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# Control of *Acanthoscelides obtectus* with *Trichoderma harzianum* applied alone or in combination with diatomaceous earth on a stored common bean

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**Abstract:** A laboratory assay was designed to determine the insecticidal efficacy of *Trichoderma harzianum* Rifai and diatomaceous earth (DE) against *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae: Bruchinae). The fungus *T. harzianum* was applied at 0.0, $3.3 \times 10^6$, $6.6 \times 10^6$ and $2.1 \times 10^7$ spores/kg of the common bean individually and mixed with 0, 200, 400 and 800 ppm of DE. The mortality counts were made after 1, 2, 4 and 7 days. All of the dead adults were removed after each count, and the vials were kept for the next 60 days to assess the emergence of any progeny. The highest mortality (93.88%) was achieved after 7 days using 800 ppm of DE and $2.1 \times 10^7$ spores/kg of *T. harzianum* and it suppressed emergence of the second generation after 60 days of treatment. These results concluded that DE can be used successfully along with *T. harzianum* against *A. obtectus* in stored common beans.

**Keywords:** Chrysomelidae; *Phaseolus vulgaris*; dry bean beetle; fungi; Bruchinae

*Acanthoscelides obtectus* Say (Coleoptera: Chryso melidae: Bruchinae), is an insect pest that causes serious damage to common beans, *Phaseolus vulgaris* Linnaeus (Thakur 2012; Vilca Mallqui et al. 2013; Gad 2019). *A. obtectus* initiates the infestation in the field and continues the infestation during storage. Populations of *A. obtectus* can grow exponentially and destroy stored grains within a few months (Gad & Abied 2019). *A. obtectus* proliferation is usually suppressed in large storage facilities by using chemical insecticides, such as phosphine and pyrethroids (Daglish et al. 1993). The application of these chemicals has increased concerns over insecticide resistance, human health and environmental contamination (Daglish 2008), and has been questioned by environmental movements that seek sustainable alternatives to control insects (Regnault-Roger et al. 2012). Diatomaceous earth (DE) is the fossilised remains of diatoms (phytoplankton); a number of formulations have been introduced throughout the world and used effectively against a wide range of stored-product insects (Ling et al. 2000; Wakil et al. 2010). DEs have advantages over standard insecticides, like it is non-toxic, the food is then free of toxic residues, and it is easily removed from the grains by washing during milling, even though only a very small quantity of grains in the world are being dusted with DE (Korunic et al. 1996; Dusan & Korunic 2018). DE kills insects by abrading their cuticles, adsorbing the cuticular lipids and causing water loss through desiccation (Korunic 1998). Although DE is safe on stored products and effective at high doses

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that often exceed 1.0 to 3.5g/kg grain (Fields & Korunic 2000), it negatively affects the bulk density of the grains, the flowability, and it leaves visible dust residues (Golob 1997; Korunic et al. 1998). Researchers have concluded after several studies on the combined effect between DE and other control methods that it offers many options including a degree of efficacy comparable with chemical pesticides and compatibility with most of the other components of IPM (integrated pest management) strategies. According to several reports, the combined use of DE and fungi has proven to have a higher insecticidal effect than when it was exploited when fungal preparations were used alone and when decreased DE dosage rates were used (Akbar et al. 2004; Kavallieratos et al. 2006).

Fungi have been identified as effective alternatives to traditional residual insecticides due to their high level of virulence against stored-product insects and low mammalian toxicity (Batta 2004; Wakil & Ghazanfar 2010). They also have the ability to reintroduce the inoculum from dead cadavers and can persist in grains for long periods of time (Athanassiou et al. 2008). Furthermore, the insecticidal activity of the fungal conidia may be able to be augmented with different carriers, such as inert dusts, mineral oils, and botanical insecticides (Akbar et al. 2005).

*Trichoderma harzianum* Rifai has been used for the biological control of different pests of crop plants and for its ability to produce effective antimicrobial agents to control plant diseases. *T. harzianum* is an environmentally friendly plant health management method compared with the use of chemical pesticides. This approach has the advantages of being pollution-free, residue-free, difficult to produce resistance to it, and conducive to human and animal safety, as well as having the advantage of helping environmental protection (Fiorentino et al. 2018).

