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EFFICACY OF THREE ENTOMOPATHOGENIC FUNGI ALONE OR IN COMBINATION WITH DIATOMACEOUS EARTH MODIFICATIONS FOR THE CONTROL OF THREE PYRALID MOTHS IN STORED GRAINS

Magda Mahmoud Sabbour[1], *Shadia El-Sayed Abd-El-Aziz*[1]*, *Marwa Adel Sherief*[2]

[1] Pests and Plant Protection Department, National Research Centre
El- Behouth St., P.O. Box 12622, Dokki, Cairo, Egypt
[2] Inorganic Chemistry Department, National Research Centre
El- Behouth St., P.O. Box 12622, Dokki, Cairo, Egypt

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Abstract: The efficacy of Natural diatomaceous (DE) alone and combined with three fungal pathogens: *Beauveria bassiana, Metarhizium anisopliae* and *Isaria fumosorosea* against three moth species, *Plodia interpunctella*, *Ephestia cautella* and *E. kuehniella* 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. *E. kuehniella* achieved the highest tolerant to tested DEs. *B. bassiana* was the most effective fungus alone against the three tested insects. Ca-DE and Na-DE treatments strongly enhanced the potency of the tested fungi. *E. kuehniella* was more susceptible to *M. anisopliae* and *B. bassiana*. Larvae of *P. interpunctella* and *E. cautella* were more tolerant to *I. fumosorosea* alone than *E. kuehniella*. In most cases, DE combinations with tested fungi had synergistic effects, while in *E. cautella*, modified diatoms with Aluminium hydroxide (Al-DE) decreased the efficacy of *M. anisopliae* and *I. fumosorosea*. Also, Al-DE and DE impaired the efficacy of *I. fumosorosea* against *E. kuehniella*. The egg production was highly suppressed by combination of Ca-DE followed by Na-DE with tested fungi in comparison to untreated control. The combination of Ca-DE/*Beauveria bassiana* strongly suppressed the number of deposited eggs of *P. interpunctella* (54.6±5.8 eggs/female), in comparison to untreated control (288.3±3.4 eggs/female).
The most effective DEs modification were Ca-DE and Na-DE had insecticidal, repellent and ovicidal effects against tested insects and they had synergistic effects on the potency of tested fungi.

Key words: *Plodia interpunctella, Ephestia kuehniella, Ephestia cautella, Beauveria bassiana, Metarhizium anisopliae, Isaria fumosorosea, Diatomaceous earths*

INTRODUCTION

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).

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). The basic disadvantage of using DEs for protecting grains is their high application rates which negatively affect the physical properties of the grain, especially bulk density and grain flowability (Fields 1999).

DEs should be applied at application rates which are considered immensely high and cause a considerable reduction in bulk density (Subramanyam and Roesli 2000; Abd El-Aziz and Sherief 2010). Many studies evaluated the efficacy of the combination of DEs with different species of fungi, as *Beavuceria bassiana* (*B.b.*) (Lord 2001; Akbar *et al*. 2004); *Metarhizium anisopliae* (*M.a.*) (Moor 2000; Kavallieratos *et al*. 2006) and *Isaria fumosorosea* (*I.f.*) (Michalaki *et al*. 2006, 2007).

The Indian meal moth, *Plodia interpunctella*, is a cosmopolitan and common pest species found frequently in food warehouses. Its larvae feed on cereals, grains, dried fruits, nuts and dried insect remains. It spins a silken web amongst the foodstuff and larvae in packaged goods often escape notice and cause significant losses (Cox and Bell 1991). The Mediterranean flour moth, *Ephestia kue-*

*Corresponding address:
shadia_abdelaziz2009@hotmail.com

hniella (Lepidoptera: Pyralidae), is a serious pest of stored food products, especially whole and milled grains. Its close association with human foods makes it a prime target for control methods other than chemical pesticides. The almond moth, *E. cautella* is a major worldwide pest of stored foods. It occurs both in tropical and temperate regions and commonly attacks grains, nuts, dried fruits and a great variety of other stored products (Horak 1994).

This work aims to evaluate the efficacy of DEs alone and combined with three fungal pathogens: *Beauveria bassiana, Metarhizium anisopilae* and *Isaria fumosorosea* against three moth species, *P. interpunctella*, *E. cautella* and *E. kuehniella*.

