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Compatibility of some botanicals and the entomopathogenic fungus, *Beauveria bassiana* (Bals.), against the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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Abstract

Background: The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is a serious pest of stored grain commodities worldwide which results in considerable losses to stored wheat, *Triticum aestivum* (Linn.).

Main body: The present laboratory bioassay was carried out to examine the efficiency of the fungus, *Beauveria bassiana* (Bals.) formulation (RacerTM), and Neem extract (NE), Eucalyptus extract (EE), and Tobacco extract (TE) against 3rd instar larvae of *T. castaneum*. The fungal formulation was applied at 0.3×10^8 , 0.6×10^8 , and 0.9×10^8 conidia kg^{-1} of the crushed wheat grains; as well, it was mixed separately with 5% concentrations of each plant extract under the laboratory conditions of $30 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ RH. Mortality rate of the tested larvae was enumerated after regular intervals of times. Mortality of the larvae increased at the highest combined concentrations of the fungal conidial formulation and the plant extracts rather than their single treatments. The highest mortality rate of the larvae (71.32%) was recorded at the highest concentration binary mixture of (RacerTM + NE), whereas relatively the lowest mortality rate (15.54%) was enumerated in the combined treatments of (RacerTM + NE). Furthermore, the highest separate concentration of *B. bassiana* (0.9×10^8 conidia/ml) persistently resulted in more larval mortality (32.68%) of 3rd instar larvae of *T. castaneum* than the plant extracts (7.52, 9.89, and 14.61%), respectively. A noticeably greater rate of mycosis and sporulation was counted in the larvae of the insect in separate treatments of *B. bassiana* than in its combined applications with the plant extracts. The highest mycosis (85.13%) and sporulation (160.12 conidia/ml) was detected in the treatment, where the lowest concentration of *B. bassiana* (0.3×10^8 conidia/ml) was used, alone.

Conclusion: Hence, it was concluded that integrated use of these two bio-pesticides plus the fungus can be helpful in the integrated pest management program of *T. castaneum*.

Keywords: *Tribolium castaneum*, *Beauveria bassiana*, Eucalyptus, Neem, Mortality, Sporulation

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Background

Stored grains commodities are vulnerable to be attacked by numerous insect pests. Among them, the red Flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is known as main insect pest of stored agricultural cereals and wheat flour worldwide (Buckman et al. 2013). Larval and adult stages of *T. castaneum* feed on damaged grains by other stored grain insects (Sarwar 2015). It is a polyphagous insect pest and typically attacks the stored broken wheat grains, wheat flour, and other grinded cereals (Bosly and Kawanna 2012). The pest adults are very energetic and capable to complete their growth even in less food reserves (Campbell and Runnion 2003).

Control of stored commodities insect pests is typically carried out by traditional synthetic insecticides (Athanasios and Palyvos 2006). In a number of countries, control of stored commodities insect pests is mostly accomplished by grain fumigants (Zettler and Arthur 2000). Fumigation by phosphine is the main approach for the operative control of the insect pests (Chadda 2016). But the application of phosphine fumigation has become restricted as some stored products insect pests like *Trogoderma granarium* Everts have developed resistance against it (Pimentel et al. 2010). To combat the resistance issues in stored grain insect pests, there is utmost desire to move substitute strategies of stored grain insect pest management which are environment-friendly, cost effective, and socially appropriate (Nboyine et al. 2015).

Application of plant-derived materials (i.e., plants powders, extracts, oils) as fumigants for the control of stored commodities insect pests and their products is potential substitute approach for the control of insect pests (Rehman et al. 2018). The plant extracts are operative fumigants and have no hazardous effects to human and animals health (Rajendran and Sriranjini 2008) and are also biodegradable (Negahban et al. 2007). Numerous plants have now been defined for their toxicological properties of which *Azadirachta indica* (A. juss.) is a promising one (Asawalam et al. 2007). The leaf extract of *Azadirachta indica* has been operative for the stored commodities' insect pests mainly for the environment-friendly control of *T. castaneum* (Islam and Talukder 2005). The extract of *Datura innoxia* (Mill.) and *A. indica* proved effective against different stored grains' insect pests (Ali et al. 2017). *Nicotiana tabacum* along with *A. indica* have been found effective against *T. castaneum* (Hanif et al. 2016).

