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Effect of the Combined Use of *Beauveria bassiana* (Balsamo) Vuillemin and Diatomaceous Earth for the Control of *Tribolium castaneum* (Herbst) and *Trogoderma granarium* Everts

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ABSTRACT

The red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae) are important insect pests of stored commodities in Pakistan and other parts of the world. The laboratory study was carried out to investigate the insecticidal properties of *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) in combination with commercial diatomaceous earth (DE) formulation Inert-PMS alone and in integrate manners against these pests. *Beauveria bassiana* was applied at 1×10^6 and 1×10^7 conidia/ml alone, or in combination with DE at 50 and100 ppm. Adult insects were exposed to the treated wheat for 5, 10 and 15 days at 27±2 °C and 65±5% r.h. in an incubator. The mortality of *Tribolium castaneum* adults was 100% even after 10 days of exposure at the dosages in combination of highest dose of *B. bassiana* and DE. While, after last exposure 100% mortality was achieved at highest dose rate of *B. bassiana* and both doses of DEs. Contrarily, 100% mortality of *Trogoderma granarium* was recorded after 15 days of exposure at highest dose rate of *B. bassiana* and both DEs. The progeny emergence was significantly lower in combined treatments of *B. bassiana* and DE at highest dose rate for both insect species, while highest was recorded at low dose of *B. bassiana*.

INTRODUCTION

The stored commodities are attacked by a number of primary and secondary insect pests which may cause 10-40% damage, and in cases of severe infestation this may exceed up to 50% (Upadhyay and Ahmad, 2011). Primary insect pests have capability to attack whole or unbroken kernels, while secondary pests attack only already damaged grains, frass and milled products. Among stored product insect pests, the khapra beetle *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae) and the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) have been primarily found to be of economic importance that ravage stored products (Boyer *et al*., 2012). *Trogoderma granarium* is one of the most destructive insect pest and pest of quarantine importance globally which is considered as one of the 100 worst invasive species in the world (Athanassiou *et al*., 2018). The larvae are damaging stage which causes both quantitative and qualitative losses in stored cereals. Also, contaminated food with barbed hairs and molted skin of larvae pose serious threat to human health (Borzoui *et al*., 2015). *Tribolium castaneum* is another important cosmopolitan insect pest, both larvae and adult of this pest feed on grain frass and already damage or broken grains. Beetles secrete quinone excretions which are carcinogenic in nature (Karunakaran *et al*., 2004; Michalaki *et al*., 2006).

The control of these insect pests is currently based on two broad categories of insecticides: Residual chemical insecticides and fumigants. Injudicious use of these insecticides causes hazardous effects on human health and environment, most importantly the insect pests has developed resistance against these insecticides (Finkelman

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et al., 2002). These issues had lead researchers to evaluate alternative grain protectant methods which are compatible to environment and safer for human health, and leave no residues on grains. Insect pathogenic fungi are potent alternative to the conventional grain protectant, as they have low mammalian toxicity and effective against a variety of stored product insect pests (Moore *et al*., 2000; Lord, 2001). Several entomopathogenic fungi have been tested against stored product pests with contradictory results (Moore *et al*., 2000). Among these, *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) found to be very effective against stored grain insect pests and self-perpetuating (Lord, 2001, 2005; Akbar *et al*., 2004).

The desiccant dusts, particularly diatomaceous earths (DEs) are another potential alternative to the chemical insecticides to control stored product pests (Subramanyam and Roesli, 2000; Arthur, 2004; Fields and Korunic, 2000). DEs can be removed easily from the grains, and have low mammalian toxicity (Korunic *et al*., 1996, 1998). Also, resistance is unlikely to develop against DEs, as their action is strictly mechanical and abrasive through which body fluid ooze out of the body and ultimately insect die due desiccation (Athanassiou *et al*., 2005; Vayias *et al*., 2006). Integrated use of both agents can be very effective and could be a mean of reducing application rates (Lord, 2001; Akbar *et al*., 2004; Vassilakos *et al*., 2006; Wakil *et al*., 2011). Akbar *et al*. (2004) reported that the combined application of *B. bassiana* and the DE Protect-It act synergistically against larvae of *Tribolium castaneum*. Similar findings were also reported by Wakil *et al*. (2011) for DEBBM and *B. bassiana* against *Rhyzopertha dominica* F. (Coleoptera: Bostrychidae). Additionally, both agents persist on the grains which may provide long term are persistent inertion against stored product insect pests (Wakil *et al*., 2011).

