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Research Article
Diatomaceous Earth and Kaolin as Promising Alternatives to the Detrimental Chemicals in the Management of *Spodoptera exigua*

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Abstract
Background and Objective: Insecticidal properties of diatomaceous earth and kaolin as eco-friendly inert powders have been studied on some of the most important agricultural pests in recent years. This study investigated the toxicity of kaolin and diatomaceous earth (Sayan®) against Spodoptera exigua. **Materials and Methods:** In the present study, contact toxicity of kaolin and diatomaceous earth (Sayan®) as environmentally compatible materials was determined against the larvae of beet armyworm, *Spodoptera exigua*, after 24, 48 and 72 h exposure times. **Results:** Higher mortality was observed with diatomaceous earth compared to kaolin on the *S. exigua* larvae. The highest mortality rate (59.25%) was observed with a concentration of 20% from diatomaceous earth after 72 h and the lowest mortality rate (18.12%) was detected with a concentration of 5% kaolin after 24 h exposure times. In fact, a positive correlation was observed between increasing the concentration of both compounds and the exposure time with pest mortality. **Conclusion:** According to the results of this study, kaolin and diatomaceous earth can be considered as suitable alternatives for chemical pesticides in the management of *S. exigua*.

Key words: Bioassay, diatomaceous earth, kaolin, *Spodoptera exigua*, ecofriendly materials, pest mortality, agriculture pest, army worm

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Beet armyworm, *Spodoptera exigua* (Hübner), is a polyphagous insect pest which, by feeding on host leaves, can reduce the product or in some cases cause complete degradation. *Spodoptera exigua* larvae feed on different crops such as sugar beet, alfalfa, rice, onions, cotton, corn and many other plants but they prefer sugar beet, alfalfa and maize[1,2]. At present, the most important strategy for management of such pests is the chemical control. *Spodoptera exigua* has been resistant to many insecticides due to overusing and the lack of proper management of insecticides application[3,4]. Further, the demands for residue-free crops have led to researches to find eco-friendly alternative to the chemical pesticides.

Diatomaceous earth and its related materials are considered as conventional natural agents to alternate chemical protectants especially residual pesticides[5-7]. Diatomaceous earth is a non-toxic material with a unique mode of action against insect pests: desiccation[8]. The insecticidal effects of diatomaceous earth from different commercial formulations have been investigated against several insect pests. For example, the promising toxicity of diatomaceous earth was approved against *Tribolium castaneum* Herbst[9], *Tribolium confusum* Jacquelin du Val[10], *Rhyzopertha dominica* (F.)[11], *Sitophiluszeamais* Motschulsky[12], *Sitophilusoryzae* (L.)[13], *Callosobruchus maculatus* (F.) and *Sitophilus granaries* (L.)[14]. Kaolin as a well-known mineral composed from aluminum silicate, with mechanisms of repellency, disturbance of ovipositional and feeding activity and negative effects on longevity and survivorship has been exhibited promising pesticidal effects against different damaging pests such as aphids, beetles, psyllids and mites[15-19]. Along with great potential in the pests' management, both diatomaceous earth and kaolin are non-toxic to humans and don't have detrimental residues on the environment[8,20,21]. To the best of authors' knowledge, no study has been made on the toxicity of diatomaceous earth (Sayan®) and kaolin against *S. exigua*. Therefore, considering the criteria such as environmental compatibility, economic justification and safety which are the main objectives of integrated pest management, in the present study, the controlling effects of diatomaceous earth (Sayan®) and kaolin were evaluated against *S. exigua* to find the appropriate alternatives to the chemical pesticides.

MATERIALS AND METHODS

Insecticides: In this research, diatomaceous earth (Sayan®: Kimia Sabzavar Company, Iran) in powder form with an average size of 50 μm and kaolin (kaolin®: Konakori Company, Guinea) in powder form with an average size of 53 μm was used for bioassays.

Insect rearing: The host plant used in this study was Sugar beet (2285ZB cultivar), which was planted in a research farm and also in the pots in the greenhouse of Department of Entomology, College of Abouraihan, University of Tehran, Iran from May-July, 2018. Pest larvae were collected from sugar beet fields (Bojnord, Iran) and after identification of the species, they were located on the host plant. In mid-stage breeding, a number of larvae were collected from the other fields (Mashhad, Iran) in order to increase the genetic diversity of the insect population and entered in the rearing cycle.

Rearing was carried out on an artificial diet consisting of soaked beans, yeast, agar, ascorbic acid, formaldehyde and distilled water under 16: 8 h of light: Darkness, 26±2°C and 65±5% relative humidity. In order to increase fertility and prevent the effects of artificial food on the physiology of the pest, third and fourth instar larvae were fed with sugar beet leaves previously planted in the field. A cylinder container (40 cm in length and 15 cm in diameter), with its inner surface fully covered by filter paper, considered as a rearing chamber. For feeding the moths, 25 mL of honey 20% and a cotton wool was also placed. Finally, the 3 rd instar larvae were selected for bioassays.

Contact toxicity of diatomaceous earth and kaolin: The experimental units were sugar beet pots that sprayed with 5, 10, 15 and 20% of both insecticides. Then, 20 larvae of sugar beet were transferred to the pots and the mortality rate was counted after 24, 48 and 72 h[5]. The control treatment was a sugar beet plant in a pot that was sprayed with distilled water. Experiments were performed separately for diatomaceous earth and kaolin with 4 replications. All experiments were performed in a greenhouse under 16: 8 h of light: darkness, 26±2°C and 5±65% relative humidity.

Statistical analysis: Analysis of variance (one-way ANOVA) was done using SPSS software version 24. If necessary, the data were normalized with the formula. To compare the effects of independent factors (concentration and exposure time) on the insect pest mortality, the ω^2 comparison was used. Mortality means were compared using Tukey's test at $\alpha = 5\%$.

RESULTS

In treated leaves with different concentrations of diatomaceous earth and kaolin, significant mortality was observed in *S. exigua* larvae. According to Table 1, the different concentrations of both insecticides, exposure times of the pest and interactions of the concentration-time had statistical significant effects in observed mortality. Also, according to the ω^2 values in Table 1, among the mentioned factors, the effect of the concentration of both insecticides on larval mortality was more important.

Results of the compare means of *S. exigua* larvae mortality alongside different concentrations of diatomaceous earth and kaolin are shown in Table 2. Mortality in all concentrations had a significant difference with control groups. Further, increasing the time and concentrations of both insecticides cause a significant increