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Simultaneous Use of Entomopathogenic Fungus *Beauveria bassiana* and Diatomaceous Earth against the Larvae of Indian Meal Moth, *Plodia interpunctella*

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Abstract

The suppressive ability of entomopathogenic fungus *Beauveria bassiana* alone and in combination with diatomaceous earth (DE) was studied against the larvae of Indian meal moth, *Plodia interpunctella* (*Hübner*) (Lep., Pyralidae). This study clearly showed that simultaneous use of *B. bassiana* and DE against larvae of *P. interpunctella*, not only could reduce the required concentration of fungal conidia or DE, but also could shorten the time need for showing insecticidal effects. The LC_{50} value of fungus at 7 d after treatment was 9.8×10^5 conidia mg^{-1} diet. Larvae showed a dose response to *B. bassiana*, and the addition of diatomaceous earth at 500 and 2000 ppm resulted in a significant increase in mortality. Larval mortality reached to the maximum of 28.3% and 71.7% after 7 d exposure to 500 and 2000 ppm DE concentrations, respectively. The LC_{50} value for *B. bassiana* in the presence of DE 500 ppm was 4.6×10^4 con. mg^{-1} diet and of DE 2000 ppm was 1.65×10^3 con. mg^{-1} diet. According to our results, *B. bassiana* and DE can be considered as two suitable candidates for integration into IPM strategy.

Keywords

Stored Product Pest, *Beauveria bassiana*, Synergism, Diatomaceous Earth, Bioassay

1. Introduction

Entomopathogenic fungi are among the important biological control agents of insect pests, by causing lethal in-

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fections and regulating insect and mite populations in nature by epizootics [1]-[3]. These biocontrol agents infect a wide range of insect orders including Hemiptera, Coleoptera and Lepidoptera which are of great concern in worldwide agriculture but some strains of these fungi could be host specific with a very low risk of attacking non-target organisms or beneficial insects [4].

The entomopathogenic as comycete, *Beauveria bassiana* (Balsamo) Vuillemin is an important pathogen of insects and it has been developed as a microbial insecticide for use against many major arthropod pests [5] [6]. It has been developed as a microbial insecticide for use against many major pests, including lepidopterans. It is reported to be non-toxic to humans and other vertebrates, so it can be applied on commodities [7].

Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae), is a serious pest of raw and processed food products worldwide [8]-[11]. Some reports have pointed out to the promising control effects of *B. bassiana* on Indian meal moth [12] [13]. However the fungi encountered some limiting factors to show adequate level of pest control.

The use of *B. bassiana* alone for the control of many stored product pests requires a high application rates. The action of fungal penetration to the host integument is dependent to physicochemical properties of cuticle including thickness, sclerotization, and presence of fatty acids as well as cuticle destroying enzymes [14]-[16].

The presence of cuticular lipids in insect integument could protect the insect from pathogenic microorganisms. If any agent could damage this barrier, greater penetration and higher pathogen virulence would be expected.

Diatomaceous Earth (DE), a natural product composed of the fossils of diatoms, can exhibit such role with its special properties like integument scarification and adsorbing wax from cuticle layer [17], causing the release of subcuticular compounds that have a synergistic effect on conidial attachment and virulence of entomopathogenic fungi [18] [19].

Several studies document that DE formulations are very effective against a wide range of stored products pests, persist on the product for a long period and can be easily removed from the grains [20]-[22].

It is generally accepted that these materials act as desiccants on insect cuticle, and that insects exposed to DE particles die from rapid water loss [17] [23] [24]. Low mammalian toxicity is another important characteristic of DE [25], rendering it a potential and safe method for pest control.

Our objective in this study was to determine the effects of *B. bassiana* and DE, alone or in combination, on the Indian meal moth larvae as well as clarify interactions of these two agents.

2. Materials and Methods

2.1. Insect Rearing

Indian meal moth, *P. interpunctella* was acquired from a laboratory population at the Department of Plant Protection, University of Tehran and reared under laboratory conditions (25˚C ± 1˚C, 65% ± 10% RH and 16:8 L:D) in Polyethylene boxes (30 × 20 × 15 cm) covered with a piece of fine cloth.

