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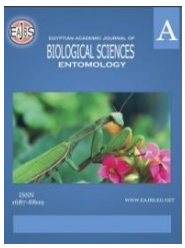

Efficacy of Two Entomopathogenic Fungi in Combination with Diatomaceous Earth Against *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae)

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ABSTRACT

The current investigation was conducted to study the efficacy of natural diatomaceous earth (DE) alone and combined with two fungal pathogens: *Metarhizium anisopliae* and *Beauveria bassiana*, as a new formulation against *Spodoptera littoralis* third instar larvae under laboratory conditions. When *M. anisopliae*, *B. bassiana*, and DE were applied alone, the mortalities of larvae were concentration-dependent. The LC_{50} values were 299.2, 391.5, and 572 ppm, respectively. The combination of DE with *M. anisopliae* and with *B. bassiana* enhances the efficacy of both. The LC_{50} values were 133.77 and 189 ppm, respectively, indicating a toxic potentiation effect. The results demonstrated that the application of DE in a binary combination with fungi significantly reduced the immune enzyme activity (phenoloxidase, prophenoloxidase, lysozyme) and protein concentration compared to the control. These findings are consistent with the theory that diatomaceous earth increases the effectiveness of entomopathogenic fungi against insects by causing cuticle damage, which increases conidial attachment and increases the availability of nutrients for conidial germination. It concluded that adding DE to entomopathogenic fungi helps in improving their quality and efficiency in reducing insect outbreaks, and it suggested participating in sustainable pest management of *S. littoralis*.

INTRODUCTION

Spodoptera littoralis (Boisd.) larvae attack different valuable cultivated plants in Egypt, including cotton, peanut, maize, wheat, rice, and vegetables (Pineda *et al.,* 2007). Because *S. littoralis* larvae have developed resistance to various synthetic chemical pesticides, suitable alternatives are required. The widespread use of insecticides to control *S. littoralis* has had negative consequences for humans, beneficial insects, and the ecosystem (Chaudhuri *et al.,* 1999). The challenges and hazards associated with the use of traditional insecticides have prompted the urgent demand for substitute insecticides. Microbial control agents are one of these, and they include bacteria, fungi, and viruses (Dent, 2000). A crucial component of a long-term pest management strategy is using entomopathogenic fungi in biological plant protection. When compared to traditional insecticides, entomopathogens have a number of advantages as bioagents. These include being affordable, highly effective, and safe for non-target organisms, minimizing environmental residues, and boosting biodiversity in human-managed settings (Lacey *et al.,* 2001). Fungal biocontrol agents invade in a distinctive way. They may directly infect

their host through the cuticle. Because of this, the entomopathogenic fungus can infect insects' non-feeding stages, such as their eggs and pupae, and due to its complex mode of action, it's very difficult for an insect to grow resistance against these pathogens (Nguyen *et al.,* 2007).

The most prevalent entomopathogenic fungus species employed as biological control agents against insect pests is thought to be *Metarhizium anisopliae* (Barra *et al.,* 2013). It is extensively distributed in nature and frequently isolated from soil or diseased insects (Razinger *et al.,* 2014).

Beauveria bassiana is an extremely virulent entomopathogenic fungus that infects a broad spectrum of insect hosts (Tanada and Kaya, 1993). It lives in soil and is found all over the world (Klingen *et al.,* 2002). The results of previous research revealed that this fungus is one of the most effective microbial control agents for battling various insect pests (Lacey and Goettel, 1995). *B. bassiana* has the capability to invade the insect through its cuticle and reach the hemocoel, inhibiting the host's immunological response (Götz, 1991). Diatomaceous earth (DE) is a natural substance that poses no hazard to the environment. It is shown to do no harm to beneficial insects and wildlife. It is simple to use and leaves no residue in the food or in the ecosystem. Because of these characteristics, DE is considered a perfect option for use as an insecticide (Korunic, 1998). Carlson and Ball (1962) reported insecticidal impacts of diatomaceous earth when applied to eight different species of stored grain insect pests. An insect dies when DE is absorbed into the cuticle's wax layer, leading to water loss and dryness (Ebeling, 1971; Quarles, 1992). The Environmental Protection Agency in the United States announced DE as a safe substance.

The combined effects of DE with fungi pathogens, *Beauveria bassiana* and *Metarhizium anisopliae*, have been observed in many studies (Athanassiou and Steenberg, 2007; Lord, 2007; Athanassiou *et al.,* 2008; Riasat *et al.,* 2011; Wakil *et al.,* 2011; Wakil *et al.,* 2012; Luz *et al.,* 2012; Hanif *et al.,* 2022). These researchers found that the rough attributes of DE improved the effectiveness of entomopathogenic fungi as insecticides. It enhanced the pathogenicity of both fungi. It has not yet been investigated how DE interacts with Egyptian isolates of *M. anisopliae* and *B. bassiana* and how this affects *Spodoptera littoralis*.

The immune system of insects is relatively effective. Both humoral and cellular defense responses make up an insect's immunity. Nodule production, encapsulation, and phagocytosis are all aspects of cellular immunity (Lavine and Strand, 2002). Antimicrobial peptides and the phenoloxidase system play significant roles in humoral defense. An essential component of the insect immune system, phenoloxidase plays a crucial role in the coagulation, melanization, and wound healing processes. In addition to the activation via the prophenoloxidase cascade, prophenoloxidase is the inactive form of phenoloxidase. It is carried out in the cuticle or in the hemolymph of the insect in response to injury. The enzyme that cleaves prophenoloxidase into phenoloxidase is believed to be a clip-domain serine protease (Ashida, 1990). To create melanin, these quinones polymerize. Fungi are affected by quinones and melanin. In the hemolymph, melanin deposition darkens parasites, and foreign bodies are encapsulated in nodules caused by hemocyte melanization (Söderhall and Cerenius, 1998). Phenoloxidase, a major enzyme in the production of melanin, is located in the hemolymph, midgut, and cuticle. Melanin and its precursors are used directly in the immune system. It is believed to play a significant part in the encapsulation of bigger organisms (Cotter *et al.,* 2004). The chemical nature of melanin itself may also hinder the growth of fungi (St. Leger *et al.,* 1988).

This work aims to explore the efficacy of two fungal pathogens, *Metarhizium anisopliae* and *Beauveria bassiana*, alone and combined with a natural control agent DE, against *Spodoptera littoralis* larvae and their effects on some biochemical elements.

MATERIALS AND METHODS

Rearing Technique of The Experimental Insect:

A laboratory strain of cotton leafworm, *S. littoralis* (Lepidoptera: Noctuidae), was reared on fresh leaves of the castor oil plant. The culture was kept under constant conditions of 27±2°C, photoperiod of 14 hours light and 10 hours dark, and 65±5% R.H. Egg-masses obtained from the Plant Protection Research Institute (PPRI) Dokki, Egypt. The larvae were daily provided with fresh castor leaves. The emerged adults were fed on a 10% sugar solution in clean jars to allow mating. Clean filter papers were used for egg laying.

