

Grains

Untreated, uncontaminated, and infestation-free grains of wheat (var. Galaxy) were used in the tests. Wheat grains were obtained from the 2017 harvest. The grain moisture content, as determined by the Grain Moisture Tester RICETER f505 (Kett Electrical Laboratory, Tokyo, Japan), was 11.7%. The grains were held at an ambient condition for 7 days to equilibrate to the desired RH before use in the experiments.

Fungal formulations

The strain of *B. bassiana* isolate used in this study was obtained from dead cadavers of rice leaf folder, *Cnaphalocrocis medinalis* (Guenée) (Rizwan et al. 2019). The fungus was then sub-cultured on Potato Dextrose Agar (PDA) plates for bulk generation of the fungal conidia. For bulk conidial production, plates were incubated for 14 days at 20 ± 1 °C and 16 h light per day. Then, the conidia were collected by scraping the conidial layers developed on the plate surface by a sterilized scalpel. The collected conidia were mixed into 100 ml sterile distilled water and filtered through muslin cloth. The fungal conidia and dust carrier were mixed at 1:4 ratio for formulation preparation (Kavallieratos et al. 2006). The conidia collected from strain were comprehensively mixed with the inert carrier ash in screw-capped bottles. Fungal conidia concentration was determined in the conidial suspension, using a hemocytometer. Two concentrations of *B. bassiana* were prepared, containing 1×10^6 and 1×10^8 conidia kg^{-1} of wheat grains (Michalaki et al. 2006 and Kavallieratos et al. 2006).

DE formulation

The DE formulation Diafil 610, used in this study, was manufactured by Celite Corporation (Lompoc, CA, USA). It contains 89% amorphous SiO_2, 4.0% aluminum oxide (Al_2O_3), 1.7% iron oxide (Fe_2O_3), 1.4% CaO, < 1% MgO and K_2O, and 3% moisture. This DE was used at concentrations of 200 and 400 ppm (equivalent to 0.20 and 0.40 g/kg of wheat grains, respectively).

Grain treatment

Eight concentrations of EPF, DE, and their combinations were tested, i.e., the lowest concentration (1×10^6 conidia kg^{-1} of wheat) of the fungus alone, the highest concentration (1×10^8 conidia kg^{-1} of wheat) of the fungus alone, the lowest concentration (200 ppm) of DE, the highest concentration (400 ppm) of DE alone, and the combinations of low fungal concentration (1×10^6 conidia kg^{-1} of wheat) + low DE concentration (200 ppm), low fungal concentration (1×10^6 conidia kg^{-1} of wheat) + high DE concentration (400 ppm), high fungal concentration (1×10^8 conidia kg^{-1} of wheat) + low DE concentration (200 ppm), and high fungal concentration (1×10^8 conidia kg^{-1} of wheat) + highest DE concentration (400 ppm). For each grain treatment replication, lots of 1000 g wheat grains were organized and the particular quantity of fungus (1 g for each concentration corresponding to 1×10^6 and 1×10^8 conidia kg^{-1} of wheat)

and DE (0.20 and 0.40 g) were added. These lots were introduced in plastic jars (24 × 14 × 14 cm), and the jars were shaken manually for approximately 5 min to attain an equal dispersal of the dust on the whole grain mass. There was an additional untreated lot which served as control. All jars were kept in a laboratory at 28 ± 2 °C and 65 ± 5% RH during the whole experimental period.

Bioassays

The post treatment efficacy for F_1 was observed for the time period of 2 months. Wheat batches of 1 kg were used to apply different treatments, viz., *B. bassiana* alone, DE alone, and their combinations. Nine samples (eight treatments and one control), each of 50 g, of wheat were used in this experiment. Each sample was placed in a cylindrical plastic jar (24 × 14 × 14 cm) with a top covered with muslin cloth for aeration, and 40 adults of *T. castaneum* were introduced into each jar. These jars were placed in a laboratory at room temperature and 65 ± 5% RH (Kavallieratos et al. 2006). The desired RH in the laboratory was maintained by using a humidifier. The number of dead adults was counted after (7, 14, and 21 days) in treated and untreated jars. The adults were classified as dead (unable to move even with stimulus) and alive (moving/or showing signs of activity). Thus, the data for mortality was recorded. The adults were removed from the jars after final observation. The mycosis data were recorded from the dead cadavers of *T. castaneum* that were collected from each treatment upon mortality assay termination. These cadavers were washed (two to three times) by 0.05% sodium hypochlorite solution, followed by three to four washings in distilled water, and then placed on PDA plates. These were incubated at 25 ± 2 °C and 75 ± 5% RH for 1 week and then observed under a microscope for white fungal growth.

