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RESEARCH

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Aptness of entomogenus fungi with diatomaceous earth against various stored grain insect pests

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Abstract

Background: Numerous biotic and abiotic factors are responsible for losses of grains quality and quantity during storage. Insecticides used to control stored grain insect pests are not only hazardous to mammals and environment but also induce resistance in insect pests towards these synthetic chemicals. A current trial was conducted, during 2020, in a stored grain laboratory at the College of Agriculture, BZU, Bahadur Sub Campus, Layyah, Punjab, Pakistan. A diatomaceous earth (DE) formulation enhanced with bitterbarkomycin (DEBBM) and combined with *Beauveria bassiana* (Balsamo) Vuillemin was evaluated against *Cryptolestes ferrugineus,* (Stephens) (Coleoptera: Laemophloeidae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae) under laboratory conditions.

Results: DEBBM was applied at the rate of 50 mg/kg, DE (150 mg/kg) and *Beauveria bassiana* (1.5×10^8 and 1.5×10^{10} conidia/kg) alone as well as in combination with wheat, rice and maize. Treated adult mortality was recorded at 7, 14 and 21 days after exposure. Results of the current study showed that insect pest mortality was maximum in the case of combined application of DEBBM with *B. bassiana* (high concentration) at prolonged exposure time as compared to their alone application. Mortality of *C. ferrugineus* was maximum in wheat and rice (100%) over maize (97%), while, *R. dominica* exhibited high mortality in wheat (100%) followed by rice (97%) and maize (94%) at combined application of DEBBM with *B. bassiana* (high concentration) after 21 days. Regarding *T. castaneum* mortality was high in wheat (100%) followed by rice (93%) and maize (88%) in case of combined application of DEBBM with *B. bassiana* (high concentration) at prolonged exposure time (21 days).

Conclusion: In crux, the current trial showed that a mixture of DEBBM and *B. bassiana* is helpful in controlling tested insect pests.

Keywords: Diatomaceous earth (DE) formulation, DE with Bitterbarkomycin (DEBBM), *Beauveria bassiana*, Stored commodities

Background

Wheat (*Triticum aestivum* L.) (Family: Poaceae) is considered as a chief staple food among all cereal grains and Pakistan stands production and consumption wise at the 8th level (Shuaib et al. 2007). Wheat covers 37% of the total cultivated area in the country (Jilani 2007). Rice is the second chief food of Pakistan that covers 11% of the food production area and is cultivated on more than 2.5 million hectares (Chaudhry 1994). Maize (*Zea mays* L.) is also considered a food crop after wheat and rice. The Annual cultivation of maize is more than one million hectares (GOP 2003).

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Rhyzopertha dominica (F.) (Coleoptera: Bostrichidae) female lays eggs externally on wheat kernel, while, larvae feed and pupate inside the kernel. (Athanassiou et al. 2011). *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) is primary stored grain pest which attack germs of seed. This insect has white color having size 3 mm in length (Mason 2003). *Tribolium castaneum* (Coleoptera: Tenebrionidae) is secondary pest of stored grain. Both larval and adult stages cause damage and complete life cycle on grain (Karunakaran et al. 2004).

Numerous biotic factors are responsible for losses of grains quality and quantity during storage (Ahmed et al. 2008). Therefore, grain storage losses due to insects may increase 10–20% in Pakistan (Khan et al. 2010). In extreme conditions, losses may occur up to 25.5% (Irshad et al. 1988). Stored grains or products are attacked by the most important 23 species and 75% from all these species are belong to only one order Coleoptera (Viñuela et al. 1993).

For the preservation of stored grains since the 1950s, synthetic residual insecticides have been used to control stored grain insect pests. Continuous use of these insecticides had made the insect pest resistant to these chemicals. Additionally, these chemicals are also toxic to animal and hazardous to the environment (Pacheco et al. 1990; Arthur 1996). Now a days the use of new chemistry products is prevailing all over the world. Because these have lower mammalian toxicity and can control the insects effectively. Different insect pest of field crops can be effectively controlled by the use of bio-nicotine pesticides (Ye et al. 2014).