The spore suspension and metabolites of this fungus showed a high pathogenic effect on the Egyptian cotton leaf worm, *Spodoptera littoralis* larvae (Ashraf & Momein 2007) and *A. obtectus* (Rodríguez-González et al. 2017, 2018, 2019). The fungi can be successfully blended with DE that may enhance the efficacy of the fungi against different stored-product insects, as previously studied by several researchers (Vassilakos et al. 2006; Athanassiou et al. 2008). The use of DE and fungi in this way not only decreases the application rates of DE, but also provides an easy way to incorporate the fungal conidia in the host commodity. This study was, therefore, aimed at investigating the insecticidal effect of a local fungal isolate of *T. harzianum* alone and in combination with a DE formulation against *A. obtectus*.

## MATERIAL AND METHODS

### Insect collection and rearing

*A. obtectus* adults were collected from naturally infested common beans. The specimens of *A. obtectus* were maintained at the Laboratory of Plant Protection Department, the Faculty of Agriculture, Al-Azhar University, Egypt. The common bean (*Phaseolus vulgaris*) (var. Nebraska) was used to feed the *A. obtectus* adults, which were placed into glass jars (1 L) and covered with a cloth to allow air to enter. Every three days, the A. *obtectus* adults were removed from the glass jars with the beans to maintain a population of young adults (1–3 days old) for use in the experiments. The *A. obtectus* adults, before and after the treatments, were kept in a chamber under laboratory conditions at 28 ± 2 °C and 70 ± 5% RH (relative humidity).

### Treatments materials

*DE formulation.* The DE formulation was obtained from Al-Ahram Mining Co., Giza, Egypt and the composition of the DE formulation was identified at the Central laboratory sector (XRF LAB) – The Egyptian Mineral Resources Authority (EMRA) - the Ministry of Petroleum- Egypt, which was used in the tests. It was composed of 46.37% of $SiO_2$, 8.04% of $Al_2O_3$, 1.23% of $Fe_2O_3$, 1% of $Na_2O$, 17.72% of CaO, 1.68% of MgO and 0.68% of $K_2O$.

*Fungal culture.* The *T. harzianum* strain that was isolated from the Egyptian soil, was identified and maintained in the Plant Pathology Department, the Faculty of Agriculture, Al-Azhar University, Egypt. The fungal isolate was grown on a potato dextrose agar (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). The fungal cultures were incubated at 24 ± 1 °C, 60 ± 5% RH, and placed under a photoperiod of 16 h of light (luminous intensity of 1 000 lx) and 8 h of darkness for 7 days. To collect the spores, 10 ml of autoclaved distilled water was poured over the plates, and the surface of the medium was scrubbed with a brush. It was then placed under a microscope at a magnification of × 200. The spore concentration was determined using a haemocytometer and adjusted to $3.3 \times 10^6$, $6.6 \times 10^6$ and $2.1 \times 10^7$ spores/mL. The spore suspensions were placed in microcentrifuge tubes (1.5 mL; Eppendorf

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AG, Germany), which were stored at 20 ± 1 °C for a few hours until use.

*Bioassay*. One-kg of the common bean grain (var. Nebraska) was prepared to apply the different treatments, and then the treatments were evaluated, namely: the fungus alone ($3.3 \times 10^6$, $6.6 \times 10^6$ and $2.1 \times 10^7$ spores/kg), the DE alone (200, 400 and 800 mg/kg) and combinations of each fungus with each DE. For each treatment, three 50-g common bean samples were treated with the above fungus/DE combinations. An additional series of samples with the untreated common bean was prepared, which served as a control. The samples were treated individually with the respective fungus/DE quantity. Each sample was placed in a plastic jar (5 cm in diameter × 7.9 cm in height) and the jars were shaken manually for approximately 5 min to attain the equal dispersal of the dust and or fungal spores on the whole grain mass. Then, 10 sexed adults (5♂ + 5♀) of *A. obtectus* were introduced into each sample, and all the samples were placed in incubators set at 28 ± 2 °C and 70 ± 5% RH. Each treatment was repeated independently three times. The mortality data were assessed after 1, 2, 4 and 7 days of exposure by inverting all of the material into trays to separate the dead and live insects. The dead beetles were removed after each reading, and the live beetles were placed back into the jars. After 12 days of exposure, all the insects were eliminated, and the treated units were held under the same environmental conditions for an additional 60 days to record the progeny production by counting the insects in the treated and control tests. The grains were sieved with different mesh sizes to remove the insects only, while the dust was returned to each vessel. After the progeny were counted, the whole grains and damaged grains were counted and weighed on an analytical balance for each sample (50 g) of treated or untreated common bean grains to obtain the damaged grain and weight loss percentages.