Entomopathogenic fungi (EPF) are a specialized group of fungi comprising of numerous genotypes (Araújo and Hughes 2016). Owing to a great stress on application of bio-control for the environment pleasant control of stored commodities insect pests and field crops, the EPF have gained huge consideration in recent few years (Hyde et al. 2019). *Beauveria bassiana* (Bals.) Vuillemin (Hypocreales: Clavicipitaceae) has been extensively studied in this respect. Efficacy of EPF highly depends upon

ambient moisture (Lord 2007). The effect of moisture on effectiveness of *B. bassiana* against insect pests has been found to range from no effect to a straight correlation between efficacy and ambient moisture (Haraprasad et al. 2001).

The present research worked on evaluating the efficacy of three plant extracts and *B. bassiana* against *T. castaneum* under laboratory conditions.

Main text

Materials and methods

Studies were carried out at the Grain Research, Training and Storage Management Cell, Department of Entomology, University of Agriculture Faisalabad, Pakistan.

Collection and rearing of test insects

Tribolium castaneum adults of mixed age were collected from different grain markets situated in the district of Faisalabad, Punjab province, Pakistan. The insect population was brought back to the laboratory and sustained at sterilized plastic jars placed in an incubator at 30 ± 2 °C and $65 \pm 5\%$ RH according to process stated by Ali et al. (2012). Sterilized crushed wheat grains were used for the mass rearing of *T. castaneum*. Adults of the tested insect pest were released on fresh, sterilized wheat grains (800 g) for the purpose of egg-laying. Jars were concealed by muslin cloth for ventilation and tighten up with rubber bands to avoid the insects' escape. To get the first homogenous generation (F_1), pupae of the identical age were collected on day-to-day basis in separate plastic jars having preferred diet of the target insect. The 3rd instar larvae of the test insect were used for further bioassays.

Source of bio insecticides

Formulation (RacerTM) of the EPF, *Beauveria bassiana* (1 g of RacerTM containing 10^8 conidia) was acquired from Agri Life, Medak District, Hyderabad, India. Leaves of neem (*Azadirachta indica*), eucalyptus (*Eucalyptus globulus*), and tobacco (*Nicotiana tabacum*) were collected from different localities in University of Agriculture Faisalabad, Pakistan.

Extraction of plant materials

Plant leaves were cleaned by distilled water and placed in shade for drying. Dried plant leaves were then ground in an electrical grinder (Mamba 1/16 oz, USA Patanasetagit Company Ltd.) to create a powdered form. Extracts were prepared by using a Rotatory shaker (IRMECO, OS 10) by immersing 50 g of each plant powder separately in 250 ml acetone in conical flasks, according to the process elaborated by Ahmad et al. (2006). Solvent from the crude extracts was evaporated by a rotary evaporator (Hei-VAP, Heidolph, Germany) and stored in a refrigerator at 4 °C before use.

Bioassay studies

Mortality rates of *T. castaneum* larvae were checked, using Whatman filter papers (diam. 125 mm). Filter papers were treated distinctly with 5% concentrations of every plant extract and permitted to get dry before starting the bioassay. Treated filter papers were transferred into Petri-dishes. One Petri-dish was kept as a control for each treatment containing untreated diet. Thirty larvae of 3rd instar of *T. castaneum* were released in each Petri-dish containing crushed wheat grains and covered with perforated lids. Three replications for each treatment were used. Larval mortality data was recorded periodically. *B. bassiana* formulation (Racer™) was applied at three concentrations of 0.3×10^8 , 0.6×10^8 , and 0.9×10^8 conidia/kg of wheat grains against *T. castaneum* (Khashaveh et al. 2011). The sterilized food was treated with each concentration rate of entomopathogenic fungi and fed to target insect pests. Samples of 50 g of treated commodity for each concentration rate were placed into 3 separate sterilized small plastic jars (replicates). Three jars with untreated food were used as control. Data regarding larval mortality was recorded periodically (Kavallieratos et al. 2006).

