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Efficacy of Diatomaceous Earth in Combination with Cestain Entomopathogenic Fungi againg of *Sitotroga cerealella* (Olivier) and *Rhyzopertha Dominica* (Fab.) in Paddy During Storage

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ABSTRACT

The talc formulations of two entomopathogenic fungi *viz., Beauveria bassiana* and *Metarhizium anisopliae* containing 1 x 10^9 CFU g^{-1} (5 g. kg^{-1}) were used alone and in combination with two different concentrations (750mg. kg^{-1} and 1000 mg. kg^{-1}) of Diatomaceous earth (DE) against *S. cerealella* and *R. dominica* on paddy (BPT-5204) during storage. Among the grain treatments, 100 % adult mortality rate of *R. dominica* was recorded in paddy, treated with higher concentration of DE *i.e.* 1000mg. kg^{-1} in combination with *B. bassiana* and *M. anisopliae* after 14 days of exposure. Significantly less *R. dominica* and *S. cerealella* F_1 individuals were recorded in paddy treated with the higher concentration of Diatomaceous earth in combination with *B. bassiana* (9.33 and 37.33 numbers respectively) and *M. anisopliae* (11.33 and 41.67 numbers respectively).

Key words : Beauveria bassiana, Diatomaceous earth, Metarhizium anisopliae, Rhyzopertha dominica, Sitotroga cerealella.

The use of residual insecticides as grain protectants and seed treatments is a common strategy for protection of stored product insect pests (Arthur, 1996). But the residues of these insecticides reduce the consumer acceptability for which there is an increasing public demand of residue-free food. Moreover, resistance development in insect pests to widely used insecticides (Arthur and Zettler, 1992; Irshad and Gillani, 1992), environmental concerns, and health hazards have necessitated the assessment of new alternatives for stored grain insect pest management.

Usage of entomopathogenic fungi (EPF) is the most promising alternative to the traditional pesticides. The fungal conidia get attached and penetrate through the insect's cuticle, causing the insect's death. Entomopathogenic fungi are naturally occurring organisms, environmentally safe with low mammalian toxicity (Cox and Wilking, 1996). The use of diatomaceous earth particles (DE) of fossilized remains of phytoplanktons (diatoms) which inactivate the epicuticular lipids of the insect's cuticle causing internal water loss and death through desiccation was found to be another best alternative to conventional insecticides. In addition to being physical poison, DEs also are chemically inert in nature and thus cause / pose no residue problem in the environments, where they are applied.

The potential of using a DE in combination with talc formulation of entomopathogenic fungi on two major stored product insects i.e. lesser grain borer, Rhyzopertha dominica (F.) (Coleoptera: Bostrychidae) and Angoumois grain moth, Sitotroga cerealella (Olivier.) (Gelechidae: Lepidoptera) was examined in the present study.

MATERIAL AND METHODS

The experimentation was conducted with the paddy grain of variety, BPT-5204 (Samba Mashuri) which was disinfested by fumigating with Aluminium phosphide tablets @ 3 per tonne of grain for seven days to eliminate pre-existing infestation, if any, before releasing the test insects. The grain was then aerated for one day to remove phosphine residues. The pure fungal isolates of B. bassiana and M. anisopliae were procured from National Bureau of Agriculturally Important Insects (NBAII), Bangalore, Karnataka.

The two entomopathogenic fungi, B. bassiana and M. anisopliae were further sub cultured by plating them on Potato Dextrose Agar medium (PDA). Further, these cultures were mass multiplied by inoculating into a conical flask containing sterilized Potato Dextrose Broth (PDB) under aseptic conditions in Laminar Air Flow (LAF) chamber. After inoculation, the flasks were incubated at 32^0C in a BOD incubator till the profused sporulation was attained. Then the mycelial mat along with spores was thoroughly macerated in a sterile pestle & mortar. Thus the fungal cultures were mixed with the sterile talc powder in the ratio of 1: 4 (250 ml. kg^{-1} of carrier material). The required concentration (1 $x10^9$ CFU. g^{-1}) of the fungi in the talc powder formulation was determined by standard serial dilution technique using PDA.