The Tested Compounds:

Diatomaceous earth (DE) was purchased from Natural Source LLC, FL, USA as a 100% pure powder.

The two entomopathogenic fungi treatments were obtained from the commercial formulation produced by the Insect Pathogen Unit, Plant Protection Research Institute, Ministry of Agriculture, Egypt. The *Beauveria bassiana* was tested as Biover® 10% W. P. (32 million spore /g) and the *Metarhizium anisopliae* was tested as Bioranza® 10% W. P. (32 million spore /g) and 200 g per 100 liters of water/Feddan was the recommended application rate. A stock solution (1000 ppm) was prepared by dissolving ten grams of each formulated fungus in one liter of distilled water. A series of different concentrations (100, 200, 300, 400, and 500 ppm) were prepared for the DE, Biover®, and Bioranza® to facilitate the comparison between the three treatments.

Bioassay Tests:

Newly molted third instar larvae were treated with the same series (100, 200, 300, 400, and 500 ppm) of the three different materials: Diatomaceous earth (DE), *Beauveria bassiana* (Biover®), and *Metarhizium anisopliae* (Bioranza®). All the tested larvae were starved for about four hours before the treatment. For each treatment, five replicates of twenty larvae in each plastic cup were used. Three different treatments were done as *M. anisopliae*, *B. bassiana*, and diatomaceous earth, independently. Two combination treatments were done as DE + *M. anisopliae* and DE + *B. bassiana*. The mixed ratio was one-to-one for each concentration. In each treatment, discs of castor leaves were dipped in the corresponding concentration of independent or combined treatments. The different treated discs were introduced to the tested larvae. In the control replicates, discs of castor leaves were dipped in distilled water. After the larvae finished feeding on the treated leaves (24 h), fresh castor leaves were provided to the tested and control larvae. The mortalities for the five treatments were recorded after ten days.

The bioassay results were handled by PC programming LdP to draw the regression line and for the identification of the concentration which kills twenty-five percent of the larvae (LC_{25}), the concentration which kills fifty percent of the larvae (LC_{50}), and the concentration which kills ninety-five percent of the larvae (LC_{95}) values as previously described (Finney, 1952).

Evaluation of the Joint Toxicity:

The joint toxicity of combinations of diatomaceous earth with *M. anisopliae* and diatomaceous earth with *B. bassiana* was detected by using the LC_{25} obtained from each treatment independently. The LC_{25} of DE was mixed with the LC_{25} of *M. anisopliae* for the first combination test. The LC_{25} of DE was mixed with the LC_{25} of *B. bassiana* for the second combination test. The experiment was conducted as previously mentioned in the bioassay test. The observed mortalities for these two combinations were used to find the relationship between the tested compounds when applied together. The co-toxicity factor was calculated by using the following equation according to Mansour *et al*. (1966).

$$\textbf{Co} - \textbf{toxicity factor} = \frac{\text{Observed \% mortality} - \text{Expected \% mortality}}{\text{Expected \% mortality}} \times 100$$

The co-toxicity factor indicated the results. Potentiation is defined as a factor of 20 or greater; a negative factor of 20 or more is considered to be antagonism, and values that are between -20 and +20 imply additive effects.

The Biochemical Analyses:

The LC_{50} values of the different five treatments were used in the biochemical study as previously described. The hemolymph was collected from the treated and control late fifth instar larvae. The thoracic legs were pulled out from the larvae by a fine forceps. The drained hemolymph was collected by a micro syringe and placed in Eppendorf tubes. The inhibitory effect of tyrosinase was prevented as recommended by Abou El-Ghar *et al*. (1996) by adding traces of phenylthiourea.

The activity of the phenoloxidase enzyme in the diluted hemolymph was measured by the colorimetric test described by Laughton and Siva-Jothy (2011) in the presence of L-β-3,4-dihydroxyphenylalanine (L-Dopa) as a substrate. The absorption was measured by a UV spectrophotometer at 490 nm.

The activity of the prophenoloxidase enzyme in the samples was measured as described for the phenoloxidase enzyme with an extra step. The diluted hemolymph was incubated with chymotrypsin as an activator in sodium cacodylate buffer for five minutes before adding the L-Dopa. The difference between these two activity readings directly indicates the activity of the prophenoloxidase enzyme.

The lysozyme activity was quantified by using dried bacterial cell walls (*Micrococcus lysodeikticus*) in sodium phosphate buffer (10 mM - pH 5.5) according to the methodology described by Powning and Davidson (1973). The turbidity was measured by a UV spectrophotometer at 450 nm after adding sodium hydroxide. When measured against 10 mM phosphate buffer, the initial absorbance readings were between 1.5 and 1.6 under these conditions. The amount of enzyme that reduces the absorbance by 0.001 units per minute is considered to be one unit of lysozyme. Units of activity per mg of protein are used to measure the lysozyme's specific activity in the samples.

The original method of Gornal *et al.* (1949) was used to determine the total protein amounts in the hemolymph of treated and untreated samples. The change in the reaction color was measured by a UV spectrophotometer at 545 nm. The final protein concentrations were calculated as gram per deciliter.

Statistical Analyses:

The means of the mortality percentages of the larvae in the various experimental treatments were separated and statistically analyzed using ANOVA (LSD). After calculating the mean mortality percentage, the standard error of the mean (SEM) was added. A significant correlation ship was recorded when $P<0.05$, and a non-significant correlation ship was recorded when $P\geq0.05$.

RESULTS

Toxic Effects of *Metarhizium anisopliae, Beauveria bassiana*, and Diatomaceous Earth on *Spodoptera littoralis* Larvae:

Data in Table (1) and Figure (1) represent the recorded mean percentage of mortalities in *S. littoralis* larvae after treatment with *M. anisopliae*, *B. bassiana*, and DE. In all three treatments, there are significant positive relationships between the used concentration of the toxic compounds and the mean larval mortalities. The highest concentration (500 ppm) in this bioassay test induced 86.67 ± 1.67, 76.67 ± 1.67, and 43.33

± 4.41% mortalities when the larvae were treated by *M. anisopliae*, *B. bassiana*, and DE, respectively. This result indicates that *M. anisopliae* was the most effective one, followed by *B. bassiana* and then DE. These data were confirmed by the results of the regression line in Figure 1 when the LC_{50} values were obtained as 299.2, 391.5, and 572 ppm for treated larvae with *M. anisopliae*, *B. bassiana*, and DE, respectively.