Combined effects of the plant extracts and *Beauveria bassiana* against *Tribolium castaneum*

The conidia of *B. bassiana* (Racer™) were mixed with wheat grains at rates of 0.3×10^8 , 0.6×10^8 , and 0.9×10^8 conidia/kg of crushed wheat grains. Three lots of 50 g from each of the fungal conidia treated diet were transferred into separate small plastic jars and sprayed with 5% of the 3 plant extracts. The treated grains were placed into small plastic jars. Thirty larvae (3rd instar) of the tested insect pest were released in each small plastic jar containing treated diet. Three replications were used. Data regarding mortality and growth inhibition were recorded after regular intervals.

Mycosis and sporulation

Cadavers of *T. castaneum* were collected from the fungal treated experimental units, positioned on a disinfected Petri dish, and refrigerated at 4 °C in small vials. Later on, all the cadavers were superficially decontaminated by 0.05% sodium hypochlorite solution for a period of 2–3 min trailed by 2–3 washing through distilled water. Afterwards, cadavers were positioned on Potato Dextrose Agar (PDA) Petri-dishes and incubated at 26 ± 1 °C; 75 ± 5 % RH for 7 days. The insect cadavers displaying the outer fungal growth were witnessed under the microscope. For checking the sporulation, the mycosed insect cadavers from every replication were mixed in 20-ml distilled water by a drop of Tween-80 and blended for 10 min. The whole quantity of conidia/ml was counted by means of the hemocytometer under microscope (Riasat et al. 2011).

Statistical analysis

After the completion of all bioassays, corrected mortality of recorded data was calculated using Abbott's formula (Abbott 1925):

$$\text{Corrected mortality (\%)} = \frac{\text{Mo(\%)} - \text{Mc(\%)}}{100 - \text{Mc(\%)}} \times 100$$

where:

Mo = observed mortality

Mc = mortality in control

The data of % corrected larval mortality and other treatments and their combinations were statistically analyzed by using STATISTICA 8 software, and significant treatment findings were compared by Tuckey-HSD test. The percent pupae and adult inhibitions were calculated as:

$$\begin{aligned} \text{\%Pupae inhibition or Adult inhibition} \\ = 100 * (1 - t/c) \end{aligned}$$

where t is number of pupae or adult in treated grains and c is pupae or adult present in control.

Results and discussion

Mortality rates of *T. castaneum* larvae

Statistically remarkable differences were noticed in the larval mortality of 3rd instar larvae of *T. castaneum* when exposed to a separate or joint treatments of the plant extracts and *B. bassiana* (*Bb*). Furthermore, the bio-assayed instar (3rd instar) of *T. castaneum* displayed higher percent mortality of combined use of both types of treatments than their separate uses (Table 1). The highest larval mortality of 3rd instar larvae (71.32%) was recorded in the treatment combination that has maximum concentration of *B. bassiana* (0.9×10^8 conidia/ml) and Neem extract (5%). Furthermore, the highest separate concentration of *B. bassiana* (0.9×10^8 conidia/ml) persistently resulted in more larval mortality (32.68%) of 3rd instar larvae of *T. castaneum* than the neem (7.52%), eucalyptus (9.89%), and tobacco extracts (14.61%). Regarding the pupae and adult inhibition, a remarkable decrease in pupation and adult appearance was detected in bio-assayed 3rd instar larvae of *T. castaneum* when exposed to joint applications of *B. bassiana* + the plant extracts than in their separate uses (Table 1). The lowest pupation and adult emergence (9.71 and 4.89%) was recorded in treatment where maximum concentration of the *B. bassiana* (0.9×10^8 conidia/ml) was used along with NE. Though the highest pupation and adult inhibition (52.87 and 44.20%, respectively) of the *T. castaneum* larvae were noticed in the treatment where the lowest separate fungal concentration (0.3×10^8 conidia/ml) was used, generally, the highest pupation and adult emergence were recorded in the treatments with the lowest larval mortality (Table 1).