The present study was aimed to evaluate the insecticidal properties of *B. bassiana* and DE against *Trogoderma granarium* and *Tribolium castaneum* alone and through combined use of both substances. Additionally, the progeny emergence for both insects was also checked on the treated grains.

MATERIALS AND METHODS

Populations of both insects were collected from different grain storage facilities of Layyah, Punjab (Pakistan) and further colony was established in the IPM laboratory, College of Agriculture, BZU Bahadur Sub-Campus, Layyah. The adults of *Tribolium castaneum* were reared on wheat flour+5% brewery yeast at 27±2 °C and 65±5% r.h. Mass production of *Trogoderma granarium* was carried out on whole wheat and conditions were maintained at 30±2 °C, relative humidity of 65±5%, and an approximate photoperiod of 14:10 (L: D) h, as described by Seifi *et al*. (2015). The laboratory culture which was reared in the laboratory since 2014s served as control.

Enhanced DE formulation Inert-PMS® and entomopathogenic fungi *B. bassiana* were taken from Microbial Control Laboratory, Dept. Entomology University of Agriculture, Faisalabad (Pakistan). The laboratory culture was sub-cultured on Potato Dextrose Agar (PDA) by incubating at 20±5 °C and 70% r.h., and the conidia were harvested using a sterilized scalpel after 14 days. The conidia were suspended in sterile 0.05% Tween-80, enumerated with a haemocytometer and adjusted to achieve concentrations of 1×10^6 and 1×10^7 conidia/ml. Prior to the initiation of the tests, the germination rate of *B. bassiana* conidia was determined as > 90%.

Untreated, clean and uninfested grains with very little dockage wheat grains were taken from the market. The moisture contents of the grains prior to the experiment were 11% measured by moisture meter. Grains then kept for 7 days at 25 °C and 75% r.h. to equilibrate the moisture contents. The moisture contents measured by moisture meter after 7 days were 13.8%. *Beauveria bassiana* and Inert-PMS® were applied at the dose rate of 1×10^6 and 1×10^7 conidia/ml, DE at the dose rate of 50 and 100 ppm alone and their possible combinations. For each treatment, lots of 1kg wheat grains were taken and treated with respective doses of fungi and DE. Jars were shaken manually for 5 min to achieve an equal distribution of the fungal spores and dusts on the entire grain mass. There was an additional untreated grain lot which served as a control.

Twenty larvae of *Trogoderma granarium* and *Tribolium castaneum* were introduced into each treated jar separately. The jars were set at 27±2 ºC and 65±5% r.h. in an incubator for the experimental period. The numbers of dead individuals were counted after 5, 10 and 15 days post exposure. After 15 days the boxes were set for next 50 days for progeny emergence.

Mortality for each treatment was corrected for control mortality using Abbott's formula (Abbott, 1925). The data was subjected to one way Analysis of Variance (ANOVA) in Statistix 8.1 using Tukey's Kramer test (HSD) at 1% significance level (Sokal and Rohlf, 1995).

RESULTS AND DISCUSSION

After 5 days of exposure, significant difference in mortality of *Tribolium castaneum* was found among all the treatments tested. Highest mortality was recorded for combined treatments of *B. bassiana* and DE at highest dose rate, while the lowest was recorded for *B. bassiana* at low dose rate. After 10 days of exposure, significant difference in mortality of *Tribolium castaneum* was found among all the treatments. Highest mortality (100%) was recorded for combined treatments of *B. bassiana* and DE at highest dose rate, while the lowest was recorded for low dose of *B. bassiana*. After last count, also significant difference in mortality of *Tribolium castaneum* was found among all the treatments. Highest mortality (100%) was recorded for combined treatments of *B. bassiana* and DE at (*B. bassiana*: 1×10^{7} conidia/ml +DE: 100 ppm), while the lowest was recorded for *B. bassiana* at low dose rate (Table I).