Table 1: The toxic effects of *Metarhizium anisopliae*, *Beauveria bassiana*, and diatomaceous earth against 3rd instar *Spodoptera littoralis* larvae as mean mortality percent ± SE at 95% Confidence Limit**.**

Conc. (ppm)	Mortality % ± SE		
	Metarhizium anisopliae	***Beauveria bassiana***	**Diatomaceous earth**
0	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]
100	13.33 ± 1.67 [b]	6.67 ± 1.67 [b]	3.33 ± 1.67 [a]
200	23.33 ± 1.67 [c]	16.67 ± 1.67 [c]	10.00 ± 0.00 [b]
300	36.67 ± 3.33 [d]	26.67 ± 1.67 [d]	23.33 ± 1.67 [c]
400	65.00 ± 5.77 [e]	43.33 ± 4.41 [e]	36.67 ± 3.33 [d]
500	86.67 ± 1.67 [f]	76.67 ± 1.67 [f]	43.33 ± 4.41 [e]
LC_{25}	180.4 ppm	237.8 ppm	312.5 ppm
LC_{50}	299.2 ppm	391.5 ppm	572 ppm
LC_{95}	1027 ppm	1320.7 ppm	2499.4 ppm

*ANOVA and the LSD test showed that means with different letters in a column are significantly different (P<0.05).

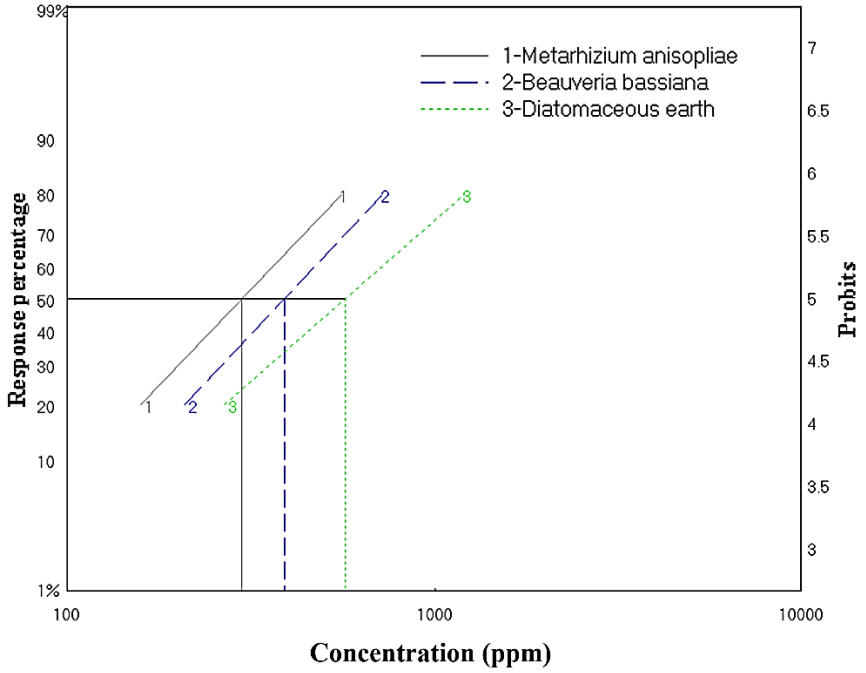

Fig. 1: The regression line of the relation between *Metarhizium anisopliae*, *Beauveria bassiana*, and diatomaceous earth application and percentage mortalities.

The Combined Toxic Effects of *Metarhizium anisopliae*, *Beauveria bassiana*, and Diatomaceous Earth on *Spodoptera littoralis* larvae:

In order to evaluate the combined toxic effect of DE with *M. anisopliae* or with *B. bassiana*, several concentrations (100, 200, 300, 400, and 500 ppm from each compound) were mixed equally for each combination in a ratio of 1:1 and were applied to 3rd instar *S. littoralis* larvae. The corresponding average percentage of mortalities was recorded after ten days of exposure in Table (2).

Data displayed in Table (2) indicates that the percentage of larvae mortality was significantly increased with the increase of the combined concentration of DE with *M. anisopliae*, or with *B. bassiana*.

The lethal concentration values (LC_{25}, LC_{50}, and LC_{95}) in Table (2) are obtained from the regression line in Figure (2). The combination of DE and *M. anisopliae* is more efficient than the combination of DE and *B. bassiana* where the mortalities percentage at 300 ppm concentration from each compound were 93.33 ± 1.67 and 71.67 ± 6.01 at the mentioned combinations above, respectively. All larvae died at 400 and 500 ppm concentrations in the two combinations. The LC_{50} values were increased from 133.77 to 189 ppm in binary combinations of DE with *M. anisopliae* and DE with *B. bassiana*, respectively. This increase in the LC_{50} value indicates the improvement of the combination between DE and *M. anisopliae* over the other combination.

Table 2: Toxicity Effects of combination between diatomaceous earth with *Metarhizium anisopliae* or with *Beauveria bassiana* on 3rd instar *Spodoptera littoralis* larvae as mean mortality percent ± SE at 95% Confidence Limit.

Conc. (ppm)	Mortality % ± SE	
	Diatomaceous earth + *Metarhizium anisopliae*	Diatomaceous earth + *Beauveria bassiana*
0 : 0	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]
100: 100	33.33 ± 4.41 [b]	15.00 ± 2.89 [b]
200: 200	68.33 ± 1.67 [c]	31.67 ± 1.67 [c]
300: 300	93.33 ± 1.67 [d]	71.67 ± 6.01 [d]
400: 400	100 ± 0.00 [e]	100 ± 0.00 [e]
500: 500	100 ± 0.00 [e]	100 ± 0.00 [e]
LC_{25}	100.14 ppm	151 ppm
LC_{50}	133.77 ppm	189 ppm
LC_{95}	271.03 ppm	327 ppm

*ANOVA and the LSD test show that means with different letters in a column are significantly different (P<0.05).

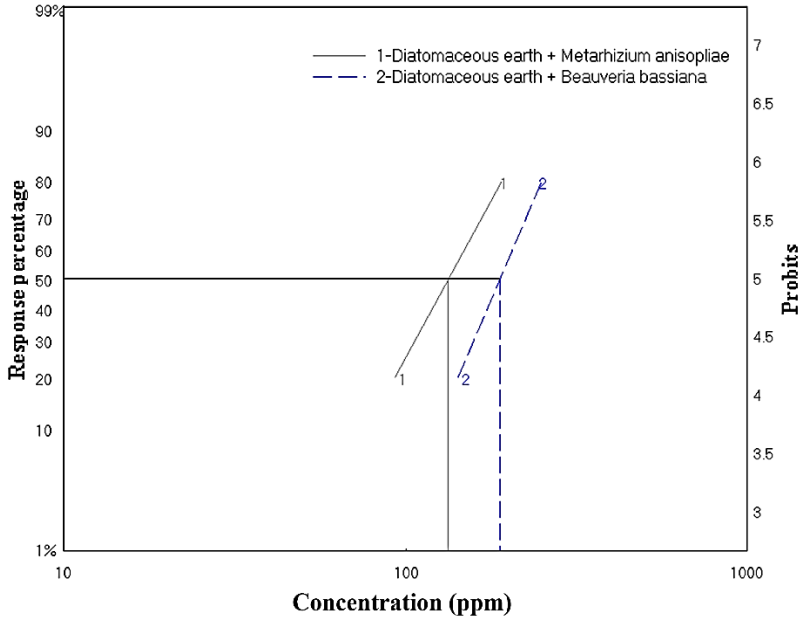

Fig. 2: Regression line of the joint toxicity of diatomaceous earth with *Metarhizium anisopliae* and diatomaceous earth with *Beauveria bassiana* correlated to the percentage mortalities.