Progeny production counts

The dead and alive adults were removed from the jars, and these jars were placed undisturbed for another 60 days to ascertain the progeny emergence. After the completion of 60 days, the emerged adults were counted in each jar.

Data analysis

The counts for mortality rates were converted into percent and then analyzed using Statistix 8.1 software; however, the control mortality was very low and was not included in the analysis. Data were analyzed in three factorial CRD design, and the means were separated using the Bonferroni test at $P = 0.05$.

Results and discussion

Mortality of *T. castaneum*

Main effects of different EPF and DE (alone and in combination), exposure intervals, and concentrations on percent mortality of *T. castaneum* were highly significant ($P < 0.05$) (Treatments: $F_{3, 71} = 602.71$, Exposure intervals: $F_{2, 71} = 551.46$, Concentrations: $F_{1, 71} = 178.32$). Moreover, highly significant effects of interactions among Treatment × Exposure intervals, Treatment × Concentrations, and Exposure intervals × Concentrations were also observed for percent mortality ($P < 0.05$) (Treatment × Exposure intervals: $F_{6, 71} = 18.73$, Treatment × Concentrations: $F_{3, 71} = 4.90$, Exposure intervals × Concentrations: $F_{2, 71} = 6.29$). Non-significant effects of interaction among Treatment × Exposure intervals × Concentrations were recorded for percent mortality ($P > 0.05$) ($F_{6, 71} = 1.43$).

Main effects of different concentrations of EPF alone and mixed with DE on percent mycosis recovered from dead cadavers of *T. castaneum* were highly significant ($P < 0.05$) (Treatments: $F_{2, 71} = 106.19$, Concentrations: $F_{1, 17} = 15.69$). Moreover, non-significant effects of interaction among Treatment × Concentrations were recorded for percent mycosis ($P > 0.05$) ($F_{2, 17} = 1.43$).

Main effects of different EPF and DE (alone and in combination) and concentrations on progeny production of *T. castaneum* were highly significant ($P < 0.05$) (Treatments: $F_{3, 35} = 30.03$, Concentrations: $F_{2, 35} = 1353.83$). Moreover, highly significant effects of interaction among Treatment × Concentrations were recoded for progeny production ($P < 0.05$) ($F_{6, 35} = 7.63$).

Mortality of *T. castaneum* in all treatments was significantly different from each other. Long exposure interval and the highest concentration had a positive effect on the mortality rate of *T. castaneum*. The combinations gave better results than single concentrations of EPF and DE. EPF-treated wheat alone gave a maximum mortality rate (31.67%) at the highest concentration (1×10^8 conidia kg^{-1} of wheat) as compared to 24.17% at the lowest concentration (1×10^6 conidia kg^{-1} of wheat). In the same way, the highest concentration of DE (400 ppm) alone gave more mortality (49.17%) than the lowest concentration (200 ppm) after a 21-day exposure interval. Similarly, combinations of EPF and DE gave maximum mortality percent (88.33%) at maximum concentration (1×10^8 conidia kg^{-1} of wheat + 400 ppm DE) after 21-day exposure time. The combinations of the lowest concentrations of EPF and DE gave less percent mortality as compared to higher concentration at combinations, but the level of *T. castaneum* mortality was high as compared to DE and the EPF alone (Fig. 1).

Mycosis and progeny production

The maximum percent mycosis (78.89%) was recorded in the treatment of low concentration of fungal pathogen