Fungi are considered biocontrol agents against insects and produce spores that cause insect mortality by penetration into the body of insects (Goettel et al. 2005). *B. bassiana* can be used as a component in integrated pest management (IPM). For birds, plants and mammals *B. bassiana* is considered ecologically non-toxic (El-Sharabasy 2015). *B. bassiana* is found in soil and decaying plant debris. Its conidia penetrate the body of the insect, by stimulating its growth and it generates a strong contact with the body layer of the host insect (Holder and Keyhani 2005). So, the fungus is a good choice in IPM, as insects have gained resistance against chemicals and other substances. The *B. bassiana* is tested against various pests on cereal crops as a biological agent (Bounechada and Doumandji 2004). *B. bassiana* has the advantage that it controls diseases by disease causing vectors of various arthropods and serves as entomopathogenic fungus (Stricker et al. 2006). Different scientists worked on the mutual effect of DE and entomopathogenic fungus (Riasat et al. 2011), or chemical insecticides and entomopathogenic fungus (Srivastava et al. 2009), DE combined with various chemical insecticides (Kavallieratos et al. 2009). In current study, the efficacy of certain control agents that affected by different parameters like concentration rate, insect species, grain type, time for storage and application method of combined form was studied.

Methods

The current study was conducted at the College of Agriculture, Bahauddin Zakariya University, Bahadur Sub-Campus Layyah Punjab Pakistan. DE, DEBBM and fungus (*Beauveria bassiana*) with different concentrations against *Cryptolestes ferruginous, Rhyzopertha dominica* and *Tribolium castaneum.*

Collection of insects

Insect species (*C. ferruginous*, *R. dominica* and *T. castaneum*) were collected from different godowns of stored grains in district Layyah.

Grain commodities

The grains used in this trial were free of chemicals and pests infestation from Wheat (var. galaxy-2013), Rice (var. Basmati-2011) and Maize (var. DK-6142). By using the Dickey-John moisture meter we measured the percentage of moisture present in the grains. The moisture present in wheat grains was 10.95% and in rice was 9.97% and 11. 17% in maize.

Diatomaceous earth and DEBBM formulation

The diatomaceous earth (DE) formulation used in the tests was DEBBM (Diatom Research and Consulting Inc., Guelph, ON, Canada). The DE constituent, which is 90% of the formulation, is from the group of fresh water DEs and is composed of 89% amorphous SiO_2,4% Al_2O_3, 1.7% Fe_2O_3, 1.4% CaO, >1% $MgO+K_2O$ and 3% H_2O. The remaining 0.05% active ingredient is Chinese plant extract (bitter barkomycin). Formulation of DE (150 mg/kg) and DEBBM (50 mg/kg) was used against test insect.

Collection of *Beuveria bassiana*

Pure culture of *B. bassiana* was taken from the first fungal culture bank of PIAS, University of the Punjab, Lahore, Pakistan. Where the pathogenicity of fungi was tested against *C. ferruginous* as described by Freeman et al. (1998) and Twizeyimana et al. (2013) in laboratory and culture was maintained in the Plant Pathology Laboratory of College of Agriculture, BZU, Bahadur campus Layyah.

Collection of spores of *B. bassiana*

One liter distilled water and 40 g PDA (Potato Dextrose Agar) was boiled in a flask till dissolved entirely. The solution was autoclaved at 120 °C for 30 min. Spores of fungi were inoculated on PDA medium aseptically, and

Table 1 Mortality (%) of *Cryptolestes ferrugineus* in various commodities treated with DE, DEBBM and *Beauveria bassiana* applied sole or in combination