Joint Toxicity Actions of Diatomaceous Earth with *Metarhizium anisopliae* and Diatomaceous Earth with *Beauveria bassiana* against 3rd instar *Spodoptera littoralis* Larvae:

Two combinations of the three tested compounds were applied to the larvae to evaluate the joint toxicity. The first combination was DE with *M. anisopliae* and the second combination was DE with *B. bassiana*. In these combinations, the LC_{25} for each compound was used.

The observed and expected mortalities were used to calculate the joint toxic action of the two combinations, which was then presented as a Co-toxicity factor for each combination (Table 3). Data in Table 3 revealed that the obtained mortality percent was increased when the mixture of DE with *M. anisopliae* or the mixture of DE with *B. bassiana* was applied. This indicates that using the two compounds together is more efficient. As a result, the larval mortality was enhanced by the combination rather than using each one independently. The two binary mixtures showed potentiating effects.

Table 3: Joint action of diatomaceous earth with *Metarhizium anisopliae* and diatomaceous earth with *Beauveria bassiana* mixtures against 3rd instar *Spodoptera littoralis* larvae:

LC-levels (Bi-mixture)	Mixing Ratio	Expected Mortality	Observed Mortality	C. F.	Joint action
LC_{25} Diatomaceous earth + LC_{25} *Metarhizium anisopliae*	1:1	50	86.67	73.34	P
LC_{25} Diatomaceous earth + LC_{25} *Beauveria bassiana*	1:1	50	73.33	46.66	P

C. F. = Co-toxicity factor.
Joint action: P = Potentiate.

Changes of Some Biochemical Parameters in Treated *Spodoptera littoralis* Larvae:

The enzymatic activity of phenoloxidase, prophenoloxidase, and lysozyme (as an immune response in insects) in addition to the total protein level in the hemolymph was quantitively detected in the treated larvae with the three tested compounds independently and in two combinations. The changes in these biochemical elements were compared to their levels in the control larvae.

In Figure (3-A), the level of phenoloxidase was significantly (P<0.05) reduced in treated larvae with *M. anisopliae*, and with *B. bassiana* by -55.94% and -63.67%, respectively. When DE was added to the fungi, the erosion in the cuticle facilitated the entrance of the fungi with a high level of secreted toxins. As a result, there were significant (P<0.05) reductions in the phenoloxidase in treated larvae with *M. anisopliae* mixed with DE, and with *B. bassiana* mixed with DE by -75.51% and -77.96%, respectively.

Data in Figure (3-B) showed significant reductions (P<0.05) in the prophenoloxidase in treated larvae with *M. anisopliae*, with *B. bassiana*, with *M. anisopliae* mixed with DE, and with *B. bassiana* mixed with DE by -56.80%, -71.59%, -66.27%, and -82.84%, respectively.

In Figure (3-C), the lysozyme enzyme activity was significantly (P<0.05) reduced when larvae of *S. littoralis* were treated with *M. anisopliae*, *B. bassiana*, *M. anisopliae*+DE, and *B. bassiana*+DE. The reduction values were -51.55%, -65.01%, -63.41%, and -71.83%, respectively.

Treatment of *S. littoralis* larvae with *M. anisopliae*, *B. bassiana*, *M. anisopliae*+DE, and *B. bassiana*+DE significantly (P<0.05) decreased their total protein content. The decreased values were -63.39%, -44.87%, -76.87%, and -63.46%, respectively, as shown in Figure (1-D).

It was noticed from Figure (3) that larvae treated with DE did not show any significant reduction ($P>0.05$) in the enzymatic activity of phenoloxidase, prophenoloxidase, and lysozyme or in the total protein level in the hemolymph.

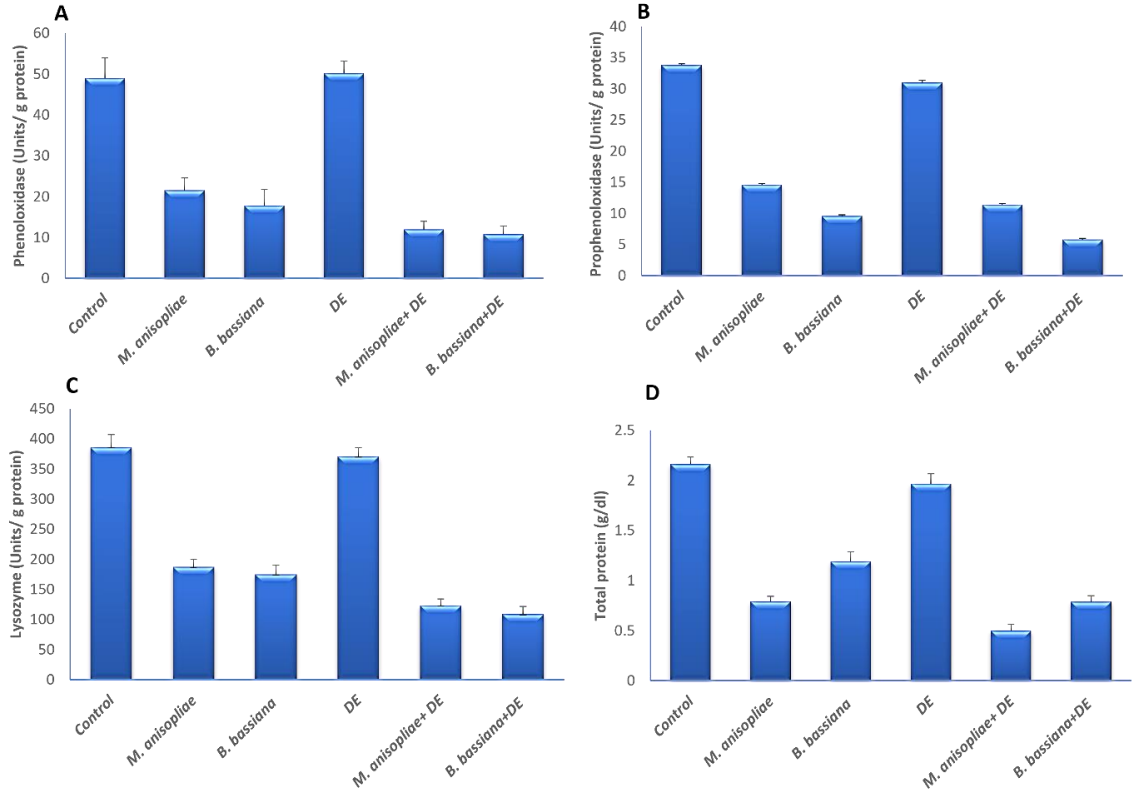

Fig. 3: Statistical histograms for the change in the activity of phenoloxidase (A), prophenoloxidase (B), lysozyme (C), and total protein (D) in *Spodoptera littoralis* larvae treated with *Metarhizium anisopliae*, *Beauveria bassiana*, or diatomaceous earth (DE), independently and in two combinations.