To determine the efficacy of diatomaceous earth in combination with entomopathogenic fungi on S. cerealella or R. dominica, the following treatments were tested with three replications in a Completely Randomized Block Design experiments. The treatments were: DE @ 750 mg. kg^{-1} of seed, DE @ 1000 mg. kg^{-1} of seed, B. bassiana @ 5 g. kg^{-1} of seed (at a concentration $1x10^9$ CFU. g^{-1}), M. anisopliae @ 5 g. kg^{-1} of seed (at a concentration $1x10^9$ CFU. g^{-1}), DE @ 750 mg + B. bassiana @ 5 g. kg^{-1} of seed, DE @ 1000 mg + B. bassiana @ 5 g. kg^{-1} of seed, DE @ 750 mg + M. anisopliae @ 5 g. kg^{-1} of seed, DE @ 1000 mg + M. anisopliae @ 5 g. kg^{-1} of seed, Untreated Control

About five hundred grams of test variety was taken in to a plastic jar of 1 L capacity and mixed with the above treatments. Moisture content of the sample was recorded before treatments. Ten pairs of one day old S. cerealella and R. dominica adults were released into each plastic jar and the cumulative mortality was recorded at 1, 2, 3, 7 and 14 days after treatment. The surviving adults were removed after 14 days and the treated seed was kept without disruption to observe for the F1 progeny adult count.

RESULTS AND DISCUSSION

Effect on released adults

Efficacy of Diatomaceous earth (DE) in combination with entomopathogenic fungi (EPF) against the mortality of adult S. cerealella and R. dominica were presented in tables 1 and 2. The results indicated that there was no significant difference between treatments and untreated control. Treatments were not significantly effective to cause mortality in the adults of S. cerealella, since both EPF and DE act only by contact. In the present experiment conducted, moths did not come in contact with paddy seeds treated with different treatments, but for egg laying which was not an enough period to get infection or cuticle abrasion with fungal formulations or DE.

Significant difference in adult mortality of R. dominica was observed between treatments at each exposure interval. At 24 hours post infection, significantly more per cent adults (15.0) were dead in paddy treated with higher concentration of DE i.e. at 1000 mg.kg^{-1} in combination with both B. bassiana and M. anisopliae than the other treatments. This trend was also evident at the 2^{nd}, 3^{rd} and 7 day mortality counts. After 14 days of exposure, 100 % of adult R. dominica were dead in paddy treated with higher concentration of DE in combination with both B. bassiana and M. anisopliae which were significantly different from other varieties followed by DE 750 mg.kg^{-1} in combination with B. bassiana and M. anisopliae with 98.15% and 96.30% adult mortalities respectively. But B. bassiana, M. anisopliae and different concentrations of DE i.e. at 750 and 1000 mg. kg^{-1} alone caused 85.19%, 81.48%, 90.74% and 94.44% of mortalities of adult R. dominica respectively.

The results found were in agreement with those of Batta (2005) who reported that the mortality of R. dominica adults reached 93.3% and 86.7% after 7 days of exposure in chickpea grains contaminated with B. bassiana and M. anisolpliae, respectively. The increased per cent mortality rate at 14 DAT when compared to mortality rate at 7 DAT might be due to the more exposure to the fungal conidia which took complete infection cycle in the insect's body. Wakil et al. (2011) and Athanassiou et al. (2008) also reported that the adult mortality was increased with increase in dosage rate and exposure period. Athanassiou and Steenberg (2007) declared that the application of entomo-pathogenic fungi alone is less effective and the toxicity increased when combined with Insecto, SilicoSec, and PyriSec DE formulations. The present findings on the enhanced toxicity of DE along with fungal

Table 1. Efficacy of entomo-pathogenic fungi in combination with diatomaceous earth on the adult mortality and F1 adult emergence of Angoumois grain moth, S.cerealella on paddy (var.BPT-5204) during storage*.

