The Effect of the Iranian Strain of *Metarhizium anisopliae* Applied Alone and in Combination with Diatomaceous Earth and Kaolin against *Plodia interpunctella*

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Abstract

Introduction: The Indian meal moth, *Plodia interpunctella* Hubner, is an important pest in pistachio stores which causes great damage to this product. Considering the importance of human health and protecting the environment, it is important to reduce the use of pesticides in pest control. One of the non-chemical methods to control this pest is the use of biological control agents such as entomopathogenic fungi combined with mineral and natural materials such as diatomaceous earth and kaolin.

Materials and Methods: The current study investigated the lethal effect of *Metarhizium anisopliae* alone and in combination with diatomaceous earth and kaolin at controlled conditions (25±1ºC, RH 85±5%, and a photoperiod of 16: 8 h L: D). The dipping test was conducted on the first instar larvae of *Plodia interpunctella* grown on pistachios of the round variety.

Results: The results showed that the lethal concentration of *M. anisopliae* (0.5×10^6 conidia ml^{-1}) caused 15, 27, 42, 50, and 55% mortality in the pest population after 24, 48, 72, 96, and 120 hours, respectively. Also, 20, 30, 40, 52, and 60% mortality were observed at LC_{50}= 486.5 mg/kg of diatomaceous earth, respectively. Moreover, kaolin powder at LC_{50}= 900 mg/kg caused 22.5, 32.5, 40, 50 and 60% mortality, respectively. However, the mortality in *M. anisopliae* treatment in combination with diatomaceous earth and kaolin powder in lethal concentration indicated the additive effect of *M. anisopliae*.

Conclusion: The results suggested that *M. anisopliae*, diatomaceous earth, and kaolin powder can be used as alternatives to chemical pesticides in the integrated management of this stored pest.

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1. Introduction

Pistachio, known with the scientific name of *Pistacia vera* and from the Anacardiaceae family, is one of the most common dry fruit trees in the world [1]. Pistachio export is of special importance in Iran's non-oil export. With a four-to-five-thousand-year history of growing pistachio trees, Iran was the largest pistachio producing country in the world until the year 2010 [2]. Pest infestation is one of the largest problems in pistachio production, processing, and storage. Due to their high reproduction power, ubiquity, and the wide host range, storage pests are one of the important problems in the storage of agricultural products [3]. Poor storage conditions and lack of product storage infrastructure, cause considerable damage by pests [4]. The Indian meal moth, *Plodia interpunctella* Hubner (Lepidoptera: Pyralidae) is one of the most important pests in pistachio storage. This pest is distributed globally and operates on a wide range of stored products such as cereals, dried fruits, legumes, pistachios, etc. The larvae of this insect spin a silk web inside and on the surface of the food. This network includes larval shell and larval excreta and causes an unpleasant smell in the infected products. Sometimes, the infected product is filled with silk nets. The created pollution can cause direct damage and indirect economic costs such as pest control costs, quality reduction and consumer complaints [5]. Fumigants pesticides are normally used to control this pest, which have irreparable effects on humans and the environment [6]. Considering that the management of storage ecosystems is simpler compared to the farm ecosystem, the use of insect pathogens or pathogen-based products can be a suitable solution for pest control in stored products such as pistachios [7]. Various species of fungi have been identified in relation to insects, which cause physiological disorders in insects and cause their destruction as the disease progresses [8]. Among the fungi that infect insects, the genus *Metarhizium* has attracted a lot of attention due to its ability to affect a wide range of pests and its ability in their biological control. This genus easily attacks a large number of insects and is also able to produce a special toxin on the surface and even the strain of the species, which plays an important role in its insecticidal efficiency. The strains of this fungus have been known as fungi that cause gradual death in low spore concentrations and have a high lethal effect [9]. The pathogenic fungus *Metarhizium anisopliae* belongs to the branch of Ascomycota, order of Hypocreales and family of Clavicipitaceae, which has a global distribution and has been isolated from a large number of insect species and is known as a biological control agent for a wide range of different orders of insects. The *Metarhizium* fungus is mostly found in the soil and as an endophyte in the rhizosphere of plants. This fungus has attracted a lot of attention in the agricultural sector for pest control, and phylogenetic studies, distribution, and ecology of the species of this genus have increased in recent years [10]. Diatomaceous earth is a combination of fossilized diatoms in the form of silica (Sio2+nH2O) along with some mineral elements. Diatomaceous earth has been successfully documented in the management of storage pests, because these natural insecticides have low toxicity to mammals and high insecticidal effects for a wide range of pests of stored products. The use of diatomaceous earth is cost-effective compared to other protection methods, so the use of diatomaceous earth has increased in the last decade [11]. By sticking to the insect cuticle and absorbing epicuticle lipids, diatomaceous earth causes the insect to lose water and eventually die [12]. Another natural factor in pest control is kaolin, a white, non-porous material with small grains which mineralogically belongs to the group of aluminum silicate minerals and is easily dispersed in water. Kaolin is a harmless and environmentally friendly material which is safe for mammals. It is a suitable material for protecting plants against pests and plant pathogens. It also protects plants from environmental stresses such as the sun's ultraviolet rays. Another property of kaolin is its repellency, which acts like a physical barrier and prevents the insect from oviposition and feeding in the product and ultimately causes the death of the insect. It is therefore considered a suitable and safe material in integrated pest control programs [13].