DISCUSSION

According to the findings of our study, there is a link between *S. littoralis* larvae mortality and a higher diatomaceous earth concentration. Mortality was reported in a significant ascending manner with the increase of the DE concentration. This death occurred as a result of DE's ability to adhere to insect cuticles and absorb their waxy, oily, outer layer, which causes water loss and promotes dryness and abrasiveness. When higher dosages are used than lower ones, the harm is more severe, causing an insect to die quickly from dehydration due to the abrasion in the cuticle layers (Athanassiou *et al.*, 2005). The killing action of DE is a mechanical one, not a chemical one, as it has microscopically razor-sharp edges, which act like rough sandpaper. There are now a lot of commercially available DE formulations, and various studies show that they work well against a variety of pest species (Subramanyam and Roesli, 2000). Mewis and Ulrichs (2001) observed high mortality in *Tribolium confusum*, *Plodia interpunctella*, and *Sitophilus granaries* treated with low dosages of DE and found that it required a long exposure period to cause higher mortality. The results stand in the same line as El-Sayed *et al.* (2010), who claimed that the treated wheat with diatomaceous earth highly reduced the population of *Sitophilus oryzae*, *Tribolium castaneum*, and *Rhyzopertha dominica*. Because of its low mammalian toxicity, worker safety, the low danger of food residues, and the formation of resistant insect

populations linked to the use of chemical pesticides, diatomaceous earth usage has increased during the past decades.

The present study illustrates the ability of the tested formulated entomopathogenic fungus, *M. anisopliae*, to show positive influences on *S. littoralis* larval mortalities. Our results were in agreement with El Husseini (2019), who tested the local isolate of the entomopathogenic fungus *M. anisopliae* against 3rd and 5th larval instars of the cotton leafworm *S. littoralis* and recorded the deaths of larvae that reached high mortality four days after treatment. Şahin and Yanar (2021) observed a high mortality rate of *M. anisopliae* when applied to *S. littoralis* larvae under laboratory conditions.

For controlling a broad range of insects with natural agents by direct penetration of the host cuticle, *M. anisopliae* is the most effective one (Santi *et al.*, 2010). Lotfy *et al.* (2016) observed positive influences on the 2nd and 4th instar larvae of *S. littoralis* treated with spores of *M. anisopliae* at different concentrations in laboratory conditions. In other studies, it was found that *Metarhizium brunneum* was very effective against *S. littoralis* and can be considered as a potential biological control agent against *S. littoralis* larvae (Funda and Yusuf, 2021; Eleawa *et al.*, 2022).

The mode of action of *B. bassiana* is through adhering to the insect cuticle, germinating, and penetrating its body. It has no effect on non-targeted insects (Goettel and Hajek, 2001). In this research, *B. bassiana* showed significant mortality effects. The correlation between the concentrations of *B. bassiana* and the mortalities was exponential. Ahmed and El-Katatny (2007) studied the effects of *B. bassiana* on both larval and pupal stages of *S. littoralis* within 5 days post-treatment. The result showed relatively high dose-dependent larval and pupal mortalities. Ramos *et al.* (2020) hypothesized that *B. bassiana* began to kill *Spodoptera frugiperda* larvae on the 4th day after treatment, with an eleven percent mortality rate. After that, mortality increased, reaching its highest mortality rate on the 9th day after treatment. Meanwhile, *M. anisopliae* started its mortality on the 3rd day after treatment with a nineteen percent larval mortality.

Because of the fungus' mode of action, it's expected that the fungus' efficacy would be enhanced in the presence of other surface-active agents. DE has an enormous surface area with sharp edges. Due to the physical structure of DE, it can induce cracks in the cuticle of the target pest. This action facilitates the entrance of the fungus into the bodies of the tested insects. In our results, a potentiation effect in the mortality rate was noticed after mixing DE with *B. bassiana* or with *M. anisopliae*. That was confirmed when the sub-lethal concentration of each in the mixture was used. The addition of DE appears to have different types of influence on the potency of fungus. According to Lord (2001), DE has a synergistic interaction with *B. bassiana* against some stored grain pests, while it has no negative effects on the fungus's germination. According to Akbar *et al*. (2004), the combination of *B. bassiana* and diatomaceous earth had good performance when used to control *T. castaneum* larvae. To decrease the amount of DE, it can be mixed with other compounds to improve their efficacy (Korunic and Fields, 1995; Subramanyam and Roesli, 2000). The use of DE in combination with other compounds is one of the potential remedies for the effects brought on by the use of DE in excessive doses. These techniques comprise a combination of entomopathogenic fungi (Michalaki *et al.*, 2007). Experimenting with these combined compounds frequently demonstrated a synergistic and improved efficacy effect (Korunic, 2007; Athanassiou and Korunic, 2007). The effectiveness of *M. anisopliae* against *S. oryzae, R. dominica*, and *T. castaneum* was enhanced by the addition of inert dust, such as DE (Batta, 2008).

In the present study, *B. bassiana* and *M. anisopliae* severely deplete the protein concentration with an average of -62.15% either when used alone or when used in combination with DE. House (1963) reached the conclusion that a great depletion in protein

content of a treated insect may be the leading factor in their mortality rates as it causes hindrance to ovulation and egg development as proteins are the key factor in these physiological processes. The finding that the fungus is accountable for the disturbance in the endocrine balance of the host and the lack of protein makes it a direct reason for its death. Highnam and Hill (1969) stated that the disturbance in the endocrine balance of insects by fungi may be responsible for the depletion in protein concentrations of the hemolymph of insects. The decrease in the protein content may be one of the main causes of death in treated insects. Spores connect to and germinate on the cuticle of an appropriate host, triggering a series of recognition and enzyme activation processes in both the host and the fungal parasite.

In the current work, the levels of phenoloxidase, prophenoloxidase, and lysozyme were significantly decreased in treated larvae with *B. bassiana* or *M. anisopliae*. These enzymes are involved in the immune response in insects, especially during the invasion of microorganisms. The recorded reductions could be due to the inhibition of these enzymes by the toxins produced by the entomopathogenic fungi (Huxham *et al.,* 1989; Cerenius *et al.*, 1990).

The phenoloxidase and other downstream enzymes are activated as a result of the defensive reactions to the fungal infection. Changes in the levels of phenoloxidase may assist in reducing microbial infection, preserving the nutritional value of the paralyzed larva as a future food supply for the parasitoid (Hagstrum, 1983).