Treatments	Dosge	Mortality					F1 Progency emerged @
		1DAT	2DAT	3DAT	7DAT	14DAT	
Diatomaceous earth	750 mg.kg^{-1}	0.00	5.00	28.33	83.33	100.00	65.33 (8.07)^{bcd}
Diatomaceous earth	1000 mg.kg^{-1}	1.67	6.67	26.67	88.33	100.00	49.33 (7.01)^{ef}
Beaveria bassiana (1x 10^{9} CFU g^{-1})	5 g.kg^{-1}	0.00	3.33	18.33	91.67	100.00	73.67 (8.57)^{bc}
Metarhizium anisopliae (1x 10^{9} CFU g^{-1})	5 g.kg^{-1}	0.00	8.33	23.33	93.33	100.00	78.00 (8.82)^{b}
Diatomaceous earth + *B. bassiana*	750 mg.kg^{-1} + 5 g.kg^{-1}	0.00	5.00	26.67	93.33	100.00	55.33 (7.40)^{dc}
Diatomaceous earth + *B. bassiana*	1000 mg.kg^{-1} + 5 g.kg^{-1}	0.00	6.67	26.67	91.67	100.00	37.33 (6.09)^{g}
Diatomaceous earth + *M. anisopliae*	750 mg.kg^{-1} + 5 g.kg^{-1}	1.67	3.33	28.33	93.33	100.00	60.67 (7.77)^{cde}
Diatomaceous earth + *M. anisopliae*	1000 mg.kg^{-1} + 5 g.kg^{-1}	0.00	8.33	35.00	98.33	100.00	41.67 (6.43)^{fg}
Control		0.00	8.33	31.67	90.00	100.00	127.33 (11.28)^{a}
SEm±		-	-	-	-	-	0.09
CD (0.05)		0.85	NS	NS	NS	NS	0.85

** Values are means of three replications
DAT - Days After Treatment
+ The values in parentheses are square root (X+0.05) transformed values
@ In each column values with similar alphabet do not vary signifcantly at 5%
NS- Non significance

isolates against R. dominica were in agreement with the results of Kavallieratos et al. (2006) who also reported that the addition of DE synergized the effectiveness of M. anisopliae and B. bassiana. Since, the inert dusts are abrasive in action and tend to cause physical damage / injury and thus might have pre-disposed the adult insects to the easy penetration and infection by entomopathogenic fungi.

Effect on progeny production

Efficacy of entomopathogenic fungi in combination with diatomaceous earth on the progeny adult development of S. cerealella and R. dominica was given in table 1 and table 2. It is evident from the tables, that extremely high number (127.33) of F1 adults of S. cerealella were recorded in untreated control when compared with all the treatments. Significantly less S. cerealella F1 individuals were recorded in paddy treated with the higher concentration of diatomaceous earth i.e. 1000 mg.kg^{-1} in combination with B. bassiana (37.33) and M. anisolpliae (41.67) which was significantly different from other treatments followed by DE i.e. 750 mg.kg^{-1} in combination with B. bassiana (55.33) and M. anisopliae (60.67) and were significantly different from the remaining treatments. While the lone treatments with B. bassiana, M. anisopliae and different concentration of DE i.e. 750 and 1000 mg.kg^{-1} also reduced the number of F1 progeny of S. cerealella viz.,73.67, 78.00, 65.33 and 49.33, respectively.

Significant differences were noted between treatments for progeny production counts.

Table 2. Efficacy of entomo-pathogenic fungi in combination with diatomaceous earth on the adult mortality and F1 adult emergence oflesser grain borer, R.dominica on paddy (var.BPT-5204) during storage*.

