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Control of *Bruchidius incarnatus* and *Rhyzopertha Dominica* using two entomopathogenic fungi alone or in combination with modified diatomaceous earth

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ABSTRACT

The efficacy of diatomaceous earth (DEs) alone and combined with two microbial pathogens *Nomuraea rileyi* and *Lecanicillium lecanii* against two species, *Bruchidius incarnatus* and *Rhyzopertha dominica* was evaluated. Modified diatoms with Calcium hydroxide (Ca-DE) and modified diatoms with Sodium hydroxide (Na-DE) were the highlight treatments against tested insects and achieved the highest mortality percentages. Ca-DE was the most effective DE and accomplished the highest mortality percentages recorded 88% and 96% after treated *R. dominica* and *B. incarnatus* with 1.0%, respectively. The lowest mortality percentage was recorded in case of Al-DE at concentration 0.5% and amounted (21and 15%), for the corresponding species, respectively. Ca-DE and Na-DE were the most effective treatments in enhancing the potency of the tested fungi. The presences of DEs seem to have different types of impact on fungal potency. In most cases, DE combinations with tested fungi had synergistic effects, while Al-DE impaired the efficacy of *N. rileyi* against tested insects. Ca-DE has insecticidal, repellent and ovicidal effects against target insects. The current results revealed that both Ca-DE and Na-DE were the most effective DEs tested and they had synergistic effects on the potency of tested fungi.

Introduction

Bruchidius incarnatus (Coleoptera: Bruchidae) is an important insect-pest, especially of faba bean, *Vicia faba* (Leguminosae) and it can infest field crops and cause severe damage in storage. Serious damage is caused to stored dry broad bean on which this pest reproduces rapidly (Fouad, 2013). The lesser grain borer, *Rhyzopertha dominica* (F.)(Coleoptera: Bostrichidae), is a major cosmopolitan insect pest of stored wheat. *R. dominica* is one of the most injurious pest of stored grains both in larval and adult stage.

The widespread and intensive use of synthetic insecticides for the control of stored-grain insects has led to serious problems including insecticide resistance of pest insects, health issues, and lethal effects on non-target organisms (Jovanovic *et al* ., 2007; Lu & Wu, 2010).Thus, there is an urgent need to develop safe alternative options for stored products pest management system.

There is a growing interest in the use of naturally occurring entomopathogenic microorganisms especially fungi for the control of stored product insects as environmentally safe and with low mammalian toxicity. The fungus attaches and penetrates through the insect's cuticle, causing the insect's death (Cox and Wilking 1996). Entomopathogenic fungi have been experimented with success against several stored product insect species in both laboratory and field tests (Moore et al. 2000; Akbar et al. 2004; Batta 2004; Kavallieratos et al. 2006; Sabbour and Abd El-Aziz 2007a, b, 2010, Sabbour *et.al.*, 2012).The fungus, *Nomuraea rileyi* is a dimorphic hyphomycete that can cause epizootic death in various insects. It has been shown that many insect species belonging to Lepidoptera including *Spodoptera litura* and some belonging to Coleoptera are susceptible to *N. rileyi*. The host specificity of *N. rileyi* and its eco-friendly nature encourage its use in insect pest management (Srisukchayakul, et.al. 2005).

Diatomaceous earths (DEs) may be successfully incorporated into Integrated Stored Grain Pest Management (ISGPM) program since they are natural insecticides of low mammalian toxicity, and have proved very effective against a wide range of species (Subramanyam and Roesli 2000). DEs are naturally occurring siliceous sedimentary mineral compound formed from the fossils of tiny phytoplanktons (diatoms) which absorb the epicuticular lipids of the insect cuticle, causing death through desiccation (Ebeling 1971). Many studies evaluated the efficacy of the combination of DEs with different species of fungi, as *Beauveria bassiana* (B.b.) (Lord 2001; Akbar *et al*. 2004); *Metarhizium anisopliae* (M.a.) (Moor *et al*.2000; Kavallieratos *et al*. 2006) and *Isaria fumosorosea* (I.f.) (Michalaki *et al*. 2006, 2007, Sabbour et al.,2012).