Exposure Interval days	Commodity	Mortality % at concentrations of *B. bassiana* (conidia kg^{-1}), DE/DEBBM (mg/kg)							
		1.5×10^8/0/0	1.5×10^{10}/0/0	0/150/0	0/0/50	1.5×10^8/150/0	1.5×10^{10}/150/0	1.5×10^8/0/50	1.5×10^{10}/0/50
7	Maize	26.23a	51.64b–d	45.45bc	60.26c–e	42.10ab	66.29ab	56.30b–e	74.21e
	Rice	31.11a	57.54b–d	53.36a–c	61.32cd	44.19ab	73.15d	61.30b–d	77.18d
	Wheat	35.12a	68.26b–d	53.18bc	71.02cd	48.14ab	75.37d	64.20b–d	77.12d
14	Maize	42.16a	61.59a–d	53.28a–c	72.32c–e	50.36ab	81.40de	66.10b–d	91.18e
	Rice	48.26a	68.20a–c	63.21a–c	77.52cd	56.36ab	88.13de	69.20b–d	97.35e
	Wheat	53.15a	72.57b–d	62.21a–c	81.25cd	60.31ab	93.34de	74.16b–d	100.00e
21	Maize	56.24a	73.23a–c	61.16ab	76.26b–d	61.29ab	86.22cd	73.13a–c	97.55d
	Rice	62.25a	77.67a–c	71.37ab	83.27b–d	66.43ab	93.14cd	77.15a–c	100.00d
	Wheat	69.26a	80.63ab	72.35ab	86.22bc	71.24a	100.00c	80.33ab	100.00c

Similar lettering within rows are not significantly different @ $p < 0.05$

kept for incubation at 26 ± 1 °C. On culture after five days Mycelium was developed. By using Whatman Filter paper No.1, the culture was filtered. Obtained mycelium grew on filter paper. In bioassay after 25 days, the conidia collected from this mycelium were used using Hemocytometer (Murali et al. 2012).

Bioassay

Mortality effect of *B. bassiana,* DE and DEBBM alone and combined against the tested insects

One kilo gram of each grain commodities viz. wheat, rice and maize were prepared and kept in plastic jars separately. Two concentrations of *B. bassiana* (1.5×10^8 and 1.5×10^{10} conidia kg^{-1} of grains), with DE (150 mg/kg and 50 mg/kg) and (50 mg/kg) of DEBBM alone or in combinations. One untreated lot was kept as control. Data were taken after 7, 14 and 21 days to check insect mortality. The corrected mortality was calculated by using Abbott's formula (1925).

$$\text{Corrected mortality (\%)} = \frac{M_o - M_c}{100 - M_c} \times 100$$

M_o = Mortality observed, M_c = Mortality in control.

Statistical analysis

The analysis was done with Minitab 13.2 (Minitab, 2002 Software Inc., Northampton, MA). The Tukey–Kramer (HSD) test at the 5% significance level was used to compare the means of adult mortality (Sokal and Rohlf 1995).

Results

Applied treatments along with their exposure time exhibited maximum mortality of *C. ferrugineus* on wheat, followed by maize. Mortality percentages were ranged between 45 and 72% where DE alone was applied, at the end of the trial, and application of DEBBM alone exhibited mortality between 60 and 87%. A combination of DE with a high concentration of *B. bassiana* exhibited mortality between 66 and 100%, while DEBBM and *B. bassiana* (high concentration) showed mortality between 74 and 100% at the end of the trial (Table 1).

The mortality of *R. dominica* was maximum on wheat as compared to maize and rice. In all cases, sole application of DE caused mortality between 39 and 67%, after 21 days of treatment, and DEBBM exhibited a high mortality between 50 and 84%. Application of DE and DEBBM with a high concentration of *B. bassiana* (1.5×10^{10} conidia kg^{-1} of grains) exerted more impact on mortality as compared to their sole application. The combined application of DEBBM with *B. bassiana* (high concentration) exhibited high mortality (68–100%), while DE with a high concentration of *B. bassiana* showed mortality between 60 and 96% after 21 days of treatments (Table 2).

Maximum mortality of *T. castaneum* was assessed on wheat followed by rice and maize. Regarding alone treatment application, DE exhibited mortality between 32 and 61% and DEBBM showed mortality between 45 and 78%. Among all cases except one, *B. bassiana* application with its high concentration exhibited maximum mortality as compared to lower dose application. Maximum mortality rate (56–100%) was observed where DEBBM was applied with a high concentration of *B. bassiana* as compared to *B. bassiana* (high concentration) alone. DE application with *B. bassiana* (high concentration 1.5×10^{10} conidia kg^{-1} of grains), after 21 days post treatment, showed mortality between 74 and 87%, while *B. bassiana* (high concentration) with DEBBM exhibited mortality between 88 and 100% (Table 3).