Treatments	Dosge	Mortality					F1 Progency emerged +@
		1DAT	2DAT	3DAT	7DAT	14DAT	
Diatomaceous earth	750 mg.kg^{-1}	8.33 (16.60)^{a}	21.67 (27.60)^{c}	40.00 (39.21)^{c}	71.35 (57.67)^{def}	90.74 (72.47)^{dc}	25.67 (5.02)^{bc}
Diatomaceous earth	1000 mg.kg^{-1}	13.33 (21.14)^{a}	31.67 (34.23)^{ab}	51.67 (45.96)^{b}	76.61 (61.34)^{cde}	94.44 (76.37)^{cd}	11.33 (3.36)^{dc}
Beaveria bassiana (1x 10^{9} CFU g^{-1})	5 g.kg^{-1}	3.33 (8.61)^{b}	5.00 (12.92)^{d}	13.33 (21.34)^{d}	66.18 (54.48)^{ef}	85.19 (67.45)^{ef}	28.33 (5.31)^{b}
Metarhizium anisopliae (1x 10^{9} CFU g^{-1})	5 g.kg^{-1}	3.33 (8.61)^{b}	3.33 (8.61)^{d}	18.33 (25.31)^{d}	58.87 (50.12)^{f}	81.48 (64.56)^{f}	31.00 (5.56)^{b}
Diatomaceous earth + *B. bassiana*	750 mg.kg^{-1} + 5 g.kg^{-1}	10.00 (18.43)^{a}	21.67 (27.71)^{c}	41.67 (40.20)^{c}	85.77 (67.90)^{bc}	98.15 (85.46)^{ab}	14.33 (3.78)^{dc}
Diatomaceous earth + *B. bassiana*	1000 mg.kg^{-1} + 5 g.kg^{-1}	15.00 (22.60)^{a}	33.33 (35.25)^{a}	56.67 (48.85)^{ab}	91.03 (72.78)a^{b}	100.00 (90.00)$^{a.}$	9.33 (3.04)^{c}
Diatomaceous earth + *M. anisopliae*	750 mg.kg^{-1} + 5 g.kg^{-1}	10.00 (18.43)^{a}	23.33 (28.86)^{bc}	50.00 (45.00)^{b}	83.82 (66.51)^{bcd}	96.30 (80.91)^{bc}	17.67 (4.19)^{cd}
Diatomaceous earth + *M. anisopliae*	1000 mg.kg^{-1} + 5 g.kg^{-1}	15.00 (22.79)^{a}	31.67 (34.23)^{ab}	60.00 (50.79)^{a}	92.79 (77.20)^{a}	100.00 (90.00)^{a}	11.33 (3.32)^{e}
Control		0.00	0.00	0.00	0.00	0.00	65.67 (8.08)^{a}
SEm±		+1.40 7.23	+1.05 5.43	+0.81 4.16	+1.65 8.47	+1.35 6.95	0.29 0.86

** Values are means of three replications
DAT - Days After Treatment
+ The values in parentheses are square root (X+0.05) transformed values
@ In each column values with similar alphabet do not vary signifcantly at 5%
NS- Non significance

Significantly less R. dominica (F1 individuals) adults were recorded in paddy treated with the higher concentration of diatomaceous earth in combination with B. bassiana (9.33) and M. anisolpliae (11.33) which was significantly different from other treatments followed by diatomaceous earth i.e. 750 mg.kg^{-1} in combination with B. bassiana (14.33) M. anisopliae (17.67) and were significantly different from the remaining treatments. But, when B. bassiana, M. anisopliae and different concentration of DE i.e. 750 and 1000 mg.kg^{-1} treated alone with paddy seeds reduced number of F1 progeny of R. dominica viz., 28.33, 31.00, 25.27 and 11.33, respectively, and they were found significantly different from untreated control (65.67).

The present results can be supported by Kavallieratos et al. (2006) who reported that the fewer F1 individuals of R. dominica and S. oryzae, were emerged from wheat treated with the highest fungal/DE dosage combination than the wheat treated with DE and fungal pathogens alone. Apart from parental mortality in the treated substrate, the avoidance of progeny production is essential for long term protection of stored grain. Both DE and fungal pathogens alone or combination substantially, suppressed progeny production since less no. of R. dominica and S. cerealella progeny were found than the untreated control.

The fact that none of the dosage rates tested was able to completely cease the progeny development of R. dominica and S. cerealella is

due to the mode of action of DE which might have caused desiccation takes place slowly so that exposed adults might tolerate short-term exposure and lay eggs before death. Since, larvae of R. dominica and S. cerealella are internal feeders and spend most of the time within the host grain thus avoiding the exposure to DE particles on the treated grain surface resulted in partial control on the development, which might imply that higher dose rate capable of rapidly desiccating R. dominica and S. cerealella first instar larvae may be required to prevent the progeny development.

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