### Data analysis

The mortality counts were corrected using Abbott's (1925) formula. The data were arcsine transformed prior to the analysis. All the data were analysed using SPSS 21.0 (version 16), with insect mortality being the response variable and the fungal/DE treatment as the main effect. For the progeny production counts, the damaged grain and weight loss, the same procedure was followed, with the number of progeny, damaged grain and weight loss as the response variables being subjected to an ANOVA, and the means were compared with the Student–Newman–Keuls (SNK) test with a significance level of 0.05

## RESULTS

### Effect of the different treatments on the mortality of *A. obtectus*

The adult mortality of *A. obtectus* was 3.40% after 1 day of exposure to the highest application of DE (800 ppm) and reached 50.00% after 7 days of exposure. In the common bean grains treated with *T. harzianum* alone, the adult mortality was (8.58%) at the highest application ($2.10 \times 10^7$ spores/kg of grains) after 1 day of exposure and reached 57.32% after 7 days of exposure. The combined effect of the DE and *T. harzianum* was enhanced by increasing the dosage rate, and significantly more beetles were dead after 7 days of exposure at $P < 0.01$ ($F = 12.17$; $df = 15.00$). In the application of 800 ppm of DE with $2.10 \times 10^7$ spores/kg on the common bean grains, the adult beetle mortality reached 93.88% (Table 1). The combination of 200 and 400 ppm of DE with all the fungal dosage rates was less effective than that of 800 ppm with all the fungal dosage rates. However, the adult mortality in the combined treatments was high compared with that in the treatments with the DE and fungus alone.

### Progeny production and reduction percentage

The progeny production of *A. obtectus* was higher in the common beans treated with DE alone than in the *T. harzianum*-treated common bean after 60 days of treatment (Table 2). In the DE-only treatment at 800 ppm, 49.34 adults/jar emerged, while the value for the fungus-only treatment was 33.00 adults/jar at $2.1 \times 10^7$ spores/kg of the common bean. In the common bean treated with the DE/*T. harzianum* combination, the progeny production was significantly suppressed, particularly when these compounds were applied at their highest dosage rates (400 ppm of DE with $2.10 \times 10^7$ spores/kg, 800 ppm of DE with $3.3 \times 10^6$ spores/kg, 800 ppm of DE with $6.6 \times 10^6$ spores/kg and 800 ppm of DE with $2.10 \times 10^7$ spores/kg).

Table 2 also shows the reduction in the adult emergence from *A. obtectus* exposed to the common bean treated with *T. harzianum* alone and mixed with DE at the different concentrations. The adult emergence of *A. obtectus* was signifi-

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Table 1. The mean mortality (% ± SE) of the *Acanthoscelides obtectus* adults exposed for 1, 2, 4 and 7 days on the common bean treated with *Trichoderma harzianum* alone and mixed with diatomaceous earth at the different concentrations