Table 2 Mortality (%) of *Rhyzopertha dominica* in various commodities treated with DE, DEBBM and *Beauveria bassiana* applied sole or in combination

Exposure interval days	Commodity	Mortality % at concentrations of *B. bassiana* (conidia kg^{-1}), DE/DEBBM (mg/kg)							
		1.5×10^8/0/0	1.5×10^{10}/0/0	0/150/0	0/0/50	1.5×10^8/150/0	1.5×10^{10}/150/0	1.5×10^8/0/50	1.5×10^{10}/0/50
7	Maize	22.32a	44.67bc	39.11ab	50.19b–d	34.28ab	60.11cd	43.13bc	68.20d
	Rice	28.23a	51.11b–d	44.25ab	55.78b–d	36.33ab	64.71cd	49.39a–c	72.23d
	Wheat	31.75a	56.80b–d	48.88a–c	63.17c–e	41.28ab	67.89de	55.34bcd	77.32e
14	Maize	34.24a	62.19c–e	47.76a–c	66.82de	44.23ab	74.52ef	56.72b–d	85.19f
	Rice	43.74a	63.71ab	50.63a	72.24bc	46.71a	77.72bc	59.63ab	86.62c
	Wheat	46.69a	67.75b–d	58.31a–c	77.12c–e	51.82ab	81.55de	61.39a–c	93.46e
21	Maize	44.38a	65.37bc	57.10ab	76.10cd	52.26ab	87.48d	68.26bc	94.33d
	Rice	52.28a	73.75b–d	63.15a–c	79.27a–c	58.22ab	92.28de	75.21b–d	97.24e
	Wheat	56.33a	77.30bc	67.36a–c	84.11c–e	62.16ab	96.17de	79.37b–d	100.00e

Similar letters within rows are not significantly different @ $p < 0.05$

Table 3 Mortality (%) of *Tribolium castaneum* in various commodities treated with DE, DEBBM and *Beauveria bassiana* applied sole or in combination

Exposure interval days	Commodity	Mortality % at Concentrations of *B. bassiana* (conidia kg^{-1}), DE/DEBBM (mg/kg)							
		1.5×10^8/0/0	1.5×10^{10}/0/0	0/150/0	0/0/50	1.5×10^8/150/0	1.5×10^{10}/150/0	1.5×10^8/0/50	1.5×10^{10}/0/50
7	Maize	16.22a	38.14bc	32.30a–c	45.34 cd	26.35ab	47.39cd	34.19bc	56.31d
	Rice	22.68a	41.43a–c	37.31a–c	49.34b–d	31.34ab	54.55cd	40.36a–c	64.28d
	Wheat	25.28a	47.34bc	42.25a–c	53.25 cd	34.79ab	56.12cd	45.41bc	68.12d
14	Maize	29.38a	52.20cd	42.91a–c	55.23c–e	34.40ab	65.41de	48.13b–d	71.18e
	Rice	32.52a	54.41b–d	47.15bc	62.01c–e	38.25ab	68.34de	53.15b–d	73.48e
	Wheat	37.53a	59.38b–d	55.43a–c	63.13cd	46.21ab	74.13de	53.43bc	83.33e
21	Maize	35.43a	61.11b–d	54.32a–c	66.19cd	45.25ab	74.81de	60.25b–d	88.20e
	Rice	43.55a	63.58bc	56.50ab	75.59cd	51.33ab	84.35de	65.41b–d	93.33e
	Wheat	48.23a	68.35bc	61.33a–c	78.25cd	56.35ab	87.35de	73.38b–d	100.00e

Similar letters within rows is not significantly different @ $p < 0.05$

Discussion

Various studies have demonstrated that insecticidal abilities of various DE preparations showed dissimilar results against specific insect species. In this illustration, three different DE formulations (Protect-It, PyriSec and Insecto) were tested on the *T. confusum* population that showed significant dissimilar in effectiveness of given DEs (Kavallieratos et al. 2009). Likewise, Collins and Cook (2006) assessed 2 DEs Diasecticide and SilicoSec at laboratory condition in contrast to *Tribolium castaneum*, *Oryzaephilus surinamensis*, *Sitophilus granaries*, *Lepidoglyphus destructor*, *Ephestia kuehniella* and mites *Acarus siro,* pests of the stored product and saw SilicoSec as the best product for all tested pest species. Athanassiou et al. (2004) have also described improved properties of Protect-It and PyriSec than Insecto for the reduction of *Rhyzopertha dominica*, *Sitophilus oryzae* and *Tribolium castaneum* stored grain pest beetles.

