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Insecticidal Effect of *Beauveria Bassiana* and *Paecilomyces fumosoroseus* alone and in Combination with Diatomaceous Earth Against Rice Weevil (*Sitophilus oryzae*) and Red Flour Beetle (*Tribolium castaneum*)

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The virulence (bioassay tests) of two fungal entomopathogens such as *Beauveria bassiana* and *Paecilomyces fumosoroseus* alone and in combination with 250 and 500 ppm of Diatomaceous earth (DE) was evaluated at laboratory scale. The results demonstrate that 50% relative humidity (RH) is more suitable to kill the insects than at 70% RH. Further, combined inoculation treatments of both the entomopathogenic fungi under test, along with higher doses of DE (500 ppm), recorded greater mortality than any other treatments followed by combined treatment of DE at 250 ppm. Nevertheless, the control treatments have recorded from 10-18% mortality. Although higher % mortality was observed with single treatment of *Beauveria bassiana* and *Paecilomyces fumosoroseus* over controls, greater differences were not observed compared to combined treatment of *Beauveria bassiana* with 250 ppm of DE. However, simultaneous use of DE with the fungus resulted an additive effect. Therefore, from the practical point of view, this substance (DE) is very good alternative to traditional insecticides.

Key words: Entomopathogens, Diatomaceous earth (DE), Bioassay, Relative humidity (RH), Stored product insects and Percent mortality.

Stored grains are vulnerable to attack by a range of insects and mite pests, which may result in damage to the cereals and subsequent economic losses, either as a result of direct damage and loss of quality, or through rejection at the point of sale. Seventy five percent of these insects are coleopterans (Vinuela *et al.,* 1993), and the most damaging species of storage insects are in the genera *Sitophilus* and *Tribolium* (Marsans., 1987; Khan and Selmon., 1988). Stored grain protection against insect pests is currently based on the use of fumigants and residual insecticides (Arthur, 1996, Bell, 2000). However, chemical based methods of pest control are now being re-evaluated, because most of them are toxic to mammals and affect the environment. Therefore, biological control using entomopathogenic fungi is one of the most promising alternative methods (Moore *et al* 2000). Among the fungal species

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tested, *Beauveria* and *Metarhizium* have shown good results for number of stored grain insect species (Lord, 2001; Ekesi et.al., 2000). Recently, one more species of entomopathogenic fungus registered in the EU according to the Plant Protection Products Directorate 91/414/EEC is *Paecilomyces fumosoroseus* (Michalaki, *et. al* 2007).

Diatomaceous earths (DEs), which are the fossils of phytoplankton (diatoms) is an other promising alternative. They have low mammalian toxicity (like fungi), and possess high effectiveness against a wide range of stored product insects (Athanassiou, *et.al* 2005; Subramanyam and Roesli, 2000; Fields and Korunic, 2000; Vayias and Athanassiou, 2004). These are natural and composed mainly of amorphous silica, which absorb the insect's cuticle, leading death through water loss (Subramaniam and Roesli, 2000). Some DE formulations are now commercially available in the market and are evaluated against stored product insects with success (Subramanyam and Roesli, 2000).

Synergistic efficacy of DE and *Beauveria bassiana* against lesser grain borer, *Rhizopertha dominica* was first reported by Lord (2001). Subsequent trials made by Akbar, et. al (2004), confirmed these results against larvae of rust red beetle, *Tribolium castaneum.* Use of combining DE with fungal entomopathogens is an appealing approach, since integrated pest management (IPM) control is a very good alternative compared to traditional pesticides in a stored product insect control strategy. In this back drop, we have evaluated two strains of fungi such as *Beauveria bassiana* and *Paecilomyces fumosoroseus* alone and in combination with 250 and 500 ppm of DE at laboratory condition.

MATERIAL AND METHODS

Insect pathogens such as *Beauveria bassiana* and *Paecilomyces fumosoroseus* were isolated from soil samples by decimal serial dilution technique (Harrigan and Mc Cance, 1990) on Veen's selective medium (Goettel and Inglis, 1997) containing (gm/lit) Peptone 10g, Glucose 20g, Distilled water 1000ml, Streptomycin 1.2 g, Tetracycline 0.1g, Dodine 0.2g, Cyclohexamide 0.05g and Agar10g. The cultures were identified according to the manual of techniques in insect pathology (Humber, 1997) considering colony morphology, color and microscopic examination. *Tribolium castaneum* and *Sitophilus oryzae* used in this study were from a colony that has been reared and maintained in our insect culture laboratory at CFTRI. Both the insects were 15 days old and maintained at 75±2 % relative humidity (RH) at 28±1^{0}C on wheat flour and whole hard wheat, respectively.