| Treatment | Concentration | Adult mortality after | | | |
|---|---|---|---|---|---|
| | | 1 day | 2 days | 4 days | 7 days |
| 0 | 0 | 0.00 ± 0.00[c] | 0.00 ± 0.00[f] | 0.00 ± 0.00[g] | 0.00 ± 0.00[g] |
| DE 1 | 200 mg/kg | 0.00 ± 0.00[c] | 6.67 ± 3.34[ef] | 10.00 ± 2.89[ef] | 20.00 ± 2.90[fg] |
| DE 2 | 400 mg/kg | 3.30 ± 3.40[c] | 6.70 ± 3.53[ef] | 10.00 ± 2.90[ef] | 40.00 ± 5.78[def] |
| DE 3 | 800 mg/kg | 3.40 ± 3.30[c] | 10.00 ± 5.78[cde] | 20.00 ± 5.03[cdef] | 50.00 ± 5.80[cde] |
| Th 1 | $3.3 \times 10^6$ | 0.00 ± 0.00[c] | 6.60 ± 1.33[ef] | 13.00 ± 06.67[ef] | 20.00 ± 5.05[fg] |
| Th 2 | $6.6 \times 10^6$ | 6.70 ± 1.67[bc] | 6.70 ± 1.16[ef] | 13.40 ± 1.67[fg] | 33.40 ± 3.13[ef] |
| Th 3 | $2.1 \times 10^7$ | 8.58 ± 0.25[ab] | 14.14 ± 2.53[abcde] | 34.59 ± 5.16[abcd] | 57.32 ± 7.89[bcd] |
| Th 1+ DE 1 | | 10.00 ± 1.73[ab] | 10.00 ± 1.67[bcde] | 13.40 ± 3.34[def] | 23.40 ± 3.00[f] |
| Th 2 + DE 1 | | 10.00 ± 1.67[ab] | 10.00 ± 1.67[bcde] | 20.00 ± 3.30[cdef] | 44.00 ± 3.40[def] |
| Th 3 + DE 1 | | 12.22 ± 2.22[a] | 19.00 ± 1.10[abcd] | 37.80 ± 2.20[abcd] | 61.11 ± 12.54[bcd] |
| Th 1 + DE 2 | | 10.83 ± 0.83[ab] | 20.00 ± 1.70[abc] | 25.83 ± 5.84[bcde] | 50.00 ± 7.27[cde] |
| Th 2 + DE 2 | | 16.70 ± 3.40[a] | 21.66 ± 5.01[abcd] | 26.70 ± 3.40[bcde] | 56.70 ± 6.60[bcde] |
| Th 3 + DE 2 | | 20.00 ± 5.80[a] | 23.00 ± 3.33[abc] | 46.66 ± 4.41[ab] | 64.00 ± 1.70[bcd] |
| Th 1 + DE 3 | | 10.00 ± 1.16[ab] | 20.00 ± 5.00a[bcd] | 36.66 ± 8.83[abcd] | 73.00 ± 3.00[bc] |
| Th 2 + DE 3 | | 20.00 ± 4.05[a] | 30.00 ± 5.00[ab] | 40.00 ± 5.80[abc] | 80.00 ± 4.62[ab] |
| Th 3 + DE 3 | | 20.00 ± 3.47[a] | 33.50 ± 3.34[a] | 60.00 ± 4.40[a] | 93.88 ± 3.10[a] |
| *F* | | 9.08 | 4.87 | 6.15 | 12.17 |
| *P* | | < 0.01 | < 0.01 | < 0.01 | <0.01 |
| *df* | | 15 | 15 | 15 | 15 |

DE 1 – 200 mg/kg; DE 2 – 400 mg/kg; DE 3 – 800 mg/kg; Th 1 – *T. harzianum* at $3.3 \times 10^6$ spore/kg; Th 2 – *T. harzianum* at $6.6 \times 10^6$ spores/kg; Th 3 – *T. harzianum* at $2.1 \times 10^7$ spores/kg; Th 1+ DE1 – *T. harzianum* at $3.3 \times 10^6$ spore/kg + 200 mg/kg; Th2 + DE1– *T. harzianum* at $6.6 \times 10^6$ spores/kg +200 mg/kg; Th 3 + DE 1 – *T. harzianum* at $2.1 \times 10^7$ spores/kg + 200 mg/kg; Th 1 + DE 2 – *T. harzianum* at $3.3 \times 10^6$ spores/kg + 400 mg/kg; Th 2 + DE2 – *T. harzianum* at $6.6 \times 10^6$ spores/kg + 400 mg/kg; Th 3 + DE 2 – *T. harzianum* at $2.1 \times 10^7$ spores/kg + 400 mg/kg; Th 1 + DE 3 – *T. harzianum* at $3.3 \times 10^6$ spores/kg + 800 mg/kg; Th 2 + DE 3 – *T. harzianum* at $6.6 \times 10^6$ spores/kg + 800 mg/kg; Th 3 + DE 3 – *T. harzianum* at $2.1 \times 10^7$ spores/kg + 800 mg/kg; values within a column sharing the same letter – not significantly different at the ($P < 0.05$) probability level; *F* – *F*-test; *df* – degrees of freedom

cantly reduced in the common beans treated with the applied compounds compared to the untreated common bean, particularly at the highest dosage rates, with a reduction of 99.38% with the application of 800 ppm of DE with $2.10 \times 10^7$ spores/kg of the common bean grains ($F$ =137.36; $P \leq 0.001$; $df$ = 15.00).