According to Trudeau *et al.* (2001), once the fungus has penetrated the cuticle of the insect, it invades the insect body and hemolymph and alters the enzyme activity and protein concentrations, where insects use their cuticle, fat body, and protein as defenses against attack by fungi. The death of *Schistocerca gregaria* and *Locusta migratoria* due to infection with *M. anisopliae* may cause hemolymph protein, phenoloxidase, and lysosome enzyme titers to decline (Gillespie *et al.*, 2000; Mullen and Goldsworthy, 2006). Proteolytic enzymes that are secreted into the insect's hemocoel and hydrolyze the host protein during parasitism may account for the decrease in total protein and loss of soluble protein from the host's hemolymph during parasitism (Gillespie *et al.*, 2000). Total protein concentrations in desert locusts infected with *M. anisopliae* drastically decreased (El-banna *et al.*, 2012). Our results were also in congruence with Gabarty *et al*. (2013), who investigated the efficacy of entomopathogenic fungi *B. bassiana* and *M. anisopliae* on the immune enzyme response of *S. littoralis* larvae. They recorded a significant decrease in the enzyme activities of phenoloxidase, prophenoloxidase, lysozyme, and protein concentration.

Conclusion

These results suggest that the use of inert powders, DE, in combination with entomopathogenic fungi, *M. anisopliae*, or with *B. bassiana*, may provide protection to agricultural crops while negatively affecting larvae of *S. littoralis*. These combinations have a great potentiation effect on *S. littoralis*. The most effective treatment was *M. anisopliae* mixed with DE, followed by *B. bassiana* mixed with DE, *M. anisopliae*, *B. bassiana*, and then DE alone. More studies in the field are needed to confirm the results and define the strategies and possible interactions of these management tools with other control methods for these species.

Ethical Approval: This research paper was approved by the research ethics committee from Faculty of Science, Ain Shams University (ASU-SCI/ENTO/2022/11/3).

REFERENCES

Abou El-Ghar, G.E.S.; Khalil, M.E.; Eid, T.M. 1996. Some biochemical effects of plant extracts in the black cutworm, *Agrotis ipsilon* (Hufn.) (Lepidoptera: Noctuidae). *Journal of Applied Entomology*, 120, 477-482.

Ahmed, A.M.; El-Katatny, M.H. 2007. Entomopathogenic fungi as biopesticides against the Egyptian cotton leaf worm, *Spodoptera littoralis*: between biocontrol promise and immune-limitation. *Journal of the Egyptian Society of Toxicology*, 37, 39-51.

Akbar, W.; Lord, J.C.; Nechols, J.R.; Howard, R.W. 2004. Diatomaceous earth increases the efficacy of *Beauveria bassiana* against *Tribolium castaneum* larvae and increases conidia attachment. *Journal of Economic Entomology*, 97, 2, 273–280.

Ashida, M. 1990. The prophenoloxidase cascade in insect immunity. *Research in Immunology*, 141, 908–910.

Athanassiou C.G.; Kavallieratos, N.G.; Vayias, B.J.; Tsakiri, J.B.; Mikeli, N.H.; Meletsis, C.M.; Tomanović, Ž. 2008. Persistence and efficacy of *Metarhizium anisopliae* (Metschnikoff) Sorokin (Deuteromycotina: Hyphomycetes) and diatomaceous earth against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) on wheat and maize. *Crop Protection*, 27, 10, 1303–1311.

Athanassiou, C.G.; Korunic, Z. 2007. Evaluation of two new diatomaceous earth formulations enhanced with abamectin and bitterbarkomycin against four stored grain beetle species. *Journal of Stored Products Research*, 43, 468-473.

Athanassiou, C.G.; Steenberg, T. 2007. Insecticidal effect of *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreaes) in combination with three diatomaceous earth formulations against *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). *Biological Control*, 40, 3, 411 - 416.

Athanassiou, U.C.G.; Vayias, B.J.; Dimizas, G.B.; Kavalieratos, N.G.; Papagregoriou, A.S.; Buchelos, C.T. 2005. Insecticidal efficacy of diatomaceous earth against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) on stored wheat: influence of dose rate, temperature and exposure interval. *Journal of Stored Products Research*. London, 41, 1, 47-55.

Barra, P.; Rosso, L.; Nesci, A.; Etcheverry, M. 2013. Isolation and identification of entomopathogenic fungi and their evaluation against *Tribolium confusum*, *Sitophilus zeamais*, and *Rhyzopertha dominica* in stored maize. *Journal of Pest Science*, 86, 217-226.

Batta, Y. 2008. Control of Main Stored-Grain Insects with New Formulations of Entomopathogenic Fungi in Diatomaceous Earth Dusts. *International Journal of Food Engineering*, 4, 1, 1-16.

Batta, Y.A. 2004. Control of the rice weevil (*Sitophilus oryzae* L., Coleoptera: Curculionidae) with various formulations of *Metarhizium anisopliae*. *Crop Protection*. 23, 2, 103–108.

Carlson, S.D.; Ball, H.J. 1962. Mode of Action and Insecticidal Value of a Diatomaceous Earth as a Grain Protectant. *Journal of Economic Entomology*. 55, 6, 964-970.

Cerenius, L.; Thornqvist, P.O.; Vey, A.; Johansson, M.W.; Soderhall, K. 1990. The effect of fungal toxin Destruxin-E on isolated crayfish haemocytes. *Journal of Insect Physiology*. 36, 785–789.

Chaudhuri, K.; Selvaraj, S.; Pal, A.K. 1999. Studies on the genotoxicity of endosulfan in bacterial systems. *Mutation Research*. 439, 63-67.

Cotter, S.C.; Hails, R.S.; Cory, J.S.; Wilson, K. 2004. Density-dependent prophylaxis and

condition-dependent immune function in lepidopteran larvae: a multivariate approach. *Journal of Animal Ecology*. 73, 283–293.

Dent, D.; 2000. Insect pest management. 2nd edition. CAB International, Wallingford, UK.

Ebeling, W. 1971. Sorptive Dusts for Pest Control. *Annual Review of Entomology*, 16, 1, 123-158.

El Husseini, M.M.M. 2019. Efficacy of the entomopathogenic fungus, *Metarhizium anisopliae* (Metsch.), against larvae of the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), under laboratory conditions. *Egyptian Journal of Biological Pest Control*. 29, 50.

El-banna, S.M.; Elhadidy, M.N.; Fayez, Semida, M.; Abdel-Rasool, T. 2012. Physiological and biochemical effect of entomopathogenic fungus *Metarhizium anisopliaeon* the 5th instar of *Schistcereca gregaria* (Orthoptera:Acrididae). *Journal of Research in Environmental Science and Toxicology*. 1, 1, 007-018.

Eleawa, M.; Waked, D.A.; Ali, M.A.; Elgohary, E.M. 2022. Efficiency of Certain Entomopathogenic Bacteria, Fungi with Pesticides for Controlling *Spodoptera littoralis*. *Journal of Plant Protection and Pathology*, Mansoura University, 13, 1, 19 – 22.

