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Efficacy of entomopathogenic fungi alone or in combination with inorganic insecticides for protecting a broad bean against certain coleopteran stored products beetles in Egypt

Magda Sabbour

Pests and Plant Protection Department, National Research Centre, El- Behouth St., P.O. Box 12622, Dokki, Cairo, Egypt

ABSTRACT

The efficacy of three entomopathogenic fungi *Paecilomyes fumosoroseus; Nomuraea rileyi* and *Verticillium lecanii* alone and with the combination with Natural diatomaceous (DE) and silica gel Cab- O-500 and Cab -O-750 evaluated against *Callosobruchus maculates* (F.), *Callosobruchus chinensis* (L.) (coleoptera: Bruchidae). Results showed that modified diatoms with Calcium hydroxide (Ca-DE) and modified diatoms with Sodium hydroxide (Na-DE) were the highlight treatments against the two tested insects and achieved the highest mortality percentages. *C. maculates* achieved the highest tolerant to tested DEs. Cab-O-Sil-750 gave highest mortality against *C. maculates* reached to 88, 50 and 13% at concentrations 1, 0.5 and 0.25, respectively. The fungus *P. fumosoroseus* was the most effective alone against *C . maculates* $LC_{50,}$ recorded 149 spore/ml. Ca-DE and Na-DE treatments strongly enhanced the potency of the tested fungi *P. fumosoroseus* and *N. rileyi* . Results showed that, *C. maculates* was susceptible to *N. rileyi* . Larvae of *C. maculates* was more tolerant to *V. lecanii* alone . In most cases, DE combinations with tested fungi had synergistic effects, while in *C. maculates* modified diatoms with Aluminium hydroxide (Al-DE) decreased the efficacy of *V. lecanii*. both silica gel and diatoms protected grain better. 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/*P. fumosoroseus* strongly suppressed the number of deposited eggs of *C. maculates* (98.5±5.3 eggs/female), in comparison to untreated control (288.6±9.4 eggs/female). Cab-O-750 with the fungus *P. fumosoroseus* significantly decreased the mean number of *C. chinensis* to 96.7 ± 5.2 eggs/ female as compared to 278±.6 9.4 in the control. The most effective DEs modification were Ca-DE and Na-DE had insecticidal, repellent and ovicidal effects against tested *C. maculates* and they had synergistic effects on the potency of tested fungi.

Key words: *Callosobruchus maculates* (F.), *Callosobruchus chinensis* (L.), *Paecilomyes fumosoroseus; Nomuraea rileyi, Verticillium lecanii,* Cab-O-Sil-750 , Cab-O-Sil-500**,** Diatomaceous earths .

Introduction

Diatomaceous earth and silica gel are used in various physical formulations with or without added pesticide. The type of silica and the formulation depend on the target pest. Diatomaceous earth and silica gel have a great potential as a grain protectant. It is non-toxic, provides good protection when grain is stored properly, can be easily separated from the grain, and possibly recycled in storage bins. Toxicity is so low that diatomaceous earth is not counted as a foreign substance when grain is rated by the USD, Korunic(1998). Ingestion of diatomaceous earth is not toxic to mammals (Bertke 1964). Dairy farms sometimes feed their animals food containing 1 to 2% diatomaceous earth to "fossil flour" to their baked goods in order to stretch their flour supply (Cummins 1975). It is so safe for use on food that the FDA has exempted diatomaceous earth from requirements of fixed residue levels when added to stored grain (Sabbour, *et al.*, 2012). According to Belforda and March, (1990), "silica gel can hold oil up to 300% of its weight used against pests

Both silica gel and diatomaceous earth are forms of amorphous silica, and they both kill insects by desiccation, not by absorbing water, but by absorbing the oily or waxy outer cuticle layer by direct contact La Hue (1970). When the thin(about 1/u) waterproof layer of the epicuticle is lost, the insect loses water, then dies. Abrasive damage to the cuticle also leads to water loss in some cases, but the effectiveness of silica as an insecticide often depends on the amount of oil it can absorb. Diatomaceous earth, and synthetic silica, have been used as insecticides for thousands of years by aboriginal peoples in North America and Africa and are also used in modern grain storage facilities (Ebeling 1971).

The ability to absorb oil or wax from an insect, is often, but not necessarily, related to surface area of the silica (Ebeling 1961). Silica gel has the advantage of a much larger surface area than diatomaceous earth, but the latter is more abrasive. Whether the one or the other is used depends on the target insect and conditions (Ebeling 1971).