Minerals such as diatomaceous earth and kaolin have been studied by many researchers in pest control in different groups, individually and in combination with other pest control agents such as insect pathogenic fungi and have proved effective in pest control [14,15].

The present study investigated the effects of the native strain of *Metarhizium anisopliae*, diatomaceous earth, and kaolin powder individually and in combination on the mortality of the Indian moth larvae. The results of this study can improve the control of the Indian moth pest in storage sites.

2. Materials and methods

In this research, Mazandaran strain of the *Metarhizium anisopliae* obtained from the biological control laboratory of the University of Tehran, the Hudson brand of diatomaceous earth, and the Sepidan brand of kaolin powder were used.

Cultivation of the Indian moth

The primary colony of the Indian moth *P. interpunctella* was obtained from the Pistachio Research Institute. Last instar larvae of the Indian moth were cultivated in containers measuring 15×25 cm and a height of 20 cm with a door covered with net with top mesh on pistachios of the round variety in the growth chamber located in the entomology laboratory of Vali-e-Asr University of Rafsanjan (Iran) with temperature conditions of 27±1 ºC, relative humidity of 65 ± 5% and a photoperiod of 16:8 hours (light: dark) and used for the experiments in the study. To make a cohort colony, adult insects were separated from the rearing colonies using an aspirator device, and the insect was released from the small opening of the funnel whose large opening was covered with a cloth net with a medium mesh. Then, the small opening was completely blocked with a cloth to prevent insects from escaping, and the funnel was placed on a dark colored paper in a tray [16]. After 24 and 48 hours, the laid eggs were collected and stored in breeding containers. After 3 to 4 days, the eggs were hatched and first instar larvae younger than 12 hours old from the time of emergence were used in the experiments.

Cultivation of pathogenic fungus *M. anisopliae*

Mazandaran strain of *M. anisopliae* was obtained from the herbal medicine department of the University of Tehran. Sabouraud's Dextrose Agar culture medium with 1% yeast extract (SDAY) was used for propagation and kept in an incubator at a temperature of 26±1 ºC and a relative humidity of 75±5%. After the sporulation of fungi in culture medium (14 days after culture), the inoculation liquid and the uniform suspension containing Tween 80 (0.02%) and sterile distilled water were prepared in sterile conditions under hood [17]. Then the number of spores was counted with a cell count slide and the desired concentration was calculated. After the conidia viability test, the required concentrations were prepared from the original suspension [14].