MATERIALS AND METHODS

Experimental insects

Larvae of *P. interpunctella, E. kuehniella* and *E. cautella* (Lepidoptera: Pyralidae) were used in the experiments. The target insects were reared under laboratory conditions on semi-artificial diet (fine wheat with some adherent endosperm) with 20% glycin and 5% yeast powder. 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) 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, *B. bassiana* and *M. anisopilae* and *I. fumosorosea* were isolated from the dead and/or infected larvae 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 dose rates (0.25, 0.50 and 1g/kg wheat) against the 3rd instar larvae of the three 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 3rd instar larvae of the three tested species were introduced into each glass jar and was covered with muslin for sufficient ventilation. Twelve replicates glass jars containing untreated wheat 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, $0.5\text{x}10^7$ spores/ml) were prepared. The target insects were fed with semi-artificial diet 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 ($0.5\text{x}10^7$ spores/kg grain) for conducting the combination tests with DEs formulations (0.5 g/kg of grains). Ten 3rd instars larvae for each insect species were kept in a glass jar (15x5 cm^2) fed on a diet containing prescribed treatments. For each case, four jars as replicates were prepared. All the experiments were repeated three times.

Ovipositional deterrent effects of DEs alone or in combination with tested fungi

The tested fungi were tested at ($0.5\text{x}10^7$ 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 and the percent repellency values were calculated according to the equation of (Lwande *et al.* 1985):

$$D = (1 - T/C) \times 100,$$

where:
T and C represent the mean number of deposited eggs per female of the treated and check set, respectively.

RESULTS AND DISCUSSION

Efficacy of DEs modifications was tested against the three tested insect larvae (Table 1). Ca-DE was the most effective DE and achieved the highest mortality percentages. Ca-DE and Na-DE were the highlight treatments against *E. cautella, P. interpunctella* and *E. kuehniella* at (5% conc.), and achieved the highest mortality percentages (78, 77 and 72%) and (76, 76 and 75%), respectively. DE – origin had moderate effect on tested insects. The lowest mortality percentage was recorded in case of Al-DE and amounted to (28, 33 and 30%), 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. *E. kuehniella* 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 *S. oryzae* (L.), *T. 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

Table 1. Mortality % of tested insect's larvae on wheat treated with DE and three DE modifications at three rates

Treatment	% of larval mortality			
	concentration	*P. interpunctella*	*E. kuehniella*	*E. cautella*
DE	1.0	47	32	41
	0.5	28	14	27
	0.25	1	0	0
Na-DE	1	77	72	78
	0.5	36	35	37
	0.25	4	3	2
Al-DE	1.0	33	30	28
	0.5	12	13	14
	0.25	0.3	0.2	.02
Ca-DE	1.0	76	75	76
	0.5	38	38	39
	0.25	5	4	2

DE – the natural diatomaceous earth

caused the complete mortality of *Callosobruchus maculatus* (100%) compared to the other tested DEs after 7 and 14 days interval (Abd-El-Aziz and Sherief 2010).

B. bassiana was the most effective fungus alone against the three 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. *E. kuehniella* was more susceptible to *M. anisopliae* and *B. bassiana*. Larvae of *P. interpunctella* and *E. cautella* were more tolerant to *I. fumosorosea* alone than *E. kuehniella*. Ca-DE and Na-DE were enhancing the efficacy of *I. fumosorosea* and decreased Lc_{50} of *P. interpunctella* and *E. cautella* from 198 and 171 to 120 and 130 and 111 and 120, 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 in *E. cautella*, Al-DE decreased the efficacy of *M. anisopliae* and *I. fumosoroseus*. Also, Al-DE and DE impaired the efficacy of *I. fumosorosea* against *E. kuehniella* where Lc_{50} increased to 144 and 137, 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. The presence of DE favors the insecticidal efficacy of *B. bassiana* against larvae 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). Būda and Pečiulytė (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 day 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).

The mean number of deposited eggs per female (egg production) of each tested species was greatly affected by the DEs/fungi combinations (Tables 3–5). In all tested insects, there were significant difference between DEs alone compared to untreated control. The combination of Ca-DE and Na-DE with tested fungi highly suppressed the moths egg production in comparison to untreated with highly significant differences. A moderate effect on suppressing the moths egg production was recorded in case of DE and Al-DE with tested fungi. *P. interpunctella* was the most susceptible moth to DE/fungi combinations followed by *E. cautella* and *E. kuehniella* moths (Tables 3–5). The combination of Ca-DE/*B.b.* strongly suppressed the number of deposited eggs of *P. interpunctella* (54.6±5.8 eggs/female), in comparison to untreated control (288.3±3.4 eggs/female),with highly significant differences (Table 3).

There were no significant differences between Na-DE and Ca-DE alone or in combinations with *I. fumosorosea* against *P. interpunctella* (Table 3). There were no significant differences between Na-DE and Ca-DE alone or in combinations with all tested fungi against *E. cautella* and *E. kuehniella* moths, respectively (Tables 4–5).