Corresponding Author: Magda Sabbour, Pests and Plant Protection Department, National Research Centre, El- Behouth St., P.O. Box 12622, Dokki, Cairo, Egypt
E-mail: sabbourm9@yahoo.com

Glob. J. Biodivers. Sci. Manag., 3(2): 183-187, 2013

Materials and Methods

Experimental insects:

Larvae of *C. maculates* and *C. chinensis* were used in the experiments., were reared on broad bean seeds *Vigna faba* (L.) at 28 ± 2^0 C and 60 ± 5 %R.H. under 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, *Paecilomyes fumosoroseus; Nomuraea rileyi, Verticillium lecanii,* 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 3^{rd} instar larvae of the two tested insect pests. 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 3^{rd} instar larvae of the two 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, 0.5x107 spores/ml) were prepared. The Experimental insects Larvae of *C. maculates* and *C. chinensis* were used in the experiments. The target insects were reared under laboratory conditions on on broad bean seeds *Vigna faba* (L.) at 28 ± 2^0 C and 60 ± 5 %R.H. under with 16 hours light and 8 hours dark.

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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, 0.5x107 spores/ml) were prepared. The Ovipositional deterrent effects of DEs alone or in combination with tested fungi The tested fungi were tested at (0.5x107 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):

$D = (1 – T/C) \times 100,$

Glob. J. Biodivers. Sci. Manag., 3(2): 183-187, 2013

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

Results and Discussions

Efficacy of DEs modifications were tested against the two tested insect larvae *C. maculates* and *C. chinensis* (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 *C. maculates* at (5% conc.), and achieved the highest mortality percentages (86, 42and 6%) and (82, 36 and 6%), respectively. DE – origin had moderate effect on tested insects. The lowest mortality percentage was recorded in case of Al-DE and amounted to (38, 22 and 1%), 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.

C. chinensis was the most tolerant species to tested DEs. Silica gel Cab-O-70 recorded a higher mortality percentage reached to 88, 50 and 13% in case of *C. maculates* and 90, 55 and 16% in case of *C. chinensis* . 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. Sabbour, *et al*., 2012, recorded that application of Ca-DE . The results also in the same line of Sabbour et al., (2012) they find, 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. The application of Ca-DE 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 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 LC50 of the target insect from (188 and 210x107) to (100 and 102x107, respectively). 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 LC50 of the target insect from (188 and 210x107) to (100 and 102x107, respectively).

Data in table (2) indicate that the LC_{50} of the tested fungus *P. fumosoroseus* decreased after the combination with NA-DE, AL-DE and Cab-O-750 and Cab-O-500, 97, 111, 114 and 105 X 10^7 spores/ml as compared to 149 X 10^7 spores/ml when the fungus applied alone. The LC_{50}s of the fungi *N. rileyi and V.lecanii* decreased to 107 X 10^7 and 118 X 10^7 spores/ ml when combined with Cab-O-750, respectively. The results also in the same line of Akbar *et a*l. (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). They tested the effect of four fungal isolates, (*B. bassiana, Lecanicillium lecanii, M. 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*, and there was no significant difference in the survival as compared to control when *B. bassiana* and *V. 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, V. lecanii* against *Bruchidius incarnatus*. Mustard oil was the most effective in enhancing the potency of *P. fumosorosea* and *N. rileyi* and decreased LC50 of the target insect from (188 and 210x107) to (100 and 102x107, respectively). These findings are in accordance with those of Sabbour *et al*., (2012).

The mean number of deposited eggs per female (egg production) of each tested species was greatly affected by the DEs/fungi combinations (Tables 3and 4). 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. *C. maculates*was the most susceptible moth to DE/fungi combinations followed by *C. chinensis* moths (Tables

Glob. J. Biodivers. Sci. Manag., 3(2): 183-187, 2013

3and 4). The combination of Ca-DE/*P.f.* strongly suppressed the number of deposited eggs of *C. maculates* (98±5.3 eggs/female), in comparison to untreated control (288.6±9.4 eggs/female),with highly significant differences. Also, the combination with P.f were significantly decreased the deposited eggs to 95.6±5.9 and 96.7±5.2 eggs/ female of *C. maculates and C. chinensis* ., respectively (Table 3and 4). The results also in the same line of sabbour *et al* ., 2012 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 crystalswith sharp and hard edges than other DEs modifications when xamined 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 LC50 of the target insect from (188 and 210x107) to (100 and 102x107, respectively). Abd El-Aziz (2001) mentioned that the treated foam with clove and eucalyptus oil vapours covering gunny sacks was the most significantly effective against *C. maculatus* infestation after 90 days of storage compared with the other applications (treated sacks or foam inside sacks)

Table 1: Mortality % of tested insect's larvae on cowpea treated with DE and three DE.