After 5 days of exposure significant difference in mortality of *Trogoderma granarium* was found among all the treatments tested. Highest mortality was recorded for combined treatments of *B. bassiana* and DE (1×10^{7} conidia/ml + 100 ppm), while the lowest was recorded for *B. bassiana* (1×10^{6} conidia/ml). After 10 days of exposure, also significant differences in mortality levels were recorded for all the treatments. 100% mortality was recorded in combined treatments of *B. bassiana* and DE (1×10^{7} conidia/ml + 100 ppm). Similar trend in mortality was recorded after 10 days as at 5 days. After last count 100% mortality was recorded for highest dose rate of *B. bassiana* and both DE concentrations (1×10^{7} conidia/ml + 50 ppm and 1×10^{7} conidia/ml + 100 ppm).

After 5 days of exposure significant difference in mortality of *Trogoderma granarium* was found among all the treatments. Highest mortality (71.47±2.09) was recorded for combined treatments of *B. bassiana* and DE (1×10^{7} conidia/ml + 100 ppm), while the lowest (4.91±0.63%) was recorded for *B. bassiana* (1×10^{6} conidia/ml). After 10 days of exposure also significant difference in mortality levels were recorded for all the treatments. Maximum (97.48±1.21%) mortality was recorded in combined treatments of *B. bassiana* and DE (1×10^{7} conidia/ml + 100 ppm). After last count 100% mortality was recorded for highest dose rate of *B. bassiana* and DE (1×10^{7} conidia/ml + 100 ppm).

Highest progeny emergence of *Tribolium castaneum* was recorded in control treatments, while the lowest was recorded for highest dose rate of *B. bassiana* and DE in combination (1×10^{7} conidia/ml + 100 ppm) (Fig. 1). Highest progeny emergence of *Trogoderma granarium* was recorded in control treatments, while the lowest was recorded for highest dose rate of *B. bassiana* and DE in combination (1×10^{7} conidia/ml + 100 ppm) (Fig. 1).

The findings of our study suggested that combined use of both *B. bassiana* and DE can be an effective mean to control *Tribolium castaneum* and *Trogoderma granarium* than their sole application. The main reason of enhanced effect is their physical mode of action, and most importantly no need to ingest to show effectiveness (Lord, 2001, 2005). Lord (2001) first time reported the insecticidal effect using DE formulation Protect-It against *R. dominica*, and *Oryzophilus surinamensis* (L.) (Coleoptera: Silvanidae). Similar findings were also reported by Akbar *et al*. (2004) against larvae of *Tribolium castaneum.* Similar results were also observed by Batta (2004), who used both *Metarhizium anisopliae* (Metschinkoff) Sorokin (Deuteromycotina: Hyphomycetes) and dust composed of several inert ingredients such as oven ash and charcoal. He subjected that the addition of these ingredients, increased the efficacy of fungus against *Sitophilus oryzae* (Linnaeus) (Coleoptera: Curculionidae) and suggested that these materials show a desiccant action. It is also suggested that the addition of DE may remove epicuticular lipid layers from the insect body that help fungal conidia to germinated fungal conidia to enter more easily into the insect body (Lord, 2001).