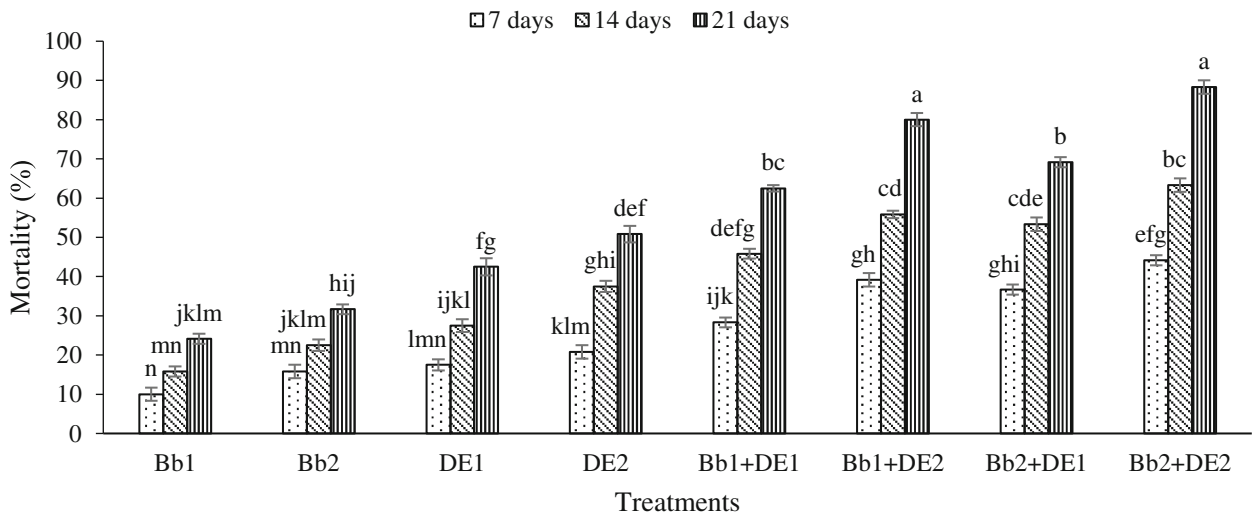

Fig. 1 Percent mortality (mean ± SE, $n = 3$) of *Tribolium castaneum* in treated wheat with two concentrations of *Beauveria bassiana* (1×10^6 and 1×10^8 conidia kg^{-1} of wheat) and DE (200 and 400 ppm) (alone and in combination) at weekly intervals

(1×10^6 conidia/kg) and DE (200 ppm), while the minimum percent mycosis (31.21%) was recorded in the treatment of higher dose combination of EPF (1×10^8 conidia/kg) with DE (400 ppm) (Fig. 2).

Maximum progeny production of *T. castaneum* (62.67 adults) in wheat grains treated with EPF and DE (alone and in combination) was recorded due to low concentration of EPF (1×10^6 conidia/kg) alone, while minimum progeny production (2.67 adults) was recorded at high concentration of EPF (1×10^8 conidia/kg) and DE (400 ppm), used in combination (Fig. 3).

EPF had been used previously for stored grain pest management with different concentrations at different time intervals. Dry conidial concentrations had different virulence against insect pests of stored grains (Kavallieratos et al. 2006). Based on the present studies, *B. bassiana* proved effectiveness against *T. castaneum*, but efficacy depended upon dose rate and exposure interval. Moreover, the addition of desiccant dust notably increased the effectiveness of EPF, when used in combination. Akbar et al. (2004) reported that the use of diatomaceous earth increased the mortality of *T. castaneum* larvae. Vassilakos et al. (2006) also reported an additive effect of *B. bassiana*, when used with DE against adults of *R. dominica* and *S. oryzae*. Athanassiou et al. (2006) stated that an addictive effect was not dependent

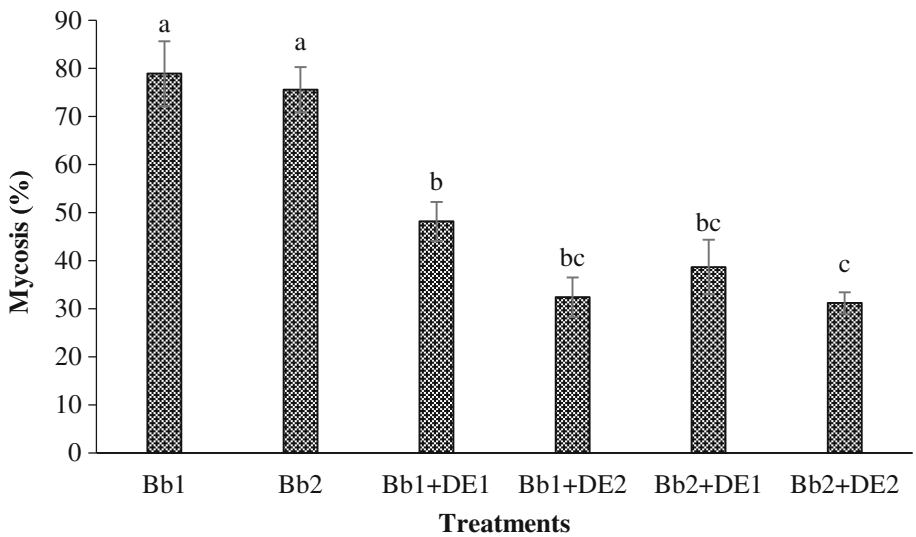

Fig. 2 Percent mycosis (mean ± SE, $n = 3$) in cadavers of *Tribolium castaneum* treated with two concentrations of *Beauveria bassiana* (1×10^6 and 1×10^8 conidia kg^{-1} of wheat) alone and mixed with DE