**Grain damage and weight loss**

The percentages of the insect-damaged grains after the treatment of the common bean with *T. harzianum* alone and mixed with DE at the different concentrations are presented in Figure 1. The highest percentage of the damaged grain was found in the untreated common bean (85.22%). The least grain damage was found when 400 ppm of DE with $2.10 \times 10^7$ spores/kg, 800 ppm of DE with $3.3 \times 10^6$ spores/kg, 800 ppm of DE with $6.6 \times 10^6$ spores/kg and 800 ppm of DE with $2.10 \times 10^7$ spores/kg were applied on the grains, and the grain damage percentage reached 8.12, 4.92, 4.88 and 2.28%, respectively. The combination of 200 and 400 ppm of DE with all the fungal dosage rates was less effective than that of 800 ppm with all the fungal dosage rates. The level of grain damage was sufficiently reduced in these combined treatments compared with the treatments with the DE and fungus alone ($F$ =16.10; $P \leq 0.001$; $df$ = 15.00).

The weight loss of the common beans in the control treatment was 43.45% after 60 days (Figure 2). The weight loss of the common beans treated with *T. harzianum* alone and mixed with DE was significantly reduced in all the treatments. Nearly the complete protection with a 1.00% weight loss

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Table 2. The mean progeny production (± SE) and the mean reduction (%) in *Acanthoscelides obtectus* exposed to the common bean treated with *Trichoderma harzianum* alone and mixed with diatomaceous earth at the different concentrations

| Treatment | Concentration | Progeny production (± SE) after 60 days | |
|---|---|---|---|
| | | No. progeny 50/g | Progeny reduction (%) |
| 0 | 0 | 600.00 ± 28.90[a] | 0.00 ± 0.00[j] |
| DE 1 | 200 mg/kg | 435.00 ± 2.89[b] | 27.50 ± 0.34[i] |
| DE 2 | 400 mg/kg | 390.00 ± 17.34[b] | 35.00 ± 2.04[i] |
| DE 3 | 800 mg/kg | 49.34 ± 1.77[fg] | 91.77 ± 0.21[de] |
| Th 1 | $3.3 \times 10^6$ | 240.00 ± 11.56[c] | 60.00 ± 1.36[h] |
| Th 2 | $6.6 \times 10^6$ | 142.00 ± 45.09[d] | 76.33 ± 5.31[g] |
| Th 3 | $2.1 \times 10^7$ | 33.00 ± 6.36[g] | 94.50 ± 0.75[bcd] |
| Th 1 + DE 1 | | 168.30 ± 10.15[d] | 71.94 ± 1.19[g] |
| Th 2 + DE 1 | | 130.00 ± 23.12[de] | 78.33 ± 2.72[fg] |
| Th 3 + DE 1 | | 18.40 ± 4.41[g] | 96.94 ± 0.52[abc] |
| Th 1 + DE 2 | | 86.00 ± 16.18[ef] | 85.66 ± 1.91[ef] |
| Th 2 + DE 2 | | 46.00 ± 17.69[fg] | 92.30 ± 2.08[cd] |
| Th 3 + DE 2 | | 14.60 ± 2.91[g] | 97.55 ± 0.35[ab] |
| Th 1 + DE 3 | | 12.00 ± 2.52[g] | 96.00 ± 0.30[ab] |
| Th 2 + DE 3 | | 7.00 ± 1.73[g] | 98.83 ± 0.20[a] |
| Th 3 + DE 3 | | 3.70 ± 0.89[g] | 99.38 ± 0.10[a] |
| *F* | | 113.78 | 137.36 |
| *P* | | < 0.01 | < 0.01 |
| *df* | | 15 | 15 |

