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Diatomaceous earth used against insect pests, applied alone or in combination with *Metarhizium anisopliae* and *Beauveria bassiana*

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Abstract: Laboratory bioassays were conducted to assess the insecticidal efficacy of the formulation SilicoSec® used alone or in combination with isolates of entomopathogenic fungi, *Metarhizium anisopliae* (Metschinkoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin. SilicoSec® is a commercial diatomaceous earth (DE) formulation. Wheat was treated with 200 mg/kg of DE, 400 mg/kg of each isolates alone or a combination of them, against *Tribolium castaneum* Herbst, *Rhyzopertha dominica* (F.), and *Oryzaephilus surinamensis* L. The experiments were carried out at 27±1°C and 65±5% relative humidity (RH) in continuous darkness. The pathogenicity of all isolates was significantly low even after 7 days of exposure, with the exception of *R. dominica*. The isolates were virulent to the beetles, but the efficacy of the isolates was enhanced in combination with the DE. *Tribolium castaneum* was the most resistant species, followed by *R. dominica*. The findings indicated that the addition of the DE to the isolates increased the pathogenicity especially at the highest exposure interval. The addition of DE may provide satisfactory control of the insect-pests of stored products.

Key words: diatomaceous earth, enhancement, pathogenicity, stored products, wheat

Introduction

The efficacy of entomopathogenic fungi has been investigated against several insect species (Adane *et al*. 1996; Batta 2005; Cherry *et al*. 2005). The mode of action of fungi is to adhere to the insect's body, germinating on and penetrating to the integument of insects (Akbar *et al*. 2004). However, lack of inoculums due to environmental contamination, high cost of production and a high application rate required for the satisfactory protection of products, limited the application of these insect pathogens (Akbar *et al*. 2004). A combination of control tactics for protecting stored products, such as an Integrated Pest Management (IPM) program, has recently been considered. One of the most effective naturally occurring dusts for protecting stored products from insect pest infestations is diatomaceous earth (DE). The wax from an insect's cuticle is absorbed by DE. The resulting death is due to desiccation and to a lesser degree by abrasion (Ebeling 1971). Diatomaceous earth has several advantages: it is generally recognized as safe, has low mammalian toxicity, does not affect grain end-use quality, provides long-term protection, is registered as a food additive, and has long been known as an alternative to synthetic pesticides. However, reduction of bulk density and grain flowability, decreases the efficacy at high humidity and DE is then unable to control the immature stages of the insects that remain within the grain *e.g*. *Sitophilus* spp. widespread use of DE is, thus, limited (Fields 1998; Korunic 1998). This limitation has encouraged the search for the optimum combination of control tactics for protecting stored products. An IPM program is one such control tactic.

There are several reports on the combined effects of different DE formulations with *Beauveria bassiana* (Balsamo) Vuillemin (Akbar *et al*. 2004; Vassilakos *et al*. 2006; Athanassiou and Steenberg 2007; Lord 2007; Riasat *et al*. 2011; Wakil *et al*. 2011; Wakil *et al*. 2012) and *Metarhizium anisopliae* (Metschinkoff) Sorokin (Kavallieratos *et al*. 2006; Michalaki *et al*. 2006; Athanassiou *et al*. 2008; Luz *et al*. 2012). According to these researchers, the abrasive activity of DE or other desiccant dusts enhanced the insecticidal efficacy of entomopathogenic fungi. So, desiccant dusts synergized the pathogenicity of *B. bassiana* and *M. anisopliae*. However, the combination of DE with Iranian isolates of *M. anisopliae* and *B. bassiana* has not yet been studied.

Red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), is categorized as a secondary pest that feeds on damaged or soon to be damaged cereal grains, flour, and related products. Throughout the world, the lesser grain borer, *Rhyzopertha dominica* F. (Coleoptera: Bostrychidae), is one of the most destructive insect pests of various food grains under storage. This species is usually found in grain storage facilities and damages harvested grains that are being stored.

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The immature development of the lesser grain borer occurs inside the grain, after which this pest bores a hole out of the grain and emerges. The saw-toothed grain beetle, *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae), is a secondary pest of cereals. This beetle is also found in spices, nuts, and dried fruits. They infest damaged cereal grains or attack the germ region of whole grains (Rees 1996; Hill 2002).

The aims of the current study were (1) to investigate the insecticidal efficacy of the Silicosec® formulation of DE, *M. anisopliae* and *B. bassiana* against adults of *T. castaneum*, *R. dominica*, and *O. surinamensi,* and (2) to evaluate the enhancement effect of DE on the tested fungi.

Materials and Methods

Insects and commodity

Beetles were obtained from cultures maintained for at least 3 years in the Plant Protection Laboratory of Urmia University. *Tribolium castaneum* was reared on wheat flour plus 5% brewer's yeast (by weight), *R. dominica* was reared on the whole wheat variety Zarin, and *O. surinamensis* on rolled oats and 5% brewer's yeast at 27±1°C and 65±5% relative humidity (RH) in continuous darkness. All adults used in the experiments were 7–14 days old.

The wheat variety, Zarin, was used for the experimentations. Wheat kernels were stored at –24°C, for at least 48 h. Before the experiments started, wheat was kept for a week in an incubator set at 27±1°C and 65±5% RH to achieve the moisture content related to environmental RH. The moisture content of wheat was measured using Dickey-John moisture meter. The moisture content was about 12.5%.

DE formulation

SilicoSec® is a freshwater commercial DE formulation obtained from Biofa GmbH (Münsingen, Germany). It is composed of 92% SiO_2, 3% Al_2O_3, 1% Fe_2O_3, and 1% Na_2O. The median particle size of SilicoSec® was between 8 and 12 µm (EGMSDS 2003).

Isolates

Iranian isolates of *M. anisopliae* Sorokin DEM and 715 and *B. bassiana* (Balsamo) Vuillemin 437 and 429 were obtained from the collection maintained by the Plant Protection Research Institute, Tehran, Iran (Table 1).

Conidial culture

Conidial culture was performed by the methods of Khashaveh and Chelav (2013) with some modifications. Isolates were cultured on Potato Dextrose Agar (PDA) and incubated in glass Petri dishes (9 cm diameter) sealed with Parafilm® for a week at 26°C, at a photoperiod of 16 : 8 h (L : D) to complete sporulation. Subsequently, a mixture of conidia and hyphae was harvested by flooding cultures with sterile water containing 0.05% (v/v) Tween 80 (Sigma Chemical, St. Louis, MO, USA) and stirred with a glass rod. All samples were vortexed for 3 min to break up the conidial chains or clumps. Conidia were separated from hyphae and substrate materials by filtering the suspension through two layers of cheesecloth. The concentration of conidia was determined using a haemocytometer (Improved Neubauer, 0.1 mm depth). Three glass slides per isolates (three replications) were placed in Petri dishes lined with moistened sterile filter paper to assess viability of conidia. Subsequently, the conidial viability was evaluated by spreading a drop of the conidial suspension on each glass slide. Germination was determined after 24 h at 25±2°C. Conidia with germ tubes equal or greater than the width were considered as germinated. Isolates were applied at a rate of 400 mg of conidia/kg of wheat.

Bioassays

Fifty grams of wheat kernels were poured into 280 ml glass vials, and treated with 200 mg/kg of DE alone, 400 mg/kg of each entomopathogenic fungi or a combination of both DE and fungi. The vials were shaken for 5 min to achieve equal distribution in the entire grain mass. Subsequently, 30 adults of each species were put into the vials and the vials were covered with muslin cloth for sufficient ventilation. Each treatment was replicated four times. Untreated wheat served as the control. The vials were placed in incubators set at 27±1°C and 65±5% RH. The mortality was counted after 2 and 7 days of exposure. When no leg or antenna movements were observed, insects were considered dead.

Data analysis

Mortality counts were corrected using Abbott's (1925) formula. Percentages of mortality were transformed to the square root of arcsine to normalize the data, but non-transformed data are presented in the tables. The data were analyzed using one-way analysis of variances. Tukey's test was used to determine significant differences between different treatments and time of exposure (SPSS 2007) at $p = 0.05$.

Results

The mean germination rate of the conidia of all isolates ranged from 90% to 94% (Table 1). Significant differences were observed between treatments and the time exposed to each treatment on three tested species (*T. castaneum*: df = 17, 54; F = 100.29; $p < 0.01$), (*R. dominica*: df = 17, 54; F = 147.40; $p < 0.01$), and (*O. surinamensis*: df = 17, 54; F = 74.30; $p < 0.01$) (Tables 2–4).

Mortality percentages were low 2 days after the introduction of *T. castaneum* adults and did not exceed 40.83% for *T. castaneum* treated with *B. bassiana* 437 + DE. However, the mortality increased when the exposure time was increased to 7 days, and after this time the presence of DE demonstrated its effectiveness. Therefore, the combination treatment was more effective than the use of isolates alone. The mortality percentage of *T. castaneum* was between 80–84% when DE was combined with different

Table 1. The origin and conidial germination of *M. anisopliae* and *B. bassiana* isolates used in the study

Isolates	Host	Location	Germination [%]
M. anisopliae 715	Locust (Orthoptera: Acrididae)	Ahvaz-Iran	90±1.9
M. anisopliae DEM	*Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae)	Saravan-Iran	94±2.2
B. bassiana 437	*Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae)	Bandar-e Anzali-Iran	91±1.8
B. bassiana 429	*Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae)	Rasht-Iran	90±1.9

Table 2. Mortality percentage of *T. castaneum* exposed to SilicoSec®, *M. anisopliae,* and *B. bassiana* alone or as a binary combination of diatomaceous earth (DE) and fungi

Treatments	Mortality percentage ±SE	
	2 days	7 days
M. anisopliae 715 + DE	25±2.15 bcd	84.16±0.83 a
M. anisopliae DEM + DE	33.33±3.04 bc	83.33±1.36 a
B. bassiana 437 + DE	40.83±3.15 b	83.33±1.36 a
B. bassiana 429 + DE	26.66±3.04 bcd	80.83±0.83 a
M. anisopliae 715 alone	0.83±0.83 e	22.50±3.69 cd
M. anisopliae DEM alone	3.33±2.35 e	14.16±4.38 de
B. bassiana 437 alone	5.00±3.19 e	15.83±3.69 de
B. bassiana 429 alone	3.33±2.35 e	28.33±8.22 bcd
DE alone	14.16±1.59 de	80.83±1.59 a

Means followed by the same letter are not significantly different; Tukey's test at $p < 0.05$

Table 3. Mortality percentage of *R. dominica* exposed to SilicoSec®, *M. anisopliae*, and *B. bassiana* alone or as a binary combination of diatomaceous earth (DE) and fungi

Treatments	Mortality percentage ±SE	
	2 days	7 days
M. anisopliae 715 + DE	14.16±4.38 def	90.83±0.83 ab
M. anisopliae DEM + DE	25.83±5.15 de	92.5±1.59 a
B. bassiana 437 + DE	26.66±2.72 d	90.00±1.36 ab
B. bassiana 429 + DE	46.66±4.08 c	92.50±2.84 a
M. anisopliae 715 alone	10.00±1.92 f	77.50±5.50 ab
M. anisopliae DEM alone	7.50±3.43 f	75.83±3.43 b
B. bassiana 437 alone	9.16±1.59 f	80.83±2.50 ab
B. bassiana 429 alone	9.16±2.09 f	85.00±0.96 ab
DE alone	10.83±2.09 ef	87.50±1.59 ab

Means followed by the same letter are not significantly different; Tukey's test at $p < 0.05$

Table 4. Mortality percentage of *O. surinamensis* exposed to SilicoSec®, *M. anisopliae,* and *B. bassiana* alone or as a binary combination of diatomaceous earth (DE) and fungi

Treatments	Mortality percentage ±SE	
	2 days	7 days
M. anisopliae 715 + DE	80.00±8.16 abc	95.00±3.19 ab
M. anisopliae DEM + DE	74.16±5.98 c	98.33±0.96 a
B. bassiana 437 + DE	87.50±2.50 abc	97.50±2.50 a
B. bassiana 429 + DE	70.83±2.50 cd	95.00±0.96 ab
M. anisopliae 715 alone	18.33±2.88 gh	53.33±5.61 de
M. anisopliae DEM alone	12.50±0.83 h	40.00±1.36 ef
B. bassiana 437 alone	27.50±1.59 fgh	75.00±3.96 c
B. bassiana 429 alone	9.16±2.09 h	31.66±3.46 fg
DE alone	78.33±4.81 bc	94.16±3.43 ab

Means followed by the same letter are not significantly different; Tukey's test at $p < 0.05$

fungi isolates, after a 7 day exposure interval. In contrast, for the application of isolates alone, the mortality did not exceed 28% even after 7 days (Table 2).

Similar results were also noted for *R. dominica*, where mortality percentages were lower after 2 days of exposure and did not exceed 46.66% for adults exposed to *B. bassiana* 429 + DE. While surprisingly, the mortality of all treatments increased after 7 days and there were no significant differences between different treatments (Table 3).

For *O. surinamensis*, the isolates of *B. bassiana* 437 + DE, and *M. anisopliae* 715 + DE caused significantly more mortality 2 days after commencement of the experiments. However, at the 7-day exposure interval, the highest mortality levels were recorded for all the combination treatments, where > 95% of the exposed individuals were dead. For application of *M. anisopliae* 715, *M. anisopliae* DEM, *B. bassiana* 437, and *B. bassiana* 429 alone, the mortality was only 53%, 40%, 75%, and 31%, respectively (Table 4). Sometimes, the application of DE alone showed the same effect as when it was combined with different isolates of entomopathogenic fungi. In most cases, there were no significant differences between different isolates of fungi applied alone against the three tested species. Moreover, *T. castaneum* seems to be more resistant, followed by *R. dominica*. *Oryzaephilus surinamensis* was the most sensitive tested species.

Discussion

Mortality was increased by increasing the time interval to 7 days especially for *T. castaneum* and *R. dominica*. Batta (2005) declared that high mortality of *R. dominica* exposed to *M. anisopliae* was obtained 7 days after treatment. The author claimed that the difference in the efficacy of isolates may be attributed to the aggression characteristics of the isolate, insect species, experimental conditions, and even the commodity that is applied for experiments.

Our findings indicated that different Iranian isolates of entomopathogenic fungi have low insecticidal toxicity against three tested species. Athanassiou and Steenberg (2007) declared that the application of *B. bassiana* entomopathogenic fungi alone is less effective and the toxicity increased when combined with Insecto®, SilicoSec®, and PyriSec® DE formulations.

It is also evident from the results that DE enhanced the toxicity of fungal isolates against *T. castaneum*, *R. dominica*, and *O. surinamensis*. This is in agreement with the results of Kavallieratos *et al*. (2006); that the addition of DE Protect-It synergized the effectiveness of *M. anisopliae*. They also stated that dry conidia of the same strain were significantly more effective than an aqueous preparation against *Sitophilus oryzae* (L.). Lord (2005) stated that the synergized efficacy of both substances could be due to the effect of both on the insect's cuticle. He also explained that the process of desiccation may change the chemistry of the cuticle and influence the ability of the conidia to stick, germinate, and penetrate the insect's cuticle. Athanassiou *et al*. (2008) proved that besides Protect-It, DE enhanced the efficacy of fungus; the persistence of *M. anisopliae* during the experiment was also increased. This contributes to DE's characteristic of being effective for over more than 8–9 months.

The efficacy of DE increased with increasing temperatures. The reason is, that at high temperatures insect locomotion and the chance of the insect having contact with DE particles is greater and water loss is faster. However, increased RH will reduce the efficacy of DE (Fields and Korunic 2000). The effectiveness of fungi decreased with increasing temperatures (Vassilakos *et al*. 2006). However, RH has no effect on the insecticidal efficacy of fungi (Akbar *et al*. 2004; Athanassiou and Steenberg 2007). Athanassiou and Steenberg (2007) tested *B. bassiana* alone on wheat against *Sitophilus granarius* L. They pointed out that the mortality was higher at 55% than 75% RH and 25°C. Vassilakos *et al*. (2006), though, stated that 26°C is the optimum temperature for *B. bassiana* effectiveness. Therefore, selecting the proper temperature and humidity rate for the combination tests of fungi with DE is very important. It seems that a temperature between 25–27°C and RH of 55–65% are appropriate for the integration of DE and fungi.

It can be concluded, that the effectiveness of fungi requires a lot of time, during which insect pests continue to infest commodities and management of the pests would not be profitable. So, a combination of fungal isolates with DE SilicoSec in the insect pest management program is recommended on a large scale, after confirmation of the obtained results at grain storage facilities. Further studies are required to make application of DE + fungi practical and acceptable on a large scale.

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