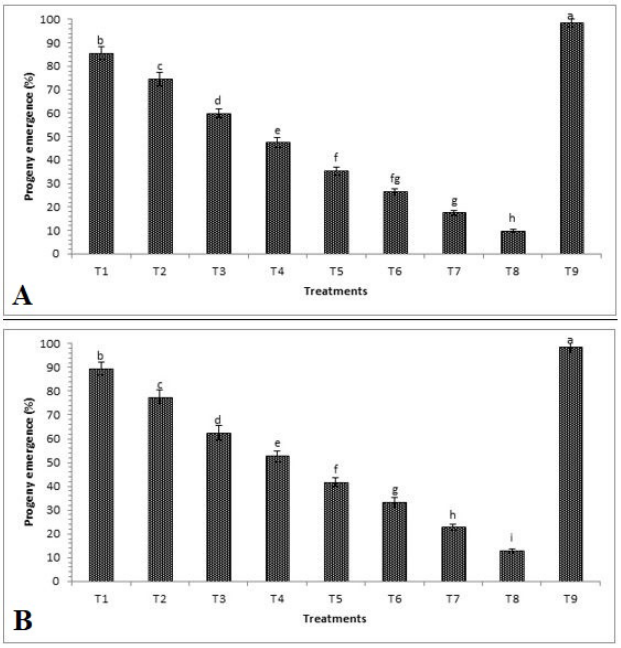

Fig. 1. Mean progeny emergence (%±SE) of *Tribolium castaneum* (A) and *Trogoderma granarium* (B) after 50 days post last exposure of application treated with *Beauveria bassiana* and diatomaceous earth (means followed by the same letters with in treatments are statistically same; HSD test at P = 1%). T_1= *B. bassiana* @ 1×10^{6} conidia/ml, T_2= *B. bassiana* @ 1×10^{7} conidia/ml, T_3= DE @ 50 ppm, T_4= DE @ 100 ppm, T_5= *B. bassiana* @ 1×10^{6} conidia/ml + DE @ 50 ppm, T_6= *B. bassiana* @ 1×10^{6} conidia/ml + DE @ 100 ppm, T_7= *B. bassiana* @ 1×10^{7}conidia/ml + DE @

50 ppm, T_8= *B. bassiana* @ 1×10^7 conidia kg^{-1} + DE @ 100 ppm and T_9= Control.

Table I. Effect of *Beauveria bassiana* and diatomaceous earth on the mortality (%±SE) of *Tribolium castaneum* and after 5, 10 and 15 days of application (means followed by the same letter are not significantly different; HSD test at *P* = 1%).

Treatment	*Beauveria bassiana* exposed for			*Trogoderma granarium* exposed for		
	5-Days	10-Days	15-days	5-Days	10-Days	15-days
1 × 10^6 conidia/ml	6.12±0.93fg	15.49±1.06fg	31.58±1.76e	4.91±0.63gh	11.71±1.11fg	26.31±1.42de
1 × 10^7 conidia/ml	9.97±1.21f	22.54±1.12f	40.21±1.87d	7.03±0.97fg	16.26±1.38f	34.58±2.58d
50 ppm	15.25±1.34e	31.54±1.89e	52.73±2.43cd	12.71±1.23ef	24.08±1.92de	46.12±2.98cd
100 ppm	24.48±1.54cde	43.369±32.17d	65.91±2.75c	19.45±1.48de	35.74±2.12d	58.24±2.37c
1 × 10^6 conidia/ml + 50 ppm	36.15±1.32cd	57.62±2.827c	81.38±2.45b	28.17±1.34d	47.85±2.48c	72.01±2.71bc
1 × 10^6 conidia/ml + 100 ppm	45.72±2.14c	72.37±3.12b	98.20±1.17ab	40.54±1.78c	61.37±2.59bc	85.35±2.44b
1 × 10^7 conidia/ml + 50 ppm	61.18±2.23b	91.32±2.459ab	100.0±0.00a	54.92±1.78b	79.19±2.81b	100.00±0.00a
1 × 10^7 conidia/ml + 100 ppm	78.63±2.47a	100.00±0.00a	-	71.47±2.09a	97.48±1.21a	-
F	34.4	63.6	30.9	39.9	56.6	82.7
P	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01