Spore viability test

In order to measure the spore viability of the desired fungi, a dilute suspension (105 conidia/ml) was prepared from the fungus and poured onto the culture medium in a Petri dish and kept at a temperature of 25 ºC. After 24 hours, 100 spores were randomly observed from each part using a light microscope with a 40x magnification [18]. Spores whose tube length was larger than half of the spore diameter were considered as germinated spores. In order to perform biometric tests, the germination percentage needs to be more than 85%. The percentage of spore germination was calculated through the following formula [19].

Percentage of spore germination = (total number of spores / the number of germinated spores) × 100

Bioassay

In order to obtain different lethal concentrations, first instar larvae of the Indian moth with a life span of less than 12 hours from the time of hatching (n = 40) were treated by being immersed in different concentrations of *M. anisopliae* (1.8×102, 1.8×104, 1.8×105, 1.8×106, and 1.8×107 conidia/ml). To treat the larvae, five milliliters of each concentrate was poured into separate Petri dishes measuring 9 cm in diameter, and 40 larvae of the first instar were exposed in them for five seconds. Distilled water with Tween 80 (0.02%) was used to treat first-instar larvae (n = 40) as the control. After being treated with certain concentrations, the tested larvae were first dried on a filter paper at room temperature and then placed inside containers with a diameter of 9 and a height of 4 cm containing pistachios. The containers were placed inside the growth chamber with temperature conditions of 27±1 ºC, a relative humidity of 65%±5%, and a photoperiod of 8:16 hours (light: dark). The mortality of the larvae was counted and recorded 24, 48, 72, 96, 120, and 144 hours after the treatment of the fungus strain. Each concentration consisted of four replicates with ten larvae. To ensure the losses caused by fungal infection, the dead larvae washed by sterile distilled water were placed in Petri dishes containing sterile agar culture medium, whose lids were closed with parafilm in the dark and at a temperature of 26 ± 1 for two weeks to prove pathogenicity [20].

Determining the lethal concentration of kaolin powder on the Indian moth

Sepidan kaolin powder WP 95% was used in this experiment. Concentrations of 200, 350, 550, 750, and 1000 mg/kg were used to determine the lethality percentage of the kaolin powder. The desired concentrations were added in containers containing 50 grams of pistachios. The containers were shaken well so kaolin is evenly mixed with pistachio. These mixtures were then divided into 4 containers (as replicates). For each replicate, a container containing pistachios without kaolin was considered as the control. Finally, 10 first instar larvae of the Indian moth with an age of less than 12 hours were released inside each container and mortality was counted and recorded after 24, 48, 72, 96, 120, and 144 hours.

Determining the lethal concentration of diatomaceous earth on the Indian moth

In this experiment, Hudson Diatomaceous Earth was used, which was obtained from Radin Chemical Company. Concentrations of 100, 200, 400, 600, and 800 mg/kg were used to determine the lethal concentration of diatomaceous earth. The steps of the experiment were the same as those followed in determining the lethal concentration of kaolin powder.

The effect of combining *M. Anisopliae* fungus with diatomaceous earth on the mortality of the Indian moth

In this experiment, the median lethal concentration (LC50) of the *M. anisopliae* fungus with 0.5×106 conidia per milliliter and diatomaceous earth with 486.5 mg/kg obtained in the first experiment was used after 24 hours [21]. To carry out the experiment, 486.5 mg/kg of diatomaceous earth was added to a container containing 50 grams of pistachio, and the container was shaken for 5 minutes so that the diatomaceous earth was evenly dispersed among the pistachio particles. This mixture was then divided into 4 containers (as replicates). Ten Indian moth larvae with an age of less than 12 hours were immersed in the fungus suspension for 2 seconds and released in containers containing pistachios and diatomaceous earth for each replicate. The four conditions of pistachio containers without *Metarhizium* fungus and diatomaceous earth, pistachio containers with diatomaceous earth without *Metarhizium* fungus, pistachio containers containing *Metarhizium* fungus without diatomaceous earth, and pistachio containers without diatomaceous earth and *Metarhizium* fungus whose insects had been killed using distilled water along with one ml of Tween 80 (0.02%) were used as the control conditions. Finally, mortalities were counted and recorded after 24, 48, 72, 96, and 120 hours.

The effect of combining *M. anisopliae* fungus with kaolin powder on the Indian moth

In this experiment, the median lethal concentration (LC50) of the *M. anisopliae* fungus with 0.5×106 conidia per milliliter and kaolin powder with the median lethal concentration of 900 mg/kg obtained in the first experiment was used after 24 hours. The steps of conducting the experiment were the same as those described in testing the combination of *M. anisopliae* fungus with diatomaceous earth.

Data analysis

The experiments were conducted in a completely randomized design, and because mortality was observed in the control experiments, the treatment and control mortalities were corrected based on Abbot’s formula. Probit analysis in SAS software was used to determine LC_{30}, LC_{50}, and LC_{90} values [22]. The results of the effects of diatom and kaolin mineral compounds as well as the *M. anisopliae* fungus on the mortality of first instar Indian moth larvae applied individually and in combination and at different times were statistically analyzed using one-way analysis of variance and SAS 9.2 statistical software. The statistical difference of means was analyzed using Tukey's test at the five percent level. The normality of the data was checked using version 16 of the SPSS software. Since the data were normally distributed, no normalization operation was performed on the data. SigmaPlot software was used to draw the diagrams.

3. Results

The germination percentage of the *M. anisopliae* fungus conidia was calculated to be 87.5%. Based on the preliminary experiments, the mortality percentage caused by different concentrations of *M. anisopliae* (1.8×102, 1.8×104, 1.8×105, 1.8×106 and 1.8×107 conidia per ml) after 24, 48, 72, 96, 120, and 144 hours from the start of the treatment, the percentage of mortality of the first instar larvae of the Indian moth also increased with the increase in concentration and the passage of time (Table 1).

Table 1. Statistical analysis of first instar of the Indian meal moth after exposure to *M. anisopliae* fungus

Metarhizium anisopliae	**control**	**Concentration $1/8\times10^{2}$conidia/ml**	**Concentration $1/8\times10^{4}$conidia/ml**	**Concentration $1/8\times10^{5}$conidia/ml**	**Concentration $1/8\times10^{6}$conidia/ml**	**Concentration $1/8\times10^{7}$conidia/ml**
P	0.009	<0.001	<0.001	<0.001	<0.001	<0.002
df	5, 18	5, 18	5, 18	5, 18	5, 18	5, 18
F	4.33	31.43	40.99	22.07	26.14.	21.47

Also, it was shown that the percentage of mortality caused by different concentrations (200, 350, 550, 750, and 1000 mg/kg) of kaolin insecticide on the first instar larvae of the Indian moth 24, 48, 72, 96, 120, and 144 hours after the start of the treatment increased significantly with the increase in kaolin concentration and also with the passage of time (Table 2).

Table 2. Statistical analysis of first instar of the Indian meal moth after exposure to kaolin

kaolin powder	**control**	**Concentration 200 mg/kg**	**Concentration 350 mg/kg**	**Concentration 550 mg/kg**	**Concentration 750 mg/kg**	**Concentration 1000 mg/kg**
P	0.210	<0.001	<0.001	<0.001	<0.001	<0.002
df	5, 18	5, 18	5, 18	5, 18	5, 18	5, 18
F	1.60	46.49	104.66	44.17	33.60	74.40

Different concentrations of diatomaceous earth insecticide on the first instar larvae of the Indian moth at different times (24, 48, 72, 96, 120, and 144 hours after the treatment) showed that the death rate of the larvae increased with the increase in the concentration (100, 200, 400, 600, and 800 mg/kg) of diatom. Also, the exposure time showed a significant effect on the mortality of larvae in the control and different concentrations (Table 3).

Table 3. Statistical analysis of first instar of the Indian meal moth after exposure to diatomaceous earth

diatomaceous	control	Concentration 100 mg/kg	Concentration 200 mg/kg	Concentration 400 mg/kg	Concentration 600 mg/kg	Concentration 800 mg/kg
P	0.004	<0.001	<0.001	<0.001	<0.001	<0.002
df	5, 18	5, 18	5, 18	5, 18	5, 18	5, 18
F	5.20	30.80	45.18	58.40	88.20	92.6

Moreover, the sub-lethal concentration (LC30) values of diatom, kaolin, and insecticidal fungus in the first 24 hours of contamination were 223.0 mg/kg, 470.2 mg/kg, and 1.8×104 conidia/ml, respectively (Table 4). According to the median lethal concentration values, diatom and kaolin had similar toxicity values.

Table 4. Susceptibility of first instar Indian meal moth larvae to diatomaceous earth, kaolin, and *M. anisopliae* insecticide compounds after 24 hours of exposure via contact toxicity

Insecticides	Replicate	χ^2	Slope ±SE	Lethal concentrations		
				LC_{30} (95% CL)	LC_{50} (95%CL)	LC_{90} (95%CL)
diatomaceous earth	240	18.6	2.25±0.35	223.0 (120.8-305.4)	486.5 (362.5-727.0)	3272.0 (1619.0-19861.0)
kaolin	240	15.1	2.7±0.4	470.2 (319.0-606.0) $1.8*10^4$	900.0 (688.0-1600.0)	4399.0 (2156.0-35838.0)
***M. anisopliae* fungus**	240	27.5	0.50±0.06	$7.1*10^4$-$1.9*10^3$	$0.5*10^6$ (1.3*2-10^5.2*10^6)	$1.6*10^9$ (1.2*3-10^8.8*10^{10})

The results of the average percentage of cumulative mortality of first instar Indian moth larvae after exposure to LC_{50} concentrations of diatom and kaolin insecticide compounds as well as *M. anisopliae* fungus in the two modes of individual and combined application are shown in Figure 1 (A-E). According to the obtained results, the mortality of larvae treated with different compounds was significantly different at the investigated times (Table 5).

Table 5. Statistical analysis of first instar Indian meal moth larvae after exposure to LC_{50} concentrations of *M. anisopliae*, kaolin, and diatomaceous earth insecticidal compounds in both individual and combined conditions

Treatment	24	48	72	96	120
P	<0.001	<0.001	<0.001	<0.001	<0.002
df	5, 18	5, 18	5, 18	5, 18	5, 18
F	23.36	42.86	55.36	78.34	108.08