DE 1 – 200 mg/kg; DE 2 – 400 mg/kg; DE 3 – 800 mg/kg; Th 1 – *T. harzianum* at $3.3 \times 10^6$ spore/kg; Th 2 – *T. harzianum* at $6.6 \times 10^6$ spores/kg; Th 3 – *T. harzianum* at $2.1 \times 10^7$ spores/kg; Th 1 + DE1 – *T. harzianum* at $3.3 \times 10^6$ spore/kg + 200 mg/ kg; Th2 + DE1– *T. harzianum* at $6.6 \times 10^6$ spores/kg +200 mg/kg; Th 3 + DE 1 – *T. harzianum* at 2.1 × 07 spores/kg +200 mg/kg; Th 1 + DE 2 – *T. harzianum* at $3.3 \times 10^6$ spores/kg + 400 mg/kg; Th 2 + DE2 – *T. harzianum* at $6.6 \times 10^6$ spores/kg + 400 mg/kg; Th 3 + DE 2 – *T. harzianum* at $2.1 \times 10^7$ spores/kg + 400 mg/kg; Th 1 + DE 3 – *T. harzianum* at $3.3 \times 10^6$ spores/kg + 800 mg/kg; Th 2 + DE 3 – *T. harzianum* at $6.6 \times 10^6$ spores/kg + 800 mg/kg; Th 3 + DE 3 – *T. harzianum* at $2.1 \times 10^7$ spores/kg + 800 mg/kg; values within a column sharing the same letter – not significantly different at the ($P < 0.05$) probability level; *F* – *F*-test; *df* – degrees of freedom

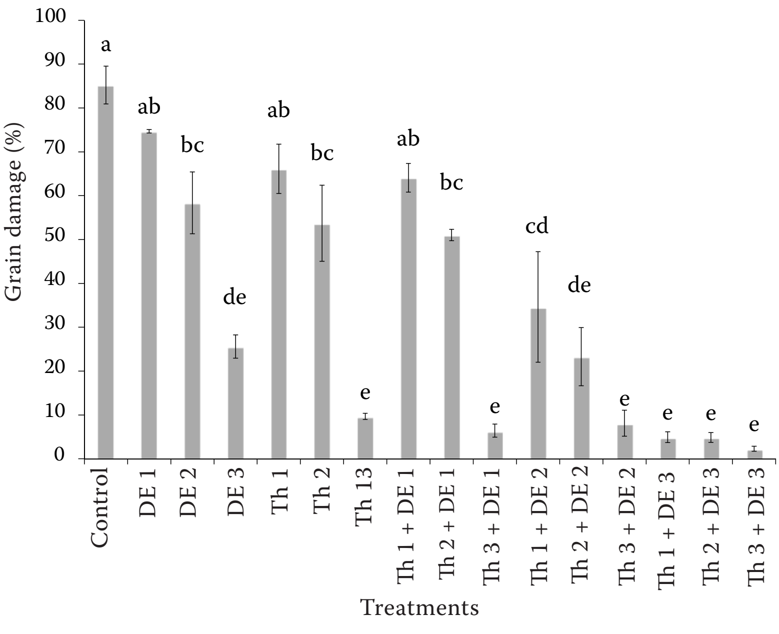


Figure 1. The grain damage percentage (% ± SE) of the common bean treated with *T. harzianum* alone and mixed with Diatomaceous earth at the different concentrations after 60 days of treatment

For abbreviations see Table 2

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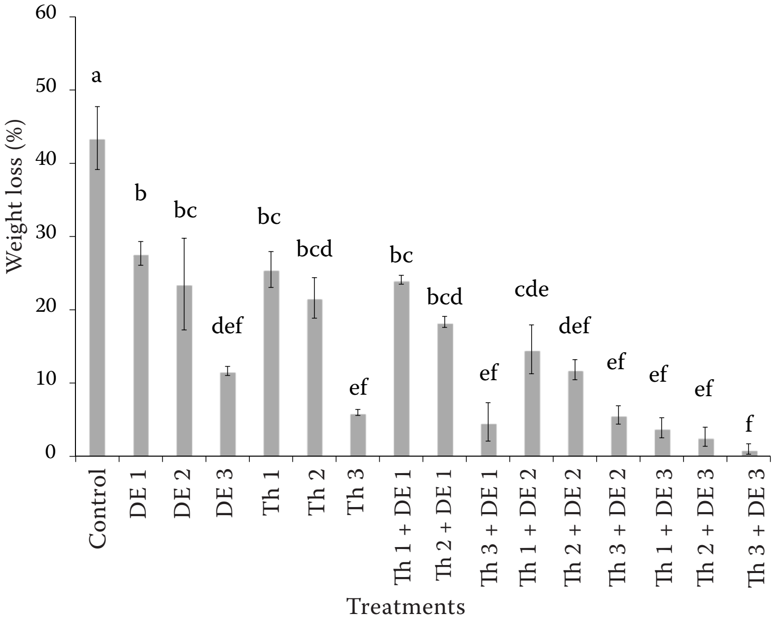


Figure 2. The grain weight loss percentage (% ± SE) of the common bean treated with *T. harzianum* alone and mixed with Diatomaceous earth at the different concentrations after 60 days of treatment

For abbreviations see Table 2

was obtained in the common bean treated with 800 pm of DE with 2.10 × $10^7$ spores/kg ($F$ =10.61; $P \leq 0.001$; $df$ = 15.00).

## DISCUSSION

The efficacy of various DE formulations and the different fungal species have been evaluated against stored-product insects in several studies (Vassilakos et al. 2006; Athanassiou et al. 2008; Rizwan et al. 2019). However, in the present study, the insecticidal effect of *T. harzianum* and DE combinations was investigated in the stored common bean for the first time. The results of the current study indicate that the treatment of the common bean with DE and or *T. harzianum* caused high insecticidal activity against *A. obtectus* with respect to the adult mortality. Our study also demonstrates that the mortality of *A. obtectus* adults treated with *T. harzianum* and DE combinations was significantly higher than the mortality of the adult beetles treated with the fungus or DE alone. The highest mortality (93.88%) was achieved after 7 days using a combination of 800 ppm of DE and 2.1 × $10^7$ spores/kg of *T. harzianum*. While the adult mortality reached 50% at DE 800 ppm and 57.32% in the concentration 2.1 × $10^7$ spores/kg of *T. harzianum*. Based on the results of the present study, DE and *T. harzianum* were effective against this insect pest, but its effectiveness varied notably according to the dosage rate. The beetles' mortality was increased by increasing the dosage rate of the DE and fungi alone or in the combinations. Moreover, the simultaneous use of DE increased the fungal efficacy at all the examined rates. These findings were supported with earlier studies in which the DE and other fungi species revealed the significant mortality of stored-product insects in the stored grains, such as Akbar et al. (2004) who also found similar results for *T. castaneum* larvae. Batta (2004) reported that the addition of several inert materials in a *Metarhizium anisopliae* dry conidial formulation increased the mortality of *Sitophilus oryzae* adults. Vassilakos et al. (2006) observed an additive effect when *B. bassiana* was used with the DE formulation in stored wheat against *R. dominica* and *S. oryzae* adults. Furthermore, Dal Bello et al. (2006) showed that *A. obtectus* insects were killed (100%) with DE alone or combined with wet or dry *B. bassiana* formulations. Kavallieratos et al. (2006) showed that the addition of DE synergised the effectiveness of *M. anisopliae*. They also stated that the dry conidia of *M. anisopliae* were significantly more effective against *S. oryzae*. Athanassiou and Steenberg (2007) declared that the application of *B. bassiana* alone is less effective and the toxicity increased when combined with DE on *S. ganarius***.** Riasat et al. (2011) described that the extended highest combined dosage rate of *B. bassiana* and DE achieved the maximum mortality of *R. dominica* (79.81%). Rizwan et al. (2019) showed that the highest concentrations of *B. bassiana* and DE in their combinations were more effective on *T. castaneum* and caused a mortality percentage of 88.13% after a 21-day exposure. DE enhanced the

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insecticidal effect of the spores of *T. harzianum* at the highest dosage. This enhancement action is due to the DE removes the insect's cuticular waxes and the insects die from desiccation (Ebeling 1971). DE may provide additional places for fungal spore penetration and germination and insect nutrients are consumed by the growing fungal hyphae; along with nutrient deficiency, various immunosuppressive and paralysing processes may also take place in the infected insects (Riasat et al. 2011).