Treatments	*concentration*	% of larval mortality *C. maculates*	*C. chinensis*
DE	1.0	41	51
	0.5	30	32
	0.25	1	3
NA-DE	1.0	82	90
	0.5	36	41
	0.25	6	13
AL-DE	1.0	38	27
	0.5	22	17
	0.25	1	1
CA-DE	1.0	86	93
	0.5	42	47
	0.25	6	14
Cab-O-Sil 500	1.0	55	62
	0.5	31	32
	0.25	1	4
Cab-O-Sil 750	1.0	88	90
	0.5	50	55
	0.25	13	16

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

Treatments	Tested DE	LC_{50} of *C. maculates*	*C. chinensis*
P. fumosoroseus	DE	139 X 10^7	176 X 10^7
	NA-DE	97 X 10^7	123 X 10^7
	AL-DE	144 X 10^7	168 X 10^7
	CA-DE	111 X 10^7	127 X 10^7
	Cab-O-Sil 500	114 X 10^7	125 X 10^7
	Cab-O-Sil 750	105 X 10^7	118 X 10^7
P. fumosoroseus alone		149 X 10^7	
N. rileyi	DE	167 X 10^7	170 X 10^7
	NA-DE	123 X 10^7	129 X 10^7
	AL-DE	165 X 10^7	165 X 10^7
	CA-DE	112 X 10^7	124 X 10^7
	Cab-O-Sil 500	125 X 10^7	131 X 10^7
	Cab-O-Sil 750	107 X 10^7	116 X 10^7
N. rileyi alone		166 X 10^7	
V.lecanii	DE	167 X 10^7	179 X 10^7
	NA-DE	145 X 10^7	158 X 10^7
	AL-DE	169 X 10^7	166 X 10^7
	CA-DE	146 X 10^7	144 X 10^7
	Cab-O-Sil 500	106 X 10^7	105 X 10^7
	Cab-O-Sil 750	118 X 10^7	118 X 10^7
V.lecanii alone		188 X 10^7	

DE – the natural diatomaceous earth

Glob. J. Biodivers. Sci. Manag., 3(2): 183-187, 2013

Table 3: Ovipostional deterrent effect of tested DEs applied alone or in combinations with tested fungi against *C. maculates* moths.

Treatment	Mean number of eggs/female ±SE of *C. maculates*			
	DE/fungi combination			
	DE-alone	DE/*P.f*	DE/*N.r*	DE/*V.l*
DE	278.0±5.9	145±7.7	146.1±2.4	195.3±6.3
Na-DE	148.0±8.6	88.5±9.6	87.6 ±4.4	161.2 ±3.9
AL-DE	201.0±3.1	148.7±8.7	154.7 ±6.5	195.9 ±8.9
CA-DE	150.0±9.9	98±5.3	100 ±8.7	161.3 ±2.9
Cab-O-Sil 500	175.1±3.6	101.8±2.8	1142.8 ±7.6	170.6 ±8.9
Cab-O-Sil 750	131.4±8.4	95.6±5.9	101.9 ±9.3	160.6 ±6.8
control	288.6±9.4			
F value LSD (0.05)	31.31 18.17			

Table 4: Ovipostional deterrent effect of tested DEs applied alone or in combinations with tested fungi against *C. chinensis* moths.

Treatment	Mean number of eggs/female ±SE of *C. chinensis*			
	DE/fungi combination			
	DE-alone	DE/*P.f*	DE/*N.r*	DE/*V.l*
DE	252.0±5.9	152±7.7	162.1±2.4	187.3±6.6
Na-DE	161.0±8.6	82.5±9.6	94.6 ±4.4	130.2 ±3.3
AL-DE	211.0±7.1	161.7±2.7	174.7 ±6.5	183.9 ±8.0
CA-DE	161.0±7.9	100±4.3	110 ±8.7	141.3 ±2.1
Cab-O-Sil 500	180.1±3.6	120.7±4.8	131.8 ±7.6	151.6 ±7.9
Cab-O-Sil 750	160.4±8.4	96.7±5.2	101.3 ±7.3	141.6 ±6.4
control	278.6±9.4			
F value LSD (0.05)	32.31 19.17			

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