Moreover, the degree of conidial attachment also increase when entomopathogenic fungi is integrated with DE. Akbar *et al*. (2004) reported more *B. bassiana* conidial attachment to the larvae of *Tribolium castaneum* when the fungus was applied with DE. Athanassiou and Steenberg (2007) declared that the application of *B. bassiana* alone is less effective and the toxicity increased when combined with PyriSec®, SilicoSec® and Insecto® DE formulations. It is also manifest from the results that DE enhanced the toxicity of fungal isolates against *Tribolium castaneum*, *R. dominica* and *O. surinamensis*. This is in conformity with the results of Kavallieratos *et al*. (2006); that the accumulation of DE Protect-It synergized the efficiency of *M. anisopliae*. Keeping in view the synergistic effect of both substances several inert resources such as clays or silicas, have been used to enhance the conidial viability of the *Metarhizium flavoviride,* Gams and Rozsypal (Deuteromycotina: Hyphomycetes) (Moore *et al*., 1996; Moore and Higgins, 1997; Horaczek and Viernstein, 2004). Desiccant dusts are being used in the fungal formulations in order to dry conidia up to the levels of 4-5% r.h., which makes conidia robust and capable of long storage (Hedgecock *et al*., 1995; Moore and Higgins, 1997). From commercial production point of view there is increasing need to control the permanence of entomopathogenic fungal formulations (Moore *et al*., 2000) but the influence of these extracts on the insecticidal effect of the fungus has not been assessed in detail.

In our case presence of both fungus and DE gave higher mortality as compare to the application of fungus or DE, and increased with the increase in in doe rates of both fungal spores and DE. This fact may suggest that fungal conidia benefit the DE efficacy only when conidial concentration exceeds. In our case mortality was high because experiment was conducted on sound grains instead of wheat flour presence of flour particles decrease the attachment of DE particles on the insect's cuticle or remove from their bodies. Contrarily, Moore and Higgins (1997) suggested that the presence of the certain clays had a harmful effect on the germinating conidia of *M. flavoviride* but in our case a positive influence in conidial viability have occurred.

CONCLUSION

It is concluded that *B. bassiana* and DEs can be successfully used against stored product pests for longer period of time, and both agents can be a successful alternate to the conventional grain protectants, and could be an effective component of IPM in stored grains.

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Ethical statement

No animals were involved for this study.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Aathanassiou, C.G., Kavallieratos, N.G., Economou, L.P., Dimizas, C.B., Vayias, B.J., Tomanovic, S.A. and Milutinovic, M., 2005. Persistence and efficacy of three diatomaceous earth formulations against *Sitophilus oryzae* (Coleoptera: Curculionidae) on wheat and barley. *J. econ. Ent*., **98**: 1404-1412. https://doi.org/10.1603/0022-0493-98.4.1404

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. *J. econ. Ent*., **18**: 265-267. https://doi.org/10.1093/jee/18.2.265a

Akbar, W., Lord, J.C., Nechols, J.R., and Howard, R.W., 2004. Diatomaceous Earth Increases the efficacy of *Beauveria bassiana* against *Tribolium castaneum* larvae and increases conidia attachment. *J. econ. Ent*., **97**: 273-280. https://doi.org/10.1093/jee/97.2.273

Arthur, F.H., 2004. Evaluation of a new insecticide formulation (F2) as a Inertant of stored wheat, maize, and rice. *J. Stored Prod. Res*., **40**: 317–330. https://doi.org/10.1016/S0022-474X(03)00023-7

Athanassiou, C.G., and Steenberg, T., 2007. Insecticidal effect of *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreaes) in combination with three diatomaceous earth formulations against (L.) (Coleoptera: Curculionidae). *Biol. Contr*., **40**: 411–416. https://doi.org/10.1016/j.biocontrol.2006.12.001

Athanassiou, C.G., Phillips, T.W., and Wakil, W., 2018. Biology and control of Khapra beetle, *Trogoderma granarium*, a major quarantine threat to global food security. *Annu. Rev. Ent*., https://doi.org/10.1146/annurev-ento-011118-111804