Overall, all the DEs have similar method of action (Korunić 2013). Therefore, the substances present in the viable DEs formulations reduced to observe efficacy changes among several formulations. In the present study, 2 DEs were tested because of their differences in composition: the Protect-It comprises silica-aerogel and the DEBBM formulation of DE is improved with the bitterbarkomycin (BBM). The DEBBM showed to be much successful as to Protect-It, at least at the lowest concentration of 50 ppm, contrary to all targeted insect species present in each product type. The efficiency of DEBBM to the *R. dominica* (Wakil et al. 2011), *C. ferrugineus, T. castaneum* and *S. zeamais* (Athanassiou et al. 2009) have been described previously. These insect dead bodies might be served as a proper substrate in the application of fungal growth. BBM retains such antifeedant features (Wu et al. 2017) consequently, boosting mutual action of these 2 ingredients are also armored with poisonous effects employed by the fungus activity, which additionally destroyed the immunity system of already starving insects.

Entomopathogens showed a synergistic mode of action and can be used as a control strategy against various stored grain insect pests for their control (Akbar et al. 2004). Similarly, Master (2001) attained significant outcomes when *B. bassiana* used for controlling the grain borer. Akbar et al. (2004) studied the application of *B. bassiana* alone and in combination with Biopesticide to control *T. granarium*, that resulted also in line with Lord (2001) who documented its synergistic impact.

The influence of combined treatments (*B. bassiana* + Biopesticide) may be accredited in a way that showed no negative impact regarding conidial germination, vegetative growth and *B. bassiana* conidiogenesis (Neves et al. 2001). Alizadeh et al. (2007) documented that biopesticide (high concentration) significantly impacted mortality when used with *B. bassiana*. Similarly, Sheeba et al. (2001) observed the maximum *S. oryzae* mortality in a case where *B. bassiana* (high concentration) was used in bioassay. A high dosage of *B. bassiana* and Biopesticide significantly enhanced mortality when used against *C. ferrugineus* and *R. dominica*. Various insecticides viz. pyrethroids (Athanassiou et al. 2004) and microbial sprays (Vayias et al. 2009) impacted mortality concerning their concentration.

The current study revealed that mortality is directly proportional to the prolonged exposure time. Results of current trials are also in accordance with previous studies (Wakil et al. 2012). Various factors viz. surface to volume ratio, concentrations, application time and exposure length impacts insect pest susceptibility (Mewis and Ulrichs 2001). Enhanced application of biopesticides increases the strength of *B. bassiana*, after 14 days, and impact is enhanced more with prolonged time of exposure against insect pests (Michalaki et al. 2007).

Conclusions

It was concluded that DEBBM along with *B. bassiana* high concentration showed maximum impact regarding to mortality of the tested insect pests. While the present trial signifies the outcomes only *in-vitro* studies. Moreover, future studies are essential as *in-vivo* environment for the expansion of an effective, non-hazardous and IPM-compatible control measure of the tested insect pests in stored commodities.

Abbreviations

DE: Diatomaceous earth; Mo: Mortality observed; Mc: Mortality in control; SE: Standard Error.

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Authors' contributions

Ch. Muhammad Shahid Hanif, Hafiz Muhammad Aatif, Muhammad Ijaz: Planned the research; Kamran Ikram, Azhar Abbas Khan, Muhammad Ismail, Muhammad Ashir: Carried out all the experiment including the bioassay tests and analytical part; Muhammad Zeeshan Mansha, Qamar uz Zaman: Analysis of data and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

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

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

This study does not contain any individual person's data.

Competing interests

The authors have no competing interests.

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