Artificial diet including wheat bran, yeast, honey, and glycerol, prepared by the method described by Sait *et al.* [26], was used for insect rearing. No antibiotic or fungicide was added to the diet.

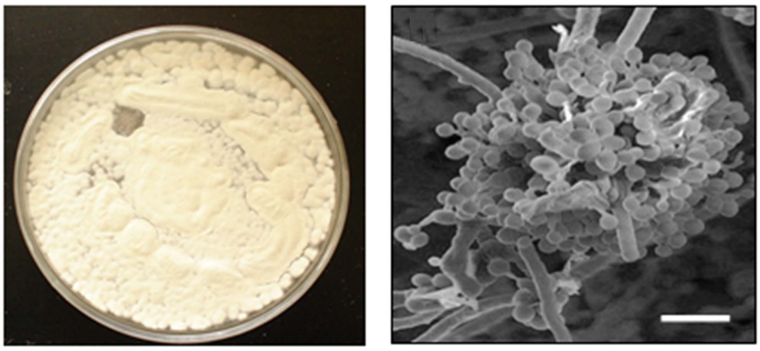
2.2. Fungal Isolate and Cultures

The isolate used in this study was *B. bassiana* ETU105, originally isolated from soil using *Galleria*-bait and preserved in Laboratory of Biological Control at the Department of Plant Protection, University of Tehran. Fungal isolate was grown on Sabouraud dextrose agar plus 1% yeast extract (SDAY) in 9 cm diameter Petri dishes (**Figure 1**) and incubated under conditions of 25˚C ± 1˚C, 75 ± 10 RH and 16:8 L:D for 14 days. Colony was preserved at 4˚C.

Fungal conidia were collected by scraping conidial layer using sterilized scalpel into 0.02% Tween 80. The conidial concentration was estimated using a haemocytometer. Each mg of collected conidia contained 4.5×10^8 spores. Five serial concentrations 10^3 - 10^7 conidia mg^{-1} diet were prepared by mixing adequate conidia with artificial diet. An electric mixer was used for 5 min to prepare an even mixture.

2.3. Diatomaceous Earth

Diatomaceous Earth (DE) was prepared from Kimia Sabz Avar Company located in Iran. It was oven-dried initially at 60˚C for two hours, and then two concentrations of 500 and 2000 mg/kg were prepared by adding sufficient DE to artificial diet. These two concentrations were selected based on the results of our preliminary test.



(a) (b)

Figure 1. *Beauveria bassiana*, (a) Fully sporulated on SDAY medium; (b) Scanning electron micrograph of conidiophores and conidia clusters (original, bar = 10 µm).

2.4. Bioassay

There were 17 treatments: five fungal dose rates alone, five fungal dose rates in combination with each of the two DE dose rates and the two DE rates alone. Five serial dose rates of fungal conidia were 10^3 - 10^7 con. mg^{-1} diet. Fifteen g of diet containing treatments were placed in series of glass Petri dishes with 9 cm in diameter. An additional series of dishes containing untreated diet served as a control.

After the preparation of the dishes, 15 third instar larvae of *P. interpunctella* were introduced into each dish. The dishes were placed in incubators set up at temperature of 25˚C ± 1˚C and RH of 65% ± 5% for a week. All the experiments were repeated four times. Larval mortality was assessed daily until 7 days after exposure.

2.5. Statistical Analysis

Mortality counts were corrected by using Abbott's formula [27]. The experiment was designed and conducted in a factorial Complete Randomized Design (CRD). The pooled data were analyzed separately for each treatment category (Fungus alone, DE alone, DE 500+ fungus, DE 2000+ fungus), by submitting the mortality counts to ANOVA's for dose rate. Means were separated using the Tukey test at $P < 0.05$. SAS software version 9.1 was used for statistical analysis. The LC_{50} values were estimated with probit analysis using POLO-Plus [28].