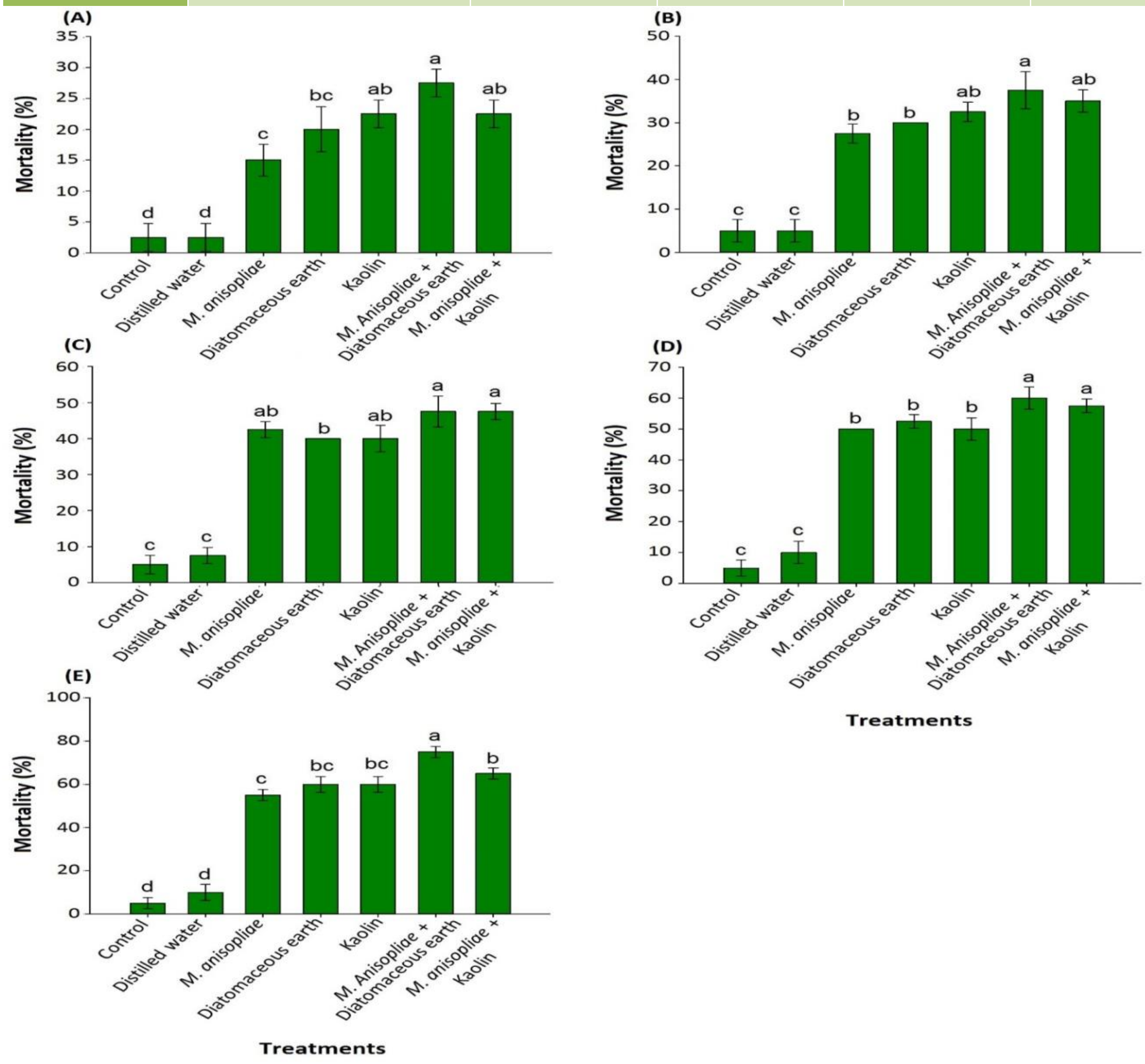

Fig1. Mean percent (±standard deviation) mortalities of first instar larvae of *Plodia interpunctella* treated with LC_{50} concentrations of diatomaceous earth, kaolin, and *Metarhizium anisopliae* alone and in combination conditions at different times 24 (a), 48 (b), 72 (c), 96 (d) and 120 (e) hours after exposure.

Means (±SE) with the same letter are not significantly different according to Tukey's test (P>0.05).