Batta, Y.A., 2004. Control of rice weevil (*Sitophilus oryzae* L., Coleoptera: Curculionidae) with various formulations of *Metarhizium anisopliae*. *Crop Inertion*, **23**: 103-108. https://doi.org/10.1016/j.cropro.2003.07.001

Borzoui, E., Naseri, B., and Namin, F.R., 2015. Different diets affecting biology and digestive physiology of the Khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae). *J. Stored Prod. Res*., **62**: 1–7. https://doi.org/10.1016/j.jspr.2015.03.003

Boyer, L., Cermolacce, M., Dassa, D., Fernandez, J., Boucekine, M., Richieri, R., Vaillant, F., Dumas, R., Auquier, P., and Lancon, C., 2012. Neurocognition, insight and medication nonadherence in schizophrenia: A structural equation modeling approach. *PLoS One*, **7**: e47655. https://doi.org/10.1371/journal.pone.0047655

Fields, P., and Korunic, Z., 2000. The effect of grain moisture content and temperature on population growth of three *Liposcelis* species (Psocoptera: Liposcelidae) infesting wheat on 12 Kansas farms. *J. Stored Prod. Res*., **37**: 221–229.

Fields, P., and Korunic, Z., 2000. The effect of grain moisture content and temperature on the efficacy of diatomaceous earths from different geographical locations against stored-product beetles. *J. Stored Prod. Res*., **36**: 1–13. https://doi.org/10.1016/S0022-474X(99)00021-1

Finkelman, S., Navarro, S., Isikber, A., Dias, D., Azrieli, A., Ridner, M., Lotan, Y., and Debruin, T., 2002. Application of vacuum to sealed flexible containers: A viable alternative to disinfestation of durable commodities with methyl bromide. In: *Proc. Int. Conf. on Alternatives to methyl bromide* (eds. T.A. Batchelor and J.M. Bolivar). pp. 145-149.

Hedgecock, S., Moore, D., Higgins, P.M., and Prior, C., 1995. Influence of moisture content on temperature tolerance and storage on *Metarhizium flavoviride* conidia in an oil formulation. *Biocontr. Sci. Technol*., **5**: 371-377. https://doi.org/10.1080/09583159550039828

Horaczek, A., and Viernstein, H., 2004. Comparison of three commonly used drying technologies with respect to activity and longevity of aerial conidia of *Beauveria brongniartii* and *Metarhizium anisopliae*. *Biol. Contr*., **31**: 65–71. https://doi.org/10.1016/j.biocontrol.2004.04.016

Karunakaran, C., Jayas, D.S. and White, N.D.G., 2004.Identification of wheat kernels damaged by the red flour beetle using X-ray image. *Biosyst. Eng*., **87**: 267-274. https://doi.org/10.1016/j.biosystemseng.2003.12.002

Kavallieratos, N.G., Athanassiou, C.G., Michalaki, M.P., Batta, Y.A., Rigatos, H.A., Pashalidou, F.G., Balotis, G.N., Tomanovic, Z., and Vayias, B.J., 2006. Effect of the combined use of *Metarhizium anisopliae* (Metschinkoff) Sorokin and diatomaceous earth for the control of threestored product beetle species. *Crop Inertion*, **25**: 1087–1094. https://doi.org/10.1016/j.cropro.2006.02.009

Korunic, Z., Cenkowski, S. and Fields, P., 1998. Grain bulk density as affected by diatomaceous earth and application method. *Postharvest. Biol. Technol*., **13**: 81-89. https://doi.org/10.1016/S0925-5214(97)00076-8

Korunic, Z., Fields, P.G. Kovacs, M.I.P., Noll, J.S., Lukow, O.M., Demianyk, C.J. and Shibley, K.J., 1996. The effect of diatomaceous earth on grain quality. *Postharvest Biol. Technol.,* **13:** 373-387. https://doi.org/10.1016/S0925-5214(96)00038-5