Table 1 Effect of *Beauveria bassiana* (Bb) and plant extracts on mortality, pupation, and adult emergence (% ± SE) of 3rd larval instars of *Tribolium castaneum*

Treatments	Mortality (%)	Pupae inhibition (%)	Adult inhibition (%)
B1	21.29 ± 2.18 g	52.87 ± 1.49c	44.20 ± 1.23b
B2	26.47 ± 2.50 g	34.43 ± 2.22f	31.90 ± 0.93 d
B3	32.68 ± 3.26f	28.75 ± 2.20 h	25.13 ± 1.11d
TE	7.521.73i	42.12 ± 2.52d	39.41 ± 2.83c
EE	9.89 ± 2.30hi	40.04 ± 1.89e	36.96 ± 1.34c
NE	14.61 ± 3.22 h	35.10 ± 2.62f	30.58 ± 1.63de
B1 × TE	15.54 ± 1.12 h	39.62 ± 2.42 g	31.08 ± 1.63f
B1 × EE	22.18 ± 2.61 g	33.14 ± 1.92f	30.08 ± 2.23de
B1 × NE	31.15 ± 3.62f	29.32 ± 2.79f	26.08 ± 2.50d
B2 × TE	38.87 ± 2.93e	25.51 ± 2.54e	22.78 ± 1.66c
B2 × EE	40.05 ± 1.11d	30.19 ± 1.85 h	26.19 ± 1.79f
B2 × NE	46.17 ± 1.92 c	23.65 ± 1.67 g	19.34 ± 1.39e
B3 × TE	50.23 ± 1.19 c	17.10 ± 1.92d	18.13 ± 2.13bc
B3 × EE	60.35 ± 1.33b	13.10 ± 1.92d	10.13 ± 2.13bc
B3 × NE	71.32 ± 1.33a	9.71 ± 2.23b	4.89 ± 2.63b
Control	-	69.81 ± 1.98a	61.10 ± 2.10a
	$F = 234.81, df = 16$	$F = 81.52 df = 16$	$F = 10.34, df = 16$

B1 0.3×10^8 conidia, B2 0.6×10^8 conidia, B3 0.9×10^8 conidia, TE tobacco extract, EE eucalyptus extract, NE neem extract. The lettering (a-g) shown that treatment means having similar letterings are not significantly different from each other

In previous studies, many researchers have shown that botanicals and EPF have entomocidal and growth inhibition effects against stored grain insect pests. As such, 3rd instar larvae of *T. castaneum* L. displayed somewhat dissimilar developmental and demise reactions to the insect control agents practiced in the presented research work. A considerable greater mortality rate was detected in the 3rd instar larvae against joint use of *Bb* and PE than their separated applications. In previous studies, joint effect of synthetic insecticides at their sub-lethal concentrations with *B. bassiana* caused an enhanced effectiveness of insect pest control strategies by synergism (Purwar and Sachan 2006). Besides the insecticides, the efficiency of EPF have been examined with plant extracts. Combined application of *B. bassiana* and plant essential oils has resulted in enhanced control of the *T. castaneum* larvae (Akbar et al. 2005). Wraight and Ramos (2005) noticed that combined use of *B. bassiana* and *Bt* resulted from greater larval death of *Leptinotarsa decemlineata* S. than their individual applications.

The improved synergistic outcome of the EPF and PE used in this research could be owing to the reality that feeding deterrence impacts of plant extracts induced starvation stress in the insects, which augmented growth period of the *T. castaneum* larvae and amplified their vulnerability to fungal invasion and multiplication because of extended inter molt times. The findings are consistent with Furlong and Groden (2003) who stated that

starvation pressure augmented the *B. bassiana* infection against the grubs of *Leptinotarsa decemlineata* (Say). The plants extracts detained the diet of insects, whereas the fungal spores quickly kill the debilitated larvae. Ali et al. (2018) checked the combined effects of botanicals and EPF against stored grains insects and noticed the combined applications resulted in greater mortality of *Sitobion avenae* (Fab.) than alone applications. Castiglioni et al. (2003) noticed that the combined application of the neem extracts and *B. bassiana* gave better results in controlling stored commodities insect pests like outcomes of this research. The compatibility of the botanicals and EPF varied among different species of the fungi. Furthermore, it also depends upon the presence of different types of the plant secondary metabolites in the extracts.