Fungi and DE formulations

Using "single spore culture" technique (Johnstone, 1969), pure cultures of fungi were grown on sterile Potato Dextrose agar (PDA) plates for 14 days in a BOD incubator (Indlab, Chennai) at 25±1^{0}C. Conidia were harvested from above fungal cultures by gently scraping the colonies with sterile inoculation loop using 10ml of Tween-80 (0.01% v/v aqueous solution). The suspension of conidia was filtered through a double layer cheese cloth to remove the debris and vortexed for 3min to breakup the conidial clumps (confirmed by microscopic examination), if any and the number of fungal conidia were estimated with an haemocytometer and adjusted to 1x10^6 conidia /mL. Diatomaceous earth (Sigma Aldrich, USA) containing 95% SiO_2 was mixed thoroughly with conidial suspension and air dried for 30min before being used at the rate of 250 and 500ppm levels.

Bioassay

The bioassays were carried out with two different RH levels (50±2 and 70±2%) at 25±1^{o}C, with little modification of the method described by Vassilakos *et.al.,* (2006). Ten grams of wheat was treated with 1ml conidial suspension of individual entomopathogens such as *B. bassiana* and *P. fumosoroseus* containing 1×10^6 conidia/ml alone or in combination with 250 and 500 ppm of DE. These treated samples were air dried for about 1h and taken in to 30 ml wide mouth glass tubes (2cm dia.), wrapped with thin muslin cloth. Thus, they were 5 fungal/DE combination treatments. For each treatment five tubes were prepared. In addition to this, a series of tubes containing untreated wheat which served as control.

After the preparation of tubes, ten adult insects (*Sitophilus oryzae)* were introduced into each tube. The tubes were placed in desiccators at 50±2 and 70±2% RH levels. The required RH was maintained by different quantity of glycerol water mix. Mean mortality was assessed on 4th and 7th day of treatment. The same procedure was followed for other insect (*Tribolium castaneum*). All the experiments were repeated three times.

RESULTS AND DISCUSSION

The percent mortality of *Sitophilus oryzae* with respect to 50 and 70% RH levels are given in Table-1. Results of the data indicate that higher levels of mortality were observed at 50% RH than 70% RH. Also, individual treatment of *Beauveria bassiana* has shown higher mortality than 250ppm of DE alone, except at 70% RH on day 7th (36.6). However, still higher percent of mortality was recorded when the weevils were treated with *Beauveria bassiana* in combination with 500ppm of DE at 50% RH (73.3 and 83.3%). The rate of mortality with individual treatment of *Beauveria bassiana* and 500 ppm of DE has not shown much difference, except at 70% RH on 7th day, in which 46.6% mortality was observed.

Table 2 shows the individual and combined effect of *Beauveria bassiana* and DE against *Tribolium castaneum*. In general, the rate of mortality in all combination treatments was slightly lesser than *Sitophilus oryzae* (Table-2). Treatment of DE at 250ppm alone has shown least activity at both RH levels throughout the experiment. Furthermore, in most of the individual treatment of *Beauveria bassiana* or combined treatment of *Beauveria bassiana* and 250ppm of DE have shown higher level of mortality than 250ppm of DE alone. Nevertheless, the combined treatment of *Beauveria bassiana* and 500ppm of DE at 50% RH has resulted 63.3% and 76.6% mortality on day 4th and day 7th, respectively. The rate of mortality in control tubes ranged from 10-18%.

Individual and combined treatments of *Paecilomyces fumosoroseus* and DE against *Sitophilus oryzae* and *Tribolium castaneum* are shown in Table-3 and 4, respectively. The percent mortality on day 4th in case of individual treatment of *Paecilomyces fumosoroseus* against *Sitophilus oryzae* was 26.6%; while 20% against *Tribolium castaneum* at 50% RH (Table 3 & 4). Similarly, on day 7th it was 33.3 and 36.6% for the same humidity level. Lower level of mortality was observed with 70% RH than 50% in both the test insects. However, the mortality rate was increased upto 70% when the wheat was treated with *Paecilomyces fumosoroseus* and 500ppm of DE against *Sitophilus oryzae* at 50% RH (Table-3). Contrary to this, the same treatment has shown lesser mortality (56.6%) against *Tribolium castaneum* (Table-4).

Moore *et.al.*, (2000) reported that the entomopathogenic fungi can act as an alternative to synthetic pesticides in control of stored product insects. The survey on insect bioassays using fungal pathogens as biological control revealed the effect of exposure interval, RH and temperature which attributes a major role on entomotoxicity of the fungus under the test condition. In addition, the toxicity also depends, the manner by which fungal conidia are attached to the insect cuticle and their ability to germinate and multiply in the test insect. Lord (2005), observed that the presence of DE particles on the insect's cuticle may inactivate few epicuticular lipids which may have negative effect on the attachment, penetration and germination of fungal spores. Similarly, Akbar *et.al.* (2004) reported that DE increased the conidial attachment on the cuticle of *Tribolium castaneum* larvae.

Although extensive literature is available on entomocidal activity of *Beauveria bassiana* on stored grain insect pests, the published literature in relation to *Paecilomyces* sp. on above mentioned insect group are very scanty. Dal-Bello *et.al.,* (2001) reported the toxicity of entomopathogenic fungi, *Paecilomyces farinosus* (Holm ex. S.F. Gray) Brown and Smith against the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculinidae). They found only 30% mortality after 14 days of exposure when fungal spore suspension (1×10^7 spores/ml) was sprayed over the test insects. Also, Kassa *et al.,* (2002) who tested *Paecilomyces* sp. against the maize weevil, *Sitophilus zeamais* (Motsch) (Coleoptera: Curculionidae) and the larger grain borer, *Prostephanus truncates* (Horn) (Coleoptera: Bostrychidae). Studies carried out by Michalaki *et al.,* (2007) in which fungal spores were added directly to stored wheat at 200 and 400ppm of DE formulation SilicoSec-R, 100% larval mortality and 34% of adult mortality was recorded in case of *Tribolium confusum*. However, the larval mortality of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) did not increase more than 56%. Further they also demonstrated, though the sp of *Paecilomyces* was effective for both *Tribolium confusum* and *Ephestia kuehniella*, its effectiveness is highly dependent on target insect, insect life stage (larvae, pupae and adult), exposure interval and temperature. The data obtained from our findings here indicate that, *Paecilomyces fumosoroseus* is more effective

Table 1. Individual and combined effect of *Beauveria bassiana* and Diatomaceous earth (DE) against Rice weevil *(Sitophilus oryzae)*

Treatments	% mortality on day 4th		% mortality on day 7th	
	50% RH	70% RH	50% RH	70% RH
Beauvaria bassiana	46.3 ± 11.5	33.3 ± 11.5	56.6 ± 11.5	33.3 ± 527
DE (250ppm)	40.0 ± 9.4	30.0 ± 7.4	43.3 ± 11.8	36.6 ± 5.7
Beauveria bassiana + DE (250ppm)	56.6 ± 5.1	50.0 ± 11.2	70.0 ± 4.7	56.6 ± 11.3
DE 500ppm	46.7 ± 5.7	36.6 ± 5.7	53.3 ± 5.3	46.6 ± 11.5
Beauveria bassiana + DE (500ppm)	73.3 ± 5.7	63.3 ± 11.5	83.3 ± 4.9	69.7 ± 5.6
Control	12.0 ± 5.8	10.0 ± 5.9	16.0 ± 7.1	14.0 ± 5.8

Values represent the mean ± standard deviation of triplicate sets

Table 2. Individual and combined effect of *Beauveria bassiana* and diatomaceous earth (DE) against Red flour beetle (*Tribolium castaneum*)

Treatments	% mortality on day 4th		% mortality on day 7th	
	50% RH	70% RH	50% RH	70% RH
Beauvaria bassiana	40.0 ± 5.9	26.6 ± 5.7	46.6 ± 11.5	30.0 ± 7.4
DE (250ppm)	30.0 ± 5.6	26.6 ± 5.1	40.0 ± 6.9	30.0 ± 8.5
Beauveria bassiana + DE (250ppm)	43.3 ± 5.7	36.6 ± 5.4	63.3 ± 5.4	50.0 ± 9.2
DE 500ppm	36.6 ± 5.3	33.3 ± 5.3	50.0 ± 8.3	36.6 ± 5.7
Beauveria bassiana + DE (500ppm)	63.3 ± 5.1	56.6 ± 11.5	76.6 ± 5.6	60.0 ± 10.3
Control	14.0 ± 3.2	10.0 ± 4.6	18.0 ± 5.9	16.0 ± 4.6

Values represent the mean ± standard deviation of triplicate sets

Table 3. Individual and combined effect of *Paecilomyces fumosoroseus* and Diatomaceous earth (DE) against Rice weevil (*Sitophilus* oryzae)

Treatments	% mortality on day 4th		% mortality on day 7th	
	50% RH	70% RH	50% RH	70% RH
Paecilomyces fumosoroseus	26.6 ± 5.7	16.6 ± 5.7	33.3 ± 5.6	23.3 ± 5.5
DE (250ppm)	40.0 ± 5.9	30.0 ± 6.3	43.3 ± 5.4	33.3 ± 5.3
Paecilomyces fumosoroseus + DE (250ppm)	46.6 ± 5.7	36.6 ± 5.7	56.6 ± 5.1	40.0 ± 6.4
DE 500ppm	46.6 ± 5.8	30.0 ± 3.4	50.0 ± 7.3	36.6 ± 5.7
Paecilomyces fumosoroseus + DE (500ppm)	56.6 ± 5.3	40.0 ± 6.5	70.0 ± 8.2	56.6 ± 5.1
Control	12.0 ± 4.1	14.0 ± 7.1	14.0 ± 5.7	18.0 ± 7.9

Values represent the mean ± standard deviation of triplicate sets against *Sitophilus oryzae* than *Tribolium castaneum*, although overall mortality is greater when *Beauveria bassiana* was combined with 500 ppm of DE. Our data also suggest, 50% RH has caused considerable higher mortality than 70% in both the insects. This is also true when fungal conidia were admixture with two doses of DE (250 and 500 ppm). However, in control treatments although not much difference in mortality was observed, the mortality rate in these treatments was from 10-18%.

In order to increase its potentiality of the insect pathogenic fungi, it is advisable to have suitable formulations with different admixtures for this specific purpose (insect control). In this direction, of the two entomopathogens tested in the present study, *Beauveria bassiana* is more effective than *Paecilomyces fumosoroseus* either singly or in combination with two doses of DE. Michalaki *et al.,* (2007) reported 34% mortality against *Tribolium castaneum,* when wheat was treated with *Paecilomyces* sp. along with Silicosec-R. In our study, combined treatment of *Paecilomyces fumosoroseus* with 500 ppm of DE against *Tribolium castaneum* resulted up to 56.6% mortality at 50% RH. Still higher % of mortality (70%) was obtained against *Sitophilus oryzae* when *Paecilomyces fumosoroseus* was combined with 500ppm of DE. It has been reported that, presence of DE seems to have varied types of impact on efficacy of entomopathogenic fungi (in some cases additive effect and in other cases the fungus might impair the efficacy of DE). The results obtained in our study corraborate this statement (additive effect). Athanassion *et al.,* (2007) used three types of DEs and *Beauveria bassiana* and found that all DEs increased the fungal effectiveness against granary weevil (*Sitophilus granaries* (L.) (Coleoptera: Curculionodae). It suggests that the effect of fungal spores was not affected by the type of DE. In the present investigation of biological control using two stored product insect pests, *Sitophilus oryzae* is more sensitive than *Tribolium castaneum* for DE formulations and DE efficacy is negatively affected at high relative humidity (70% RH).

Table 4. Individual and combined effect of *Paecilomyces fumosorous* and diatomaceous earth (DE) against Red flour beetle (*Tribolium casteneum*)

Treatments	% mortality on day 4th		% mortality on day 7th	
	50% RH	70% RH	50% RH	70% RH
Paecilomyces fumosoroseus	20.0 ± 4.8	13.3 ± 5.7	36.6 ± 5.8	23.3 ± 5.4
DE (250ppm)	36.6 ± 5.7	30.0 ± 6.2	40.0 ± 8.1	30.0 ± 5.9
Paecilomyces fumosoroseus+DE(250ppm)	40.0 ± 6.9	33.3 ± 5.7	43.3 ± 5.7	40.0 ± 8.8
DE 500ppm	40.0 ± 5.3	36.6 ± 5.1	46.6 ± 5.3	33.3 ± 5.7
Paecilomyces fumosoroseus+ DE (500ppm)	46.6 ± 5.7	36.6 ±5.5	56.6 ± 5.5	43.3 ± 5.1
Control	12.0 ± 5.1	10.0 ± 4.6	14.0 ± 5.3	14.0 ± 6.2

Values represent the mean ± standard deviation of triplicate sets

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