El-Sayed, F.M.A.; El-Zun, H.M.; Abd El-latif, A.M.; Nasr, M.E.H. 2010. Insecticidal effect of some inert dusts against three of stored grain insects at Kafr El-sheikh governorate. *Journal of Plant Protection and Pathology*, Mansoura University. 1, 12, 959 – 972.

Finney D.J. 1952. Probit analysis. 2nd Edition. Cambridge University Press, New York. 1952, 318.

Funda, S.; Yusuf, Y. 2021. Pathogenicity of some local entomopathogenic fungus isolates on the cotton leafworm larvae, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *Egyptian journal of biological pest control*. 31, 150.

Gabarty, A.; El-Sonbaty, S.M.; Ibrahim, A.A. 2013. Synergistic effect of gamma radiation and entomopathogenic fungi *Beauveria bassiana* and Metarhizium anisopliae on the humoral immune enzyme response in cotton leaf worm *Spodoptera littolaris* (Boisd). *Egyptian Academic Journal of Biological Sciences,A.Entomology,* 6(3) 1 – 10.

Gillespie, J. P.; Burnett, C.; Charnley, A.K. 2000. The immune response of the desert locust *Schistocerca gregaria* during mycosis of the entomopathogenic fungus, *Metarhizium anisopliae* Varacridum. *The Journal of Physiology*, 46, 429-437.

Goettel, M.S.; Hajek, A.E. 2001. Evaluation of Non-target Effects of Pathogens Used for Management of Arthropods. *CABI Press*, Wallingford, UK, 81–97.

Gornal, A.G.; Bardawill, G.J.; David, M.M. 1949. Determination of serum protein by means of the biuret reagent. *Journal of Biological Chemistry*, 177, 2, 751-766.

Götz, P. 1991. Invertebrate immune response to fungal cell wall components. In: Latge LP, Boucias D (eds) Fungal Cell and Immune Response. *Springer*, Berlin, 53, 317–330.

Hagstrum, D.W. 1983. Self-provisioning with paralyzed hosts and age, density, and concealment of hosts as factors influencing parasitization of *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) by *Bracon hebetor* Say (Hymenoptera: Braconidae). *Environmental Entomology*, 12, 1727–1732.

Hanif, M.; Mansha, M. Z.; Aatif, H.; Zaman, Q.; Ijaz, M.; Ikram, K.; Khan, A.; Ismail, M.; Ashir, M. 2022. Aptness of entomogenus fungi with diatomaceous earth against various stored grain insect pests. *Egyptian Journal of Biological Pest Control*, 32, 14, 1-6.

Highnam, K.C.; Hill, L. 1969. The comparative Endocrinology of the Invertebrates.

American *Elsevier*. New York.

House, H.L. 1963. Nutrition, in physiology of insects (Rockstein, ed) 2nd ed. 5, 791–821. *Academic press*, N.Y. and London.

Huxham, I.M.; Lackie, A.M.; McCorkindale, N.J.; 1989. Inhibitory effects of cyclodepsipeptides, destruxins, from the fungus *Metarhizium anisopliae*, on cellular immunity in insects. *Journal of Insect Physiology*, 35, 97–105.

Klingen I.; Eilenberg, J.; Meadow, R. 2002. Effects of farming system, field margins and bait insect on the occurrence of insect pathogenic fungi in soils. *Agriculture, Ecosystems & Environment*. 91, 191–198.

Korunic, Z. 1998. Diatomaceous earths, a group of natural insecticides. *Journal of Stored Products Research*, Philadelphia, v. 4, n. 2, p. 87-97.

Korunić, Z. 2007. Joint action of ready to use insecticide mixture of plant extract bitterbarkomycin and diatomaceous earth to control stored grain insects. *Proceedings of the 19th Seminar DDD and ZUPP 2007 Disinfection, Disinfestation, Deratization and Protection of Stored Agricultural Products*, Zagreb, Croatia: 375-387.

Korunic, Z.; Fields P.G. 1995. Diatomaceous earth insecticidal composition. USA. *Patent* 5, 773, 017.

Korunic, Z.; Fields, P.G. 1998. Diatomaceous Earth Insecticidal Composition. U.S.A. *Patent* 5,773,017.

Lacey, L.A.; Goettel, M.S. 1995. Current developments in microbial control of insect pests and prospects for the early 21st century. *Entomophaga*, 40, 3–27.

Laughton, A.; Siva-Jothy, M. 2011. A standardised protocol for measuring phenoloxidase and prophenoloxidase in the honey bee, *Apis mellifera*. *Apidologie*, 42, 2, 140-149.

Lavine, M.D.; Strand, M.R. 2002. Insect haemocytes and their role in immunity. *Insect Biochemistry and Molecular Biology*, 32, 10, 1295-1309.

Lord, J.C. 2001. Desiccant dusts synergize the effect of *Beauveria bassiana* (Hypomycetes: Moniliales) on stored grain beetles. *Journal of Economic Entomology*, 94, 367-372.

Lord, J.C. 2007. Desiccation increases the efficacy of *Beauveria bassiana* for stored-grain pest insect control. *Journal of Stored Products Research*, 43, 4, 535–539.

Lotfy, D.E.; El Sabagh, M.M; Ahmed, Y.E.; Yousef, H. 2016. Biological and histological effects of *Metarhizium anisopliae* on the cotton leafworm, *Spodoptera littoralis* (boisd.) (Lepidoptera: Noctuidae). *Egyptian Journal of Agricultural Research*, 94, 2, 343-352.

Luz, C.; Rodrigues, J.; Rocha, L.F.N. 2012. Diatomaceous earth and oil enhance effectiveness of *Metarhizium anisopliae* against *Triatoma infestans*. *Acta Tropica*. 122, 1, 29–35.

Mansour, N.A.; Eldefrawi, M.E.; Toppozada, A.; Zeid, M. 1966. Toxicological studies on the Egyptian cotton leaf worm, *Prodenia litura*. VI. Potentiation and antagonism of organophosphorous and carbamate insecticides. *Journal of Economic Entomology*, 59, 2, 307-311.

Mewis, I.; Ulrichs, C. 2001. Action of Amorphous Diatomaceous Earth against Different Stages of the Stored Product Pests *Tribolium confusum*, *Tenebrio molitor*, *Sitphilus granarius* and *Plodia interpunctella*. *Journal of Stored Products Research*, 37, 153-164.

Michalaki, M.P.; Athanassiou, C.G.; Steeberg, N.G.; T. Buchelo, C.T. 2007. Effect of *Paecilomyces fumosorroseus* (Wise) Brown and Smith (Ascohyta: Hypocreales) alone and in combination with diatomaceous earth against *Tribolium castaneum*

Jacquelin du Val (Coleoptera: Tenebrionidae) and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). *Biological Control*, 40, 280-286.

Mullen, L.M.; Goldsworthy, G.J. 2006. Immune responses of locust to challenge with the pathogenic fungus Metarhizium or high doses of Laminarin. *Journal of Insect Physiology*, 52, 2, 389-398.