The efficacy of *B. bassiana* (RacerTM) was straightly connected to the temperature and RH (Lord 2007). The less mortality rates of the target larvae after the use of *B. bassiana* were attributed to the temperature practiced in the present study. This is in line with Vassilakos et al. (2006) who noticed greater effectiveness of *B. bassiana* at 26 °C than at 30 °C. Obtained outcomes were in consistent with Khashaveh et al. (2011) who examined the exposure period and concentration depended impact of *B. bassiana* for *Sitophilus granarius* (L.), *Oryzophilus surinamensis* (L.), and *T. castaneum*. The findings of the presented study at low concentration of *B. bassiana* were in line with Akbar et al. (2004) who applied a formulation

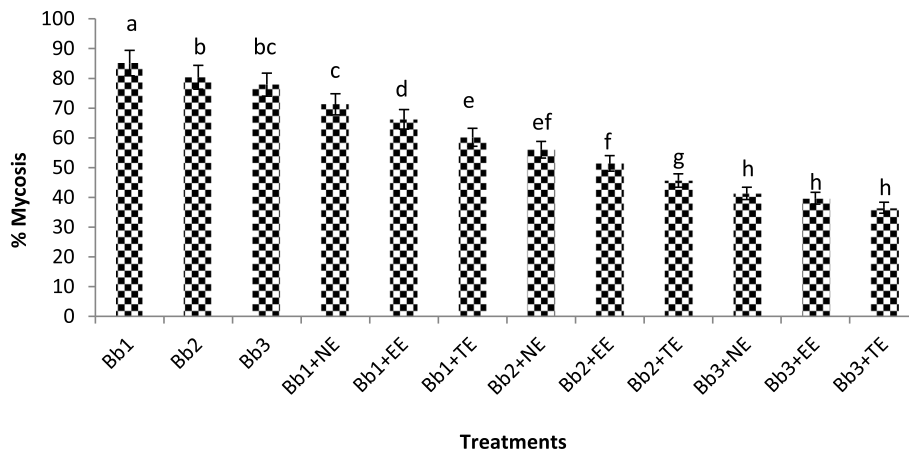


Fig. 1 Impact of the plant extracts and *Beauveria bassiana* (Bb) concentrations on (%) mycosis in cadavers of 3rd instars larvae of *Tribolium castaneum*. Bars with the same letters are not significantly different at 5% significance level. Vertical bars reflect standard error (SE). Where the value of Bb1 is 0.3×10^8 conidia/ml, Bb2 is 0.6×10^8 conidia/ml and Bb3, 0.9×10^8 conidia/ml (F cal. = 7.65, df = 11, and $P < 0.05$)

of *B. bassiana* (containing 9.4×10^{10} conidia per 2000-g wheat grains) against the *T. castaneum*. The outcomes are in agreement with the results of Inglis et al. (2001), who described that dissimilar developmental phases of insects differ in their vulnerability to fungal contagion. It has been specified the detoxication activity of enzymes variations expressively inside and among dissimilar growth phases of insects. This enzymatic activity is negligible in egg phase, intensifies by every larval phase, and then again decreases to the minimum level at pupal phase (Mullin 1988).

Mycosis and sporulation

Applied treatments affected the mycosis and sporulation in the cadavers of 3rd instar larvae of *T. castaneum*. A considerably high rate of mycosis and sporulation was enumerated in the larvae of the insect when they were exposed to separate treatments of *B. bassiana* than the combined applications with the plant extracts. The

highest mycosis (85.13%) and sporulation (160.12 conidia/ml) were detected in the treatments where the lowest concentration of *B. bassiana* (0.3×10^8 conidia/ml) was used, alone. Contradictory to dose effect for larval mortality, the maximum fungal concentration resulted in the lowest mycosis and sporulation in the 3rd instar larvae of *T. castaneum* (Figs. 1 and 2).