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 m^2/g) followed by Na-DE (11.4 m^2/g), which can absorb more lipid from insect bodies. Also, Ca-DE showed the highest number of crystals

Table 2. Efficacy of tested fungi alone or combined with DEs against the target insects

Microbial agent	Tested DE	Lc_{50} (x10^7)		
		Plodia interpunctella	*Ephestia cautella*	*Ephestia kuehniella*
Beauveria bassiana	DE	129	121	111
	Na-DE	100	115	110
	Al-DE	138	120	141
	Ca-DE	103	111	102
B. bassiana – alone		138	130	141
Metarhizium anisopliae	DE	130	111	102
	Na-DE	111	111	102
	Al-DE	140	111	102
	Ca-DE	129	111	102
M. anisopliae – alone		144	112	132
Isaria tumosorosea	DE	160	151	137
	Na-DE	130	120	122
	Al-DE	160	172	144
	Ca-DE	120	111	120
I. tumosorosea – alone		198	171	133

DE – the natural diatomaceous earth

Table 3. Ovipostional deterrent effect of tested DEs applied alone or in combinations with tested fungi against *P. interpunctella* moth

Treatment	Mean number of eggs/female ±SE			
	DE/fungi combination			
	DE – alone	DE/*B.b*	DE/*M.a*	DE/*I.f*
DE	247.0±7.9	154.4±4.4	177.9±5.8	129.0±5.6
Na-DE	175.0±8.8	99.4±4.4	124.4±7.4	99.0±5.9
Al-DE	217.0±7.9	178.4±4.4	150.0±3.5	171.0±6.1
Ca-DE	177.0±7.9	54.6±5.8	83.8±5.7	90.1±2.9
Control	288.3±3.4			
F value LSD (0.05)	31.33 18.18			

DE – the natural diatomaceous earth; *B.b. – Beauveria bassiana*; *M.a. – Metarhizium anisopilae; I.f. – Isaria fumosorosea*; SE – standard error

Table 4. Ovipostional deterrent effect of tested DEs applied alone or in combinations with tested fungi against *E. cautella* moth

Treatment	Mean number of eggs/female ±SE			
	DE/fungi combination			
	DE – alone	DE/*B.b*	DE/*M.a*	DE/*P.f*
DE	164.0±3.1	94.4±4.4	109.0±2.1	110.0±7.1
Na-DE	84.0±4.8	41.4±4.4	50.0±2.2	59.0±1.6
Al-DE	153.0±5.1	88.4±4.4	90.0±2.1	90.0±1.9
Ca-DE	88.1±3.1	44.6±5.8	51.0±4.8	59.0±2.3
Control	182.3±3.4			
F value LSD (0.05)	30.14 19.18			

Note – see table 3

Table 5. Ovipostional deterrent effect of tested Des applied alone or in combinations with tested fungi against *E. kuehniella* moth

Treatment	Mean number of eggs/female ±SE			
	DE/fungi combination			
	DE – alone	DE/*B.b*	DE/*M.a*	DE/*P.f*
DE	116.0±3.1	74.4±4.1	88.0±2.9	98.0±3.7
Na-DE	97.0±3.3	39.4±3.4	49.0±5.3	57.0±2.1
Al-DE	106.0±4.2	78.4±4.4	88.0±3.5	99.1±3.6
Ca-DE	91.0±1.2	24.6±5.8	48.5±4.5	65.5±3.2
Control	132.3±3.4			
F value LSD (0.05)	21.14 18.18			

Note – see table 3

with sharp and hard edges than other DEs modifications when examined by Transmission electron microscope (TEM). Sabbour and Abd-El-Aziz (2010) evaluated the potential activities of three essential oils (cumin, clove and mustard) alone or in combinations with three fungi species [(*I. fumosorosea, N. rileyi, L. lecanii* against *B. 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).

CONCLUSIONS

The most important factors of Integrated Pest Management (IPM) in stored products is the combinations between different control methods to achieve the highly protection levels for safe food. Our results demonstrated that *B. bassiana* is the most effective fungus than the other tested species against *P. interpunctella*, *E. cautella* and *E. kuehniella*, but its effective is influenced by several factors such as insect species. In most cases, modified DE combinations with tested fungi had synergistic effects. Ca-DE and Na-DE were the most effective treatments against *E. cautella*, *P. interpunctella* and *E. kuehniella* and achieved the highest mortality percentages and suppressing egg production. Modified DE combinations with tested fungi should be evaluated as a possible alternative control method against other stored insect species, stored products and conditions.

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