Nguyen, N.C.; Borgemeister, H.P.; Zimmermannn, G. 2007. Laboratory investigations on the potential of entomopathogenic fungi for biocontrol of *Helicoverpa armigera* (Lepidoptera: Noctuidae) larvae and pupae. *Biocontrol Science and Technology*, 17, 12, 853-864.

Pineda, S.; Schneider, M.; Smagghe, G.; Martinez, A.; Estal, P.D., Uela, E., Valle, J.; Budia, F. 2007. Lethal and sublethal effects of methoxyfenozide and spinosad on *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Journal Economic Entomology*, 100, 3, 773 - 780.

Powning, R.F.; Davidson, W.J. 1973. Studies of insect bacteriolytic enzymes - I. Lysozyme in haemolymph of *Galleria mellonella* and *Bombyx mori*. *Comparative Biochemistry and Physiology*, 45B, 669–686.

Quarles, W.; Winn, P. 1996. Diatomaceous Earth and Stored Product Pests. *IPM Practitioner*, 18, (5-6), 1-10.

Ramos, Y.; Taibo, A.D.; Jiménez, J.A. et al. (2020). Endophytic establishment of *Beauveria bassiana* and *Metarhizium anisopliae* in maize plants and its effect against *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) larvae. *Egyptian Journal of Biological Pest Control*, 30, 20.

Razinger, J.; Lutz, M.; Schroers, H.J.; Urek, G.; Grunder, J. 2014. Evaluation of insect associated and plant growth promoting fungi in the control of cabbage root flies. *Journal of Economic Entomology*, 107, 4, 1348-1354.

Riasat, T.; Wakil, W.; Ashfaq, M.; Sahi, S. 2011. Effect of *Beauveria bassiana*; mixed with diatomaceous earth on mortality, mycosis and sporulation of *Rhyzopertha dominica* on stored wheat. *Phytoparasitica*, 39, 4, 325–331.

Şahin, F.; Yanar, Y. 2021. Pathogenicity of some local entomopathogenic fungus isolates on the cotton leafworm larvae, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control*, 31, 150.

Santi, L.; Beys da Silva, W.O; Berger, M.; Guimarães, J.A.; Schrank, A.; Vainstein, M.H. 2010. Conidial surface proteins of Metarhizium anisopliae: Source of activities related with toxic effects, host penetration and pathogenesis, *Toxicon*, 55, 4, 874-880.

Söderhall, K.; Cerenius, L. 1998. Role of the prophenoloxidase-activating system in invertebrate immunity. *Current Opinion in Immunology*, 10, 23–28.

St. Leger, R.J.; Cooper, R.M.; Charnley, A.K. 1988. The effect of melanization of Manducasexta cuticle on growth and infection by *Metarhizium anisopliae*. *Journal of Invertebrate Pathology*, 52, 459–470.

Subramanyam, B.h.; Roesli, R. 2000. Inert dusts. In: Subramanyam, Bh., Hagstrum, D.W. (Eds), Alternatives to Pesticides in Stored-Product IPM. *Kluwer Academic Publishers*, Dordreecht, The Netherlands, pp. 321-380.

Tanada, Y.; Kaya, H.K. 1993. Insect pathology. *Academic Press*, London, pp 319–385.

Trudeau, D.; Washburn, J.O.; Volkman, L.E. 2001. Central role of hemocytes in *Autographaca lifornica* M *nucleopolyhedrovirus* pathogenesis in *Heliothis virescens* and *Helicover pazea*. *Journal of Virology*, 75, 996-1003.

Wakil, W., Riasat, T., Ashfaq, M. 2012. Residual efficacy of thiamethoxam, *Beauveria bassiana* (Balsamo) Vuillemin, and diatomaceous earth formulation against *Rhyzopertha dominica* F. (Coleoptera: Bostrychidae). *Journal of Pest Science*, 85,

3, 341–350.

Wakil, W.; Riasat, T.; Ghazanfar, M.U.; Kwon, Y.J.; Shaheen, F.A. 2011. Aptness of *Beauveria bassiana* and enhanced diatomaceous earth (DEBBM) for control of *Rhyzopertha dominica* F. *Entomological Research*, 41, 233–241.

ARABIC SUMMARY

فعالية اثنين من الفطريات المسببة للأمراض الحشرية في تركيبة مع التراب الدياتومي ضد دودة ورق القطن *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae)

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يهدف البحث الحالي إلى تقييم الآثار السامة لتركيبة جديدة من الفطريات المسببة للأمراض الحشرية *Metarhizium anisopliae* و *Beauveria bassiana* وغبار الأرض الدياتومي ضد دودة أوراق القطن *Spodoptera littoralis*.

تم إجراء البحث الحالي لدراسة فعالية التراب الدياتومي وحده ودمجه مع اثنين من مسببات الأمراض الفطرية للحشرات: *M. anisopliae* و *B. bassiana* كتركيبة جديدة ضد العمر الثالث ليرقات دوده ورق القطن تحت ظروف المختبر. أجريت دراسات مختبرية لتقييم فعالية التراب الدياتومي المستخدم كمعلق مائي علي حده والمرتبط بالفطريات المسببة للأمراض الحشرية *M. anisopliae* و *B. bassiana* ضد يرقات العمر الثالث ليرقات دوده ورق القطن. عندما تم تطبيق مسحوق التراب الدياتومي والفطريات بمفردهما، كانت الإماتة في اليرقات المعالجة لها زيادة طردية مع التركيز. وكانت قيم التركيز نصف المميت لكل منهما على حده 391,5 و572 و 299,2 جزء في المليون، على التوالي. مزيج من التراب الدياتومي مع *B. bassiana* ومع *M. anisopliae* يعزز فعالية كل منهما. وكانت قيم التركيز نصف المميت للمركبات المدمجة 189 و133.77 جزء في المليون، على التوالي، مما يشير إلى تأثير التقوية السام. أظهرت النتائج أن تطبيق التراب الدياتومي في تركيبة ثنائية مع الفطريات قلل بشكل كبير من نشاط الإنزيم المناعي (phenoloxidase، prophenoloxidase، lysozyme) وتركيز البروتين مقارنة بالعينات المرجعية. تدعم هذه النتائج الفرضية القائلة بأن التراب الدياتومي يعزز من فعالية الفطريات المسببة للأمراض الحشرية ضد الحشرات عن طريق إتلاف الطبقة الخارجية لجسم الحشرات، وبالتالي زيادة تعلق الفطر بالحشرات وجعل العناصر الغذائية متاحة بشكل أكبر للكونيديا لإنباتها. وخلصت الدراسة إلى أن إضافة التراب الدياتومي إلى الفطريات المسببة للأمراض الحشرية يساعد في تحسين جودتها وكفاءتها في الحد من تفشي الحشرات، واقترحت مشاركة هذه المركبات الثنائية في برامج الإدارة المستدامة للآفات.