The tenacity of a fungal insecticide in an agroecosystem relies not merely on its inoculum but also depends to some extent on the later on contagion from mycosed cadavers (Wood and Thomas 1996). In the present research, percent mycosis and sporulation attained the highest peak in treatment where unaccompanied *B. bassiana* was used at its low concentration. These findings were supported by Tefera and Pringle (2003), who described augmented percent mycosis and sporulation in lepidopteron larvae cadavers infected by least conidial concentration of *B. bassiana* than to its great application rates. Likewise, Riasat et al. (2011) also

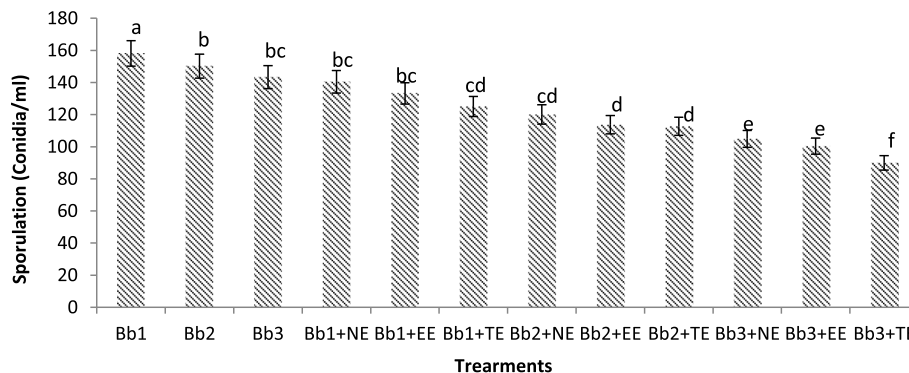


Fig. 2 Impact of the plant extracts and *Beauveria bassiana* (Bb) concentrations on sporulation in cadavers of 3rd instars larvae of *Tribolium castaneum*. Bars with the same letters are not significantly different at 5% significance level. Vertical bars reflect SE. Bb1, 0.3×10^8 conidia/ml; Bb2, 0.6×10^8 conidia/ml; Bb3, 0.9×10^8 conidia/ml (F cal. = 4.43, df = 11, and $P < 0.05$)

noticed a great mycosis and sporulation in the *Rhizopertha dominica* F. cadavers at application of less *B. bassiana* conidia. The least percent mycosis and sporulation at increased conidial concentrations can be credited to the self-inhibiting process of fungal conidia at great concentrations (Tefera and Pringle 2003).

Conclusion

Results showed that binary mixtures of botanicals, especially neem extract and *B. bassiana*, could be an economical approach for the control *T. castaneum* population. The tactic of joint uses of the plant materials and EPF assurances delivering improved efficiency than the efforts to combat the insect pest infestation with individually used treatments.

Abbreviations

NE: Neem extract; EE: Eucalyptus extract; TE: Tobacco extract; RacerTM: Trade name of *Beauveria bassiana* formulation; RH: Relative humidity; IPM: Integrated pest management; ml: Milliliter; EP: Entomopathogenic; F₁: First homogenous generation; g: Grams; PDA: Potato Dextrose Agar; Mo: Observed mortality; Mc: Mortality in Control; t: Number of pupae or adult in treated wheat grains; c: Pupae or adult present in control; Bb: *Beauveria bassiana*; B1: 0.3 × 10⁸ conidia; B2: 0.6 × 10⁸ conidia; B3: 0.9 × 10⁸ conidia; LC₅₀: Lethal concentration required to kill 50% insect population

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Authors' contributions

HR, MS, and MAF designed the experiment. HR and BM conducted the experiment and wrote the article. AR and QA helped in the statistical analysis. MAF and HUA revised the article. All authors approved the final article after reading.

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Availability of data and materials

All data of the study have been presented in the manuscript, and high quality and grade materials were used in this study.

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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