The long-term stability of DE along with the ability of the fungus to be reintroduced in the vicinity is the key factor determining the effect on the progeny production of the insect pest (Athanassiou & Steenberg 2007). Based on the results of the current study, the DE, *T. harzianum* and their combinations showed a reduction in the *A. obtectus* progeny after 60 days of treatment with *T. harzianum* at $2.1 \times 10^7$ spores and 400 mg/kg, with *T. harzianum* at concentration $3.3 \times 10^6$ spores and 800 mg/kg, *T. harzianum* at $6.6 \times 10^6$ spores, 800 mg/kg and *T. harzianum* at $2.1 \times 10^7$ spores and 800 mg/kg being the most effective combinations. The reducing of progeny may be attributed to the quick killing of the *A. obtectus* adults after the application of the treatments or to the toxic effect of the DE, *T. harzianum* and their combinations on the eggs and newly hatched neonate larvae. In the present study, out of nine tested combinations, four treatments caused the near complete inhibition of the progeny of *A. obtectus* after 60 days of treatment which indicated the long-term protection for the stored common bean treated with these combinations. It is well known that the progeny reduction is more important than the mortality of the parental beetles introduced to the treated grain, since a grain protectant should protect the grain for a long storage period (Athanassiou et al. 2008). Previous studies indicated that the progeny production was higher in the case of *S. oryzae* treated with *M. anisopliae* and DE (Kavallieratos et al. 2006; Athanassiou et al. 2008). Similar results were obtained by Riasat et al. (2011) when wheat grains infested with *R. dominica* were exposed to *B. bassiana* alone and mixed with DE at different concentrations and found that the emergence of the progeny after 60 days of treatment was also highly suppressed (20.00 adults), when the maximum dosage rate of the synergised treatments was applied. Rizwan et al. (2019) showed that the maximum progeny production of *T. castaneum* (62.67 adults) was achieved in wheat grains treated with a low concentration of *B. bassiana* ($1 \times 10^6$ conidia/kg) alone, while the minimum progeny production (2.67 adults) was recorded in wheat grains treated with a high concentration of *B. bassiana* ($1 \times 10^8$ conidia/kg) and a DE (400 ppm) combination.

This study demonstrated that the tested treatments of *T. harzianum* and the DE combinations reduced the damage to the stored common bean caused by *A. obtectus* for 60 days, particularly when these combinations were applied at their highest dosage rates (400 ppm of DE with $2.10 \times 10^7$ spores/kg, 800 ppm of DE with $3.3 \times 10^6$ spores/kg, 800 ppm of DE with $6.6 \times 10^6$ spores/kg and 800 ppm of DE with $2.10 \times 10^7$ spores/kg). At the same time, the *T. harzianum* and DE did not affect the common bean grain weight at these combinations. The near complete protection of the treated common bean observed in this study may be attributed to the high mortality of *A. obtectus* adults after treatment with the tested *T. harzianum* and DE combinations at the highest concentrations. Similar results were obtained by Dal Bello et al. (2017), who evaluated the effect of *B. bassiana*, DE and fenitrothion on the grain damage, the germination power of the wheat and the bulk density for four months and found that *B. bassiana* mixed with DE reduced the damaged insect grains by 50% in comparison with both the fenitrothion and untreated wheat. Further studies are needed to explain the potential of *T. harzianum* against other stored-product insects not only under variable environmental conditions, but also as an admixture with certain other natural control measures.

## CONCLUSION

The results demonstrated that the highest dosage rate from the DE and *T. harzianum* combination can achieve the highest mortality of *A. obtectus* and suppress the progeny production of the second generation after 60 days of treatment. The results showed that DE can be used successfully along with *T. harzianum* against *A. obtectus* in the stored common beans. This study is novel on the basis of the use of a local fungal isolate of *T. harzianum* and its efficacy against *A. obtectus* alone and in combination with DE, which is reported here for the first time. This study suggests that using a combination of 800 ppm of DE and $2.1 \times 10^7$ spores/kg of *T. harzianum* provides the enhanced long-term management of *A. obtectus* and it had a lethal and a suppressive effect against the progeny production of the pest insect.

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