Twenty-four hours after exposure, the lowest percentage of mortality was related to control treatments (2.2 ± 2.2%) and distilled water (2.2 ± 2.2%). On the other hand, the highest percentage of mortality (27.2 ± 5.2 percent) was observed in the treatment of diatom + *M. anisopliae* fungus (Figure 1 A). Forty-eight hours after exposure, the mortality of control larvae and larvae treated with distilled water was 5%. The highest percentage of mortality was related to larvae treated with diatom + *M. anisopliae* fungus (37.4 ± 5.3 percent), kaolin + *M. anisopliae* fungus (35.2 ± 0.6 percent), and kaolin (32.2 ± 5.2 percent) (Figure 1 B). Also, 72 and 96 hours after exposure, the mortality of larvae treated with diatom + *M. anisopliae* fungus (47.5 ± 2.3 and 60.0 ± 3.6%, respectively) and kaolin + *M. anisopliae* fungus (47.5 ± 2.2 and 57.5 ± 2.2 percent, respectively) were significantly higher than individual treatments (Figure 1 C, D). 120 hours after exposure, the mortality of control larvae and larvae treated with distilled water was 5% and 10%, respectively. The highest mortality (75% ± 2.6%) was related to diatom + *M. anisopliae* fungus (Figure 1 E). The results of the experiments showed that in most cases, in treatments where *M. anisopliae*, diatomaceous earth, and kaolin were combined, the mortality of larvae was significantly higher than individual and control treatments.

4. Discussion

In this research, the lethal effect of two mineral compounds including diatomaceous earth and kaolin powder and insect pathogenic *M. anisopliae* fungi was investigated alone and in combination on first instar larvae of the Indian moth. The results of the experiments show that all the investigated compounds, both individually and in combination, caused high mortality on the larvae. Based on the obtained LC_{50} values, the combination of diatom with *M. anisopliae* fungus had the highest toxicity on the larvae of Indian moth, followed by the combination of kaolin with *M. anisopliae* fungus, compared to the other investigated compounds. Diatom and kaolin had high and relatively similar toxicity in individual application. In contrast, the lowest toxicity was related to *M. anisopliae* fungus. The higher toxicity of these compounds may be related to the formulation or particle size (diatom 37 microns, kaolin 45 microns, and *Metarhizium* fungus 5 to 10 microns) of these compounds. Yang et al. [23] reported that the smaller the particle size of powder-formulation insecticides, the higher the number of pores and the ratio of the surface to the volume of the particles, and as a result, the higher the efficiency of the mineral compound. Based on the obtained LC_{50} values, there was no significant difference between the lethal toxicity of diatom and kaolin. The findings of this study showed that the sensitive population of larvae die at a low concentration of diatom, but high concentrations of the poison are required for a 50% mortality of the population. The difference between these concentrations of diatom and the concentration of kaolin required for a 50% mortality of the population was not significant.

In the present study, the reported LC_{50} values for diatom, kaolin, and *M. anisopliae* insecticides on first-instar larvae were lower than those reported by other researchers for other insects. Latifian and Rad [24] LC50 reported 759.8 ppm and 5.69×108 conidia/ml values of diatom and *M. anisopliae* fungus, respectively, for the larvae of the palm horned beetle *Oryctes elegans* Prell. (Coleoptera: Scarabaeidae). The difference in the values reported in the present study and the studies conducted by other researchers may be related to the test conditions and methods or the investigated insects. To some extent, these differences were expected, because it seems that the larvae of the Indian moth have a higher sensitivity to these insects due to their body morphology and thinner skin compared to the larvae of the palm horned beetle or the complete insects of the tobacco beetle.

The results of the present study showed that with the increase in the exposure time of first-instar Indian moth larvae, the mortality rate increased in the investigated mineral compounds, which indicates the positive effect of the exposure time on the increase in the toxicity of these compounds. In similar results, Ziaee et al. [25] reported that the increase in the activity of insects caused them to come in contact with the mineral compound, which leads to higher efficiency of the mineral compound and its greater toxicity.

Similar results were observed in the case of the insecticidal *M. anisopliae* fungus, where the mortality rate of first-instar Indian moth larvae increased as exposure time increased. The results of Mantzoukas et al. [26] who reported the mortality rate of *Trogoderma granarium* Everts (Coleoptera: Dermestidae), *Ephestia kuehniella* Zell. (Lepidoptera: Phycitidae) and *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) treated with *M. anisopliae* fungus increases with time and that after 10 days the death rate of these pests reaches 60-90% confirm the results of the present study. The present study showed that in the investigated insecticide compounds, increasing the concentration caused an increase in the mortality of first-instar Indian moth larvae. An increase in mortality caused by the concentration of the insecticide compound was observed at all times after the treatment of the larvae. It is obvious that increasing the concentration of diatom and kaolin per unit surface has an effect on the amount of contact between the insect and the particles, and more scratches are created in the body of the larvae in a shorter period of time, which in turn shortens the time required to increase the percentage of mortality in the Indian moth population.