3. Results

3.1. Effect of *B. bassiana* on *P. interpunctella* Larvae

Mortality rates of third instar larvae of *P. interpunctella* treated with *B. bassiana* increased with increasing conidial concentration and time of exposure. Differences among lethal effects established by different conidial concentrations of fungal isolate were significantly different (F = 72.49, df = 5, $P < 0.0001$).

The LC_{50} value of fungus at 7 d after treatment on *P. interpunctella* larvae was 9.8×10^5 con. mg^{-1} diet. Comparative virulence of *B. bassiana* isolate against L_3 instar of *P. interpunctella* indicated that mortality of larvae began at the third day after exposure and reached to 61.7% at the day 7. The LT_{50} value of fungus at 1×10^7 con. mg^{-1} was 6.59 d (6.23 - 7.12 days lower and upper 95% C.I., respectively).

3.2. Effect of Diatomaceous Earth on *P. interpunctella* Larvae

Larval mortality reached to a maximum of 28.3% and 71.7% after 7 d exposure to 500 and 2000 mg/kg DE concentrations, respectively. There were highly significant differences in larval mortality resulting from different concentrations of DE (F = 121.13, df = 2, $P < 0.0001$). Larval mortality ranged from 1.7 to a maximum of 71.7% over the range of tested concentrations.

3.3. Combination of *B. bassiana* and DE

Data on comparative virulence of *B. bassiana* isolate in combination with DE against L_3 instar of *P. interpunctella* is brought in **Table 1**. These results showed that there was a synergistic relationship between fungus and DE targeting larvae of *P. interpunctella*.

The LC_{50} value for *B. bassiana* plus DE 500 mg/kg was 4.6×10^4 con. mg^{-1} diet and with DE 2000 mg/kg was 1.65×10^3 con. mg^{-1} diet in contrast to the LC_{50} of 9.8×10^5 con. mg^{-1} diet for *B. bssiana* alone (**Table 2**). There were significant differences among these treatments ($F = 72.49$, $df = 5$, $P < 0.0001$).

The combination treatment of fungus with DE caused the highest mortality at 7d after treatment which was significantly greater than all other treatments. With concentration of 500 ppm DE, mortality of larvae began at the second day after exposure to 1×10^7 con. mg^{-1} of fungus and reached to 100% at the day 7.

With DE 500, LT_{50} value for fungus with concentration of 1×10^5 con. mg^{-1}, diminished to 3.9 d which was significantly lower than that of fungus alone with 6.6 d ($F = 11.48$, $df = 26$, $P < 0.0001$). This value showed a greater reduction when DE 2000 was applied, such that it was reduced to 2.1 d. The log-probit regression lines of LC_{50} had slopes of 0.34 without DE, 0.65 with DE 500 and 0.64 with DE 2000. These parameters for LT_{50s} were 8.21, 6.54 and 5.35, respectively (**Table 3**).

4. Discussion

Results from bioassays on *P. interpunctella* larvae showed that the *B. bassiana* isolate ETU105 was virulent against the pest, however, a relatively high concentration of 9.8×10^5 conidia mg^{-1} diet needs to cause 50% mortality during 7 d. These results stand somewhat in agreement with that of Buda and Pečiulytė [29] who found that after treatment with concentration of 2.6×10^6 conidia mg^{-1} of *B. bassiana*, larval mortality of the species, was reached to 50% during 5 days.

Many of the important pests in grain storage have proven to be susceptible to *B. bassiana*, but its high production costs and high application rates make it economically unreachable [30]-[36].

Table 1. Mean percent mortalities of *P. interpunctella* third instar larvae caused by *B. bassiana* in combination with diatomaceous earth.

DE concentration (ppm)	Fungal concentration (conidia mg^{-1} diet)					
	0	10^3	10^4	10^5	10^6	10^7
0	5	25	28.33	43.33	55	61.66
500	38.33	41.66	60	71.66	80	100
2000	71.66	83.33	91.66	96.66	98.33	100

Table 2. LC_{50} values of *B. bassiana* alone or in combination with two diatomaceous earth doses based on experiments with 3rd instar larvae of *P. interpunctella*.