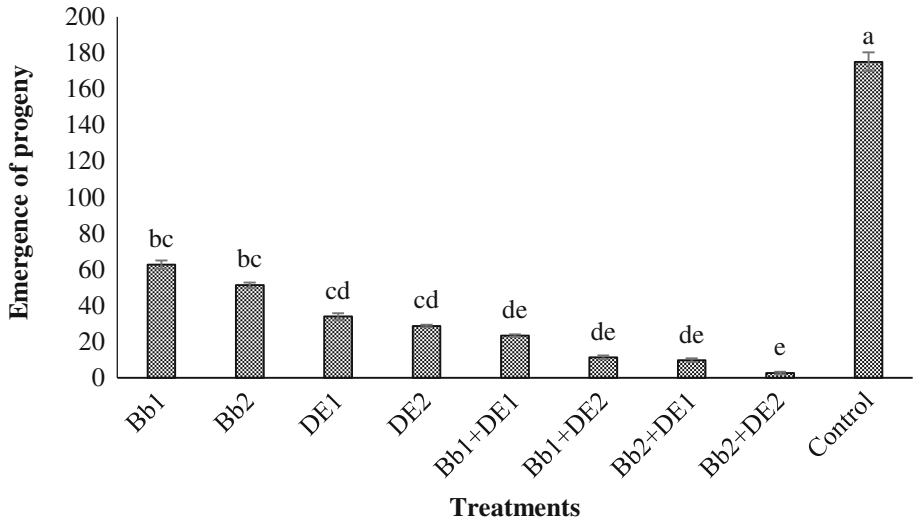

Fig. 3 Progeny production (mean ± SE, $n = 3$) of *Tribolium castaneum* treated with two concentrations of *Beauveria bassiana* (1×10^6 and 1×10^8 conidia kg^{-1} of wheat) and DE (200 and 400 ppm) (alone and in combination)

on the type and formulation of desiccant dust, which means all types of DEs can successfully be used for this purpose. Similarly, virulence of fungal strains differed remarkably against stored grain insect pests (Moore et al. 2000 and Stathers 2002), and addition of synergistic inert material was preferable than mass production of fungal strain. DE formulations produced much better results, and DEs could efficiently be least affected by the environment, which could retain their effectiveness for 8–9 months, when applied on wheat grains (Athanassiou et al. 2005), to 20 months, when applied to maize grains (Stathers et al. 2008). Vayias et al. (2006) also reported similar results for DEs on maize and wheat against *Tribolium confusum*. DEs slowly absorb moisture from the air, which reduce their efficacy (Subramanyam and Roesli 2000), while in case of fungi, spore persistence is an important distinctive characteristic (Thomas et al. 1997 and Stathers 2002). However, conidial activity declined with time (Moore et al. 2000). Batta (2004) found that the conidial viability of fungus decreased with time, but its insecticidal efficacy was not badly affected. This trend has also been reported in case of some greenhouse and field trials and pests such as the rice leaf roller, *C. medinalis* (Rizwan et al. 2019), and the whitefly *Bemisia tabaci* (Gennadius) (Batta 2003), respectively. Fungal pathogens are more advantageous in bio-control cases as these could recycle in cadavers producing conidiospores that are reintroduced into the eco-system (Thomas et al. 1997 and Stathers 2002). A high mortality rate was obtained in wheat in the case of combined use of EPF and DE against *T. castaneum* because it is a mobile pest, hence more chances of abrasion with DEs and picking fungal pathogen. Second, it might be that the use of wheat grains in this study as previous studies reported more efficacy of fungal pathogen and DEs against stored grain insect pest in case of wheat grains (Subramanyam and Roesli 2000; Athanassiou and Kavallieratos 2005 and Kavallieratos et al. 2005). DE efficacy is greatly attributed to the type of grains/cereals used for bioassay studies as wheat grains retain DE particles considerably as compared to maize (Athanassiou and Kavallieratos 2005), while Michalaki et al. (2006) reported a higher survival rate of *T. confusum* Jacquelin du Val larvae on flour rather than treated wheat with *M. anisopliae* and DE. The dead cadavers become the source of secondary infection and increase the efficacy of EPF (Thomas et al. 1997). High percent mycosis at a low dose had been recorded earlier by Tefera and Pringle (2003). This suggests that insects may resort to a less-treated stratum of grains, may oviposit and produce generations, or may have the ability to recover and continue to cause damage. Hence, the destruction of progeny is an equal important task with parental mortality.

Conclusion

This study suggested that a combination of EPF and DE provided a better long-term management of *T. castaneum*. Both substances had a lethal as well as a suppressive effect against progeny (F_1) production of the pest.

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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.

Authors' contributions
Muhammad R, BA, and AMS planned the research and designed the methodology. Muhammad R and BA conducted the experiments. ZUS helped in the data analysis. Misbah R and MH drafted the manuscript. All the authors have read and approved the final manuscript.

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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