Also, the increase in the number of conidia per unit area causes a larger number of them to sit on the larva's body, germinate, and colonize the larvae's body in a shorter time and cause their death. These findings are similar to the results reported by other researchers about the concentration-dependent effects of inorganic insecticides and plant pathogens on different pests. Farazmand et al. [27] reported that the treatment of adult pistachio psyllids *Agonoscena pistaciae* (Hem.: Psyllidae) with kaolin concentrations of 3 and 5% resulted in 40.34 and 65.64% population mortality after 7 days, respectively. In a study conducted by Ziaee [28] on the effect of different diatom concentrations on the reduction of *T. Confusum* on wheat, it was shown that at concentrations of 1000 to 2000 mg/kg, the mortality of insects fed on diatom-treated seeds increased from 15% to 81%. Also, Mohammadipour [29] investigated the effectiveness of kaolin powder in reducing the population of citrus psyllid and reported that increasing the concentration of the compound caused a significant increase in the mortality of the exposed insects, in such a way that the concentrations of 3, 5, and 7% of the compound had 33, 51, and 61% lethality, respectively. Cavallieratos et al. [30] investigated the lethal effects of *M. anisopliae* and diatoms on *Rhyzopertha dominica* (Coleoptera: Bostrichidae), *Tribolium confusum* (Coleoptera: Tenebrionidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae). Their results showed that the mortality of the developed insects of these pests increases with the increase in the concentration of *M. anisopliae* and diatom. Also, the effectiveness of both insecticide compounds increased with the passage of time.

In the concentrations investigated in the present study, the insecticidal effect of diatom and kaolin was slightly higher than that of *M. anisopliae* fungus, in such a way that the mortality of first instar larvae of the Indian moth treated with diatom (486.5 mg/kg) and kaolin (0.900 mg/kg)) reached 60% after 120 hours, and the mortality of the larvae treated with *M. anisopliae* fungus (0.5 x 106 conidia/ml) reached 56% after the same period of time.

In the present study, it was observed that combined treatments caused higher mortality of larvae at different times, which is in accordance with the findings of other researchers [31]. In another study, Riasat et al. [32] investigated the lethal effects of diatom and *Beauveria bassiana* fungus applied alone and in combination on mature *Rhyzopertha dominica*. Their results showed that the mortality of mature insects after exposure to the mixture of the two insecticide compounds was significantly higher than their individual application. In the present study, the high mortality of the first instar Indian moth larvae in a few days after the start of the treatment indicates the delayed effects of the applied compounds. On the other hand, combining each mineral insecticide with *M. anisopliae* increased their lethal effects, which is line with the results reported by Shafighi et al. [33] and Saeed [34] who investigated the effect of diatomaceous earth alone and in combination with *M. anisopliae* fungus on several storage pests and found that the effect of combining diatomaceous earth with *M. anisopliae* fungus was greater than the effect of each of these substances alone.

5. Conclusion

The general results of this experiment show that each of the mineral compounds used in this study or the *M. anisopliae* fungus applied alone or in combination can be used as a suitable alternative to chemical pesticides to reduce the damage caused the Indian moth larvae and the side effects of using pesticides in stored products. Also, the fact that the fungal strain used in this experiment is native not only reduces the cost of pesticides but can also be more efficient due to better adaptation to environmental conditions.

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Conflicts of interest

We confirm that there are no conflicts ofinterest related to this study, and there has been no substantial financial support for this work that could have influenced its outcome

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