DE concentration (ppm)	Fungus LC_{50} (con. mg^{-1} diet)	χ^2	Slope	Lower 95% confidence limit	Upper 95% confidence limit
0	0.98×10^6	4.23	0.34	0.28×10^6	0.50×10^7
500	4.06×10^4	8.86	0.65	6.11×10^3	0.12×10^6
2000	1.65×10^3	8.21	0.64	0.26×10^2	1.03×10^4

Number of treated larvae = 360.

Table 3. Values of LT_{50} for *B. bassiana* at 1×10^7 con. mg^{-1} diet alone or in combination with two doses of diatomaceous earth based on experiments with 3rd instar larvae of *P. interpunctella*.

DE concentration (ppm)	Fungus LT_{50} (d)	χ^2	Slope	Lower 95% confidence limit	Upper 95% confidence limit
DE 0	6.59	5.9	8.21	6.23	7.12
DE 500	3.92	9.68	6.54	3.69	4.15
DE 2000	2.09	8.08	5.35	1.90	2.28

Number of treated larvae = 360; df = 26.

The concentration of 500 ppm of DE, caused 28.3% mortality of *P. interpunctella* larvae with increasing the DE concentration to 2000 ppm could enhance the mortality rate to 71.7 percent. This result approved the finding of Sabbour *et al.* [13], however, the LC_{50} of fungus in their study was 1.29×10^9 con. mg^{-1} which was much greater than that of our finding, 1×10^5 con. mg^{-1} diet. This difference may be related to fungus isolate or population of *P. interpunctella*.

Our results also are in conformity with findings of Akbar *et al.* [18] who found that DE increased the efficacy of *B. bassiana* against *Tribolium castaneum* larvae. Also Michalaki *et al.* [19] found that the effectiveness of entomopathogenic fungus, *Metarhizium anisopliae* can be benefitted by the presence of DE against *T. confusum* larvae.

DE does not leave any toxic residue because of its physical effects; however, regarding the possible health issues, lower concentrations should be preferred. Low concentration of DE does not leave any harmful residue on stored commodities. If it could be prepared from good quality and suitable resources, would have very low effect on human breathing system. Also, using suitable breathing mask, such effects could be greatly diminished.

Fast and stable effectiveness, make DE an appropriate choice to substitute chemical compounds for stored product protection. Moisture increase of environment can lead to less effectiveness of DE, so it is more successful in control of stored product pests. Moreover such pests are little in size, have a higher body surface to body volume ratio, and encountered to low water content of commodities. The lack of insect resistance against DE is another important prominence which enhances its capability for use as a part of integrated pest management program.

DE does not have any significant effect on stored products by own itself and can be removed easily with air blowing or washing. This can decrease applying chemical pesticides in grain deposit. Using DE at concentration of 500 ppm, the LC_{50} value of *B. bassiana* was significantly decreased on *P. interpunctella* larvae.

The exact mechanisms by which DE interacts with *B. bassiana* are not clear but may involve a combination of increased availability of water and other nutrients, removal or mitigation of inhibitory materials, alteration of adhesive properties, and physical disruption of the cuticular barrier [18]. Lord [12] [34] proposed that lipid removal may contribute to the synergistic interaction between *B. bassiana* and DE against some stored grain pests.

Akbar *et al.* [18] showed the number of *B. bassiana* conidia attached to the larval cuticle of *Tribolium castaneum* was significantly greater with DE presence than without it. The mean counts of conidia were 212.7 with DE and 90.9 without DE.

Michalaki *et al.* [19] revealed that DE benefits the fungal efficacy only when conidial concentration exceeds a certain "active threshold". Below this "threshold", a considerable amount of conidia may be damaged by the presence of DE, or DE particles may partially lose their desiccant capacity.

5. Conclusion

Finally, this study clearly indicated that simultaneous use of *B. bassiana* and DE against larvae of *P. interpunctella*, not only reduced the required concentration of fungus or DE, but it would also shorten the time need for showing their effects. On the other hand, the greatest factor in the loss of inoculum viability of entomopathogenic fungi under field conditions is inactivation caused by UV light [37] [38], but grain storage environment does not have such disadvantage, thus it could be a suitable environment for integrated application of *B. bassiana* and DE.

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