This work aims to evaluate the efficacy of DEs alone and combined with two fungal pathogens: *Nomuraea rileyi* and *Lecanicillium lecanii* against the broad bean beetle, *Bruchidius incarnatus* and the lesser grain borer, *Rhyzopertha dominica.*

Materials And Methods

Bruchidius incarnatus* and *Rhyzopertha dominica were used in the experiments. The target insects were reared under laboratory conditions on beans seeds and wheat seeds, respectively. All cultures and experiments were held at 26±2°C and 70–80% relative humidity (RH), with 16 hours light and 8 hours dark.

Diatomaceous earths (DEs)

The natural DE and three DEs modifications were tested alone or in combinations with tested fungi. The natural diatomaceous earth (DE) was chemically modified by different mono-, di-, tri- valent metal hydroxides (MOH, M = Na, Ca, Al)

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according to (Abd-El-Aziz and Sherief 2010). The DEs were treated at the application rates of 0.25, 0.5 and 1g/kg of grains.

Isolation of tested fungi

The tested fungi species, *N. rileyi* and *L. lecanii* were isolated from the dead and/or infected adults and pupae of tested insects (Sabbour and Sahab 2005) and were identified at Microbiology Department, NRC.

Insecticidal efficacy of tested DEs

The insecticidal efficacies of DEs were tested at three rates (0.25, 0.50 and 1g/kg wheat) against adults of the two tested species. For each case, four glass jars as replicates were used. Each replicate was treated individually with the respective DE quantity and then shaken manually for one minute to achieve equal distribution of the DE. Subsequently, ten mixed-six adults of each tested species were introduced into each glass jar and were covered with muslin for sufficient ventilation. Eight replicates glass jars containing untreated wheat or beans served as control. Mortality was assessed after seven days of exposure in the treated and untreated jars and mortality has corrected according to (Abbott 1925). All tests were conducted at 27±2°C and 65±5% relative humidity (RH).

Insecticidal efficacy of tested fungi alone and with DEs

Six concentrations (in percent of v/v) for each tested fungi (16, 8, 4, 2, 1, $0.5x10^7$ spores/ ml) were prepared. The target insects were fed with wheat or beans contaminated with the different fungi rates. Mortalities were calculated after seven days, and corrected mortality according to (Abbott 1925). Also, LC_{50} variance, 95% confidence limits were calculated according to (Finney 1964). The tested fungi were tested at ($0.5x10^7$ spores/kg grain) for conducting the combination tests with DEs formulations (0.5 g/kg of grains). Ten adults for each insect species were kept in a glass jar (15x5 cm2) fed on a diet containing prescribed treatments. For each case, four jars as replicates were prepared. All the experiments were repeated three times.

Efficacy of tested DEs applied alone or in combinations with tested fungi on the mean number of deposited eggs of target insects

The tested fungi were tested at ($0.5x10^7$ spores/kg grain) for conducting the combination tests with DEs formulations (0.5 g/kg of grains).The DEs alone were used at rate (1.0 g/kg) of grains. Four replicates of 100 g grains for each treatment were used. Each replicate was treated individually with treatments and then shaken manually for 1 min to achieve equal distribution of the dust in the entire formulation quantity and was placed in glass jar. Four replicates jar containing untreated grain served as control. Subsequently, one paired of newly emerged adults were introduced into each jar. The number of deposited eggs on treated or untreated grains/female was counted.

The data was analyzed using analysis of variance (ANOVA), where significant differences between the treatments were observed. Mean values were significantly separated by using the least significant difference (LSD) test at 5% level (Sokal and Rohlf 1981).

Results and discussion

The efficacy of DEs modifications was tested against the two tested insect adults (Table 1). Ca-DE was the most effective DE and achieved the highest mortality percentages recorded 88% and 96% after treated *R. dominica* and *B.incarnatus* with 1.0%, respectively. Ca-DE and Na-DE were the highlight treatments against *R. dominica* , *B.incarnatus* at (0.5% conc.), and achieved the highest mortality percentages(77and 81%) and(67 and 72), respectively. DE – origin had moderate effect on tested insects. The lowest mortality percentage was recorded in case of Al-DE at concentration 0.5% and amounted (38 and 25%), for the corresponding pest, respectively. The decrease in DEs concentrations leads to the decrease in the larval mortality in all cases. Also, the lower concentration of DEs had no insecticidal effects against target insects. *R. dominica* was the most tolerant species to tested DEs. This results stands in agreement with Korunic and Mackay (2000) who reported that the treated wheat with 0.5 and 0.75 g of diatomaceous earth Protect-It® per kg of wheat, reduced the population of *Sitophilus oryzae* (L.), *Tribolium castaneum* (Herbst) and *R. dominica* (Fabricius) by 98 to 100% with respect to controls due to the repellent properties of diatomaceous earth, and probably has very good dispersal capacity in the grain mass. The application of Ca-DE caused the complete mortality of *Callosobruchus maculatus* (100%) compared to the other tested DEs after 7 and 14 days intervals (Abd-El-Aziz and Sherief 2010).

Table 1. Mortality % of tested insect's adults on wheat and beans treated with DE and three modified DEs at three rates

Treatments	*Concentration*	% of adult mortality	
		R. dominica	*B. incarnatus*
DE	1.0	59	50
	0.5	33	31
	0.25	1	3
NA-DE	1.0	85	93
	0.5	67	72
	0.25	4	11
AL-DE	1.0	38	25
	0.5	21	15
	0.25	1	0
CA-DE	1.0	88	96
	0.5	77	81
	0.25	3	10

N. rileyi was the most effective fungus alone against the two tested insects; also the presence of DEs increased the fungal efficacy (Table 2). Ca-DE and Na-DE were the most effective treatments in enhancing the potency of the tested fungi. Adults of *B.incarnatus and R. dominica* were more tolerant to *L. lecanii* alone than combinations . Ca-DE and Na-DE were enhancing the efficacy of *L. lecanii* and decreased LC_{50} of *R. dominica* and *B.incarnatus* from 254 and 279 to (152 and 133) and (157and 145), respectively (Table 2).The presences of DEs seem to have different types of impact on fungal potency. In most cases, DE combinations with tested fungi had synergistic effects. While Al-DE decreased the efficacy of *N. rileyi* where LC_{50} of *R. dominica* and *B.incarnatus* reached 257 and 267 . The current results revealed that both Ca-DE and Na-DE were the most effective DEs tested and they had synergistic effects on the potency of tested fungi. The presence of DE favors the insecticidal efficacy of *N. rileyi* against adults of the lesser grain borer, *R. dominica*, (Lord 2005). The addition of many inert dust types such as charcoal, ash or DE increased the potency of *M. anisopliae* against *S. oryzae* (Batta 2004 and Kavallieratos *et al*. 2006). Akbar *et al*. (2004) mentioned that DE significantly increased the attachment of *B. bassiana* conidia on the cuticle of *T. castaneum* larvae. This attachment resulted to damage the epicuticle lipids of insects (Moore *et al*. 2000). Buda and Peèiulyte (2008) tested the effect of four fungal isolates, (*B. bassiana*, *Lecanicillium lecanii*, *M. anisopliae var. anisopliae and I. farinosa*) on adults of Indian meal moth, (*P. interpunctella*) and one species tested on mature larvae of the pest. All the fungal isolates tested were pathogenic, however, with a different effectiveness. During the first three days period after spraying, the highest mortality (35–40% versus control) was caused by *I. farinosa* and *M. anisopliae* var *anisopliae*, and there was no significant difference in the survival as compared to control when *B. bassiana* and *L. lecanii* were used. Sabbour and Abd-El-Aziz (2010) evaluated the potential activities of three essential oils (cumin, clove and mustard) alone or in combinations with three fungal species (*I. fumosorosea*, *Nomuraea rileyi*, *L. lecanii* against *Bruchidius incarnatus*. Mustard oil was the most effective in enhancing the potency of *I. fumosorosea* and *N. rileyi* and decreased LC_{50} of the target insect from (188 and $210x10^7$) to (100 and $102x10^7$, respectively).

Table 2. The calculated of LC_{50} of the tested fungi alone or combined with DEs against the target insects

Treatments	Tested DE	LC_{50} for *R. dominica*	*B. incarnatus*
N. rileyi	DE	144	150
	NA-DE	101	109
	AL-DE	257	267
	CA-DE	99	101
N. rileyi alone		240	255
L.lecanii	DE	177	187
	NA-DE	133	145
	AL-DE	176	180
	CA-DE	152	157
L.lecanii alone		254	279

The mean number of deposited eggs per female (egg production) of each tested species was greatly affected by the DEs/fungi combinations (Table 3). In all tested insects, there were significant differences between DEs alone compared to untreated control. The combination of Ca-DE and Na-DE with tested fungi highly suppressed the adult's egg production in comparison to untreated with highly significant differences. A moderate effect on suppressing the adult's egg production was recorded in case of DE and Al-DE with tested fungi. *B.incarnatus* was the most susceptible to DE/fungi combinations followed by *R. dominica* adults (Tables 3–4). The combination of Ca-DE/Nr. strongly suppressed the number of deposited eggs of *R. dominica* (11.7 ±6.5 eggs/female), in comparison to untreated control (148.6±9.4eggs/female), with highly significant differences (Table 3). There were no significant differences between Na-DE and Ca-DE alone or in combinations with *N. rileyi* against *B.incarnatus* (Table 3). There were no significant differences between Na-DE and Ca-DE alone or in combinations with all tested fungi against *R. dominica* and *B.incarnatus*, respectively. The current results revealed that both Ca-DE and Na- DE were the most effective DEs tested and they had synergistic effects on the potency of tested fungi. Abd-El-Aziz and Sherief (2010) tested the insecticidal effects of modified diatomaceous earth (DE) with different hydroxides (MOH, M = Na, Ca, Al) against *C. maculatus* (F.) beetles on stored cowpea grains. Ca-DE has insecticidal, repellent and ovicidal effects against *C. maculatus*. These effects are due to the modification by using Ca-DE (divalent metal hydroxide) and had the biggest surface area (12.6 m2/g) followed by Na-DE (11.4 m2/g), which can absorb more lipid from insect bodies. Also, Ca-DE showed the highest number of crystals.

The current results revealed that both Ca-DE and Na-DE were the most effective DEs tested and they had synergistic effects on the potency of tested fungi. Ca-DE has insecticidal, repellent and ovicidal effects against tested insects. Even though tested insect adults could land on grain, treated with DEs/fungi when no choice was given, fewer eggs were laid. DEs/fungi treatments may be a useful alternative for management of *R. dominica and B. incarnates* in the store.

Table 3. Efficacy of tested DEs applied alone or in combinations with tested fungi on the mean number of deposited eggs of *R. dominica* and *B.incarnatus*

Treatment	**Mean number of eggs/female ±SE of *R. dominica***			
	DE/fungi combination			
	DE-alone	DE/*N.r*		DE/*L.l*
DE	57.0±5.9	36.1±2.4		44.3±6.3
Na-DE	25.0±8.6	18.6 ±4.4		20.2 ±3.9
AL-DE	77.0±9.9	55.1 ±8.7		61.3 ±2.9
CA-DE	21.0±3.1	11.7 ±6.5		19.9 ±8.9
Control	148.6±9.4			
F value LSD (0.05)	32.11 19.17			
	Mean number of eggs/female ±SE of *B. incarnatus*			
	DE/fungi combination			
	DE-alone	DE/*N.r*		DE/*L.l*
DE	36.0±5.9	26.1±2.4		30.3±6.6
Na-DE	35.0±8.1	28.6 ±4.4		30.2 ±3.3
AL-DE	37.0±7.1	26.7 ±6.5		29.9 ±8.0
CA-DE	27.0±7.9	11±4.3	21 ±8.7	20.3 ±2.1
Control	139.9±4.4			
F value LSD (0.05)	3. 32 19.10			

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