Lord, J.C., 2001. Desiccant dusts synergize the effect of *Beauveria bassiana* (Hyphomycetes: Moniliales) on stored-grain beetles. *J. econ. Ent*., **94**: 367–372. https://doi.org/10.1603/0022-0493-94.2.367

Lord, J.C., 2005. Low humidity, moderate temperature, and desiccant dust favor efficacy of *Beauveria bassiana* (Hyphomycetes: Moniliales) for the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bruchidae). *Biol. Contr*., **34**: 180–186. https://doi.org/10.1016/j.biocontrol.2005.05.004

Michalaki, M.P., Athanassioua, C.G., Kavallieratos, N.G., Batta, Y.A. and Balotis, G.N., 2006. Effectiveness of *Metarhizium anisopliae* (Metschinkoff) Sorokin applied alone or in combination with diatomaceous earth against three stored grain beetle species. *Crop Inertion*, **25**: 1087–1094. https://doi.org/10.1016/j.cropro.2006.02.009

Moore, D., Douro-Kpindou, O.K., Jenkins, N.E., and Lomer, C.J., 1996. Effects of moisture content and temperature on storage of *Metarhizium flavoviride* conidia. *Biocontr. Sci. Technol*., **6**: 51–61. https://doi.org/10.1080/09583159650039520

Moore, D., and Higgins, P.M., 1997. Viability of stored conidia of *Metarhizium flavoviride* Gams and Rozsypal, produced under different culture regimes and stored with clays. *Biocontr. Sci. Technol*., **7**: 335–343. https://doi.org/10.1080/09583159730749

Moore, D., Lord, J.C. and Smith, S.M., 2000. Pathogens. In: *Alternatives to pesticides in stored- product IPM* (eds. B.H. Subramanyam and D.W. Hagstrum). Kluwer Academic Publishers, Dordreecht, pp. 193–227. https://doi.org/10.1007/978-1-4615-4353-4_8

Seifi, P., Henry, R.S., and Ingham, J.M., 2015. *Preliminary test results of precast concrete panels with grouted connections*. Proceedings of the NZSEE Annual Conference, Rotorua, April 10-12.

Sokal, R.R., and Rohlf, F.J., 1995. *Biometry,* 3rd Eds. Freedman and Company, New York.

Subramanyam, B., and Roesli, R., 2000. Inert dusts. In: Alternatives to pesticides in stored product IPM (eds. B. Subramanyam and D.W. Hagstrum). Kluwer Academic Publishers, Norwell, MA, USA, pp. 321–380. https://doi.org/10.1007/978-1-4615-4353-4_12

Upadhyay, R.K. and Ahmad, S., 2011. Management strategies for control of stored grain insect pests in farmer stores and public ware houses. *World J. agric. Sci*., **7**: 527-549.

Vassilakos, T.N., Athanassiou, C.G. Kavallieratos, N.G. and Vayias, B.J., 2006. Influence of temperature on the insecticidal effect of *Beauveria bassiana* in combination with diatomaceous earth against *Rhyzopertha dominica* and *Sitophilus oryzae* on stored wheat. *Biol. Contr*., **38**: 270–281. https://doi.org/10.1016/j.biocontrol.2006.03.009

Vayias, B.J., Athanassiou, C.G., Kavallieratos, N.G., Tsesmeli, C.D. and Buchelos, C.T., 2006. Persistence and efficacy of two diatomaceous earth formulations and a mixture of diatomaceous earth with natural pyrethrum against *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) on wheat and maize. *Pest Manage. Sci*., **62**: 456–464. https://doi.org/10.1002/ps.1185

Wakil, W., Riasat, T., Ghazanfar, M.U., Kwon, Y.J. and Shaheen, F.A., 2011. Aptness of *Beauveria bassiana* and enhanced diatomaceous earth (DEBBM) for control of *Rhyzopertha dominica (*F.). *Entomol. Res*., **41**: 233–241. https://doi.org/10.1111/j.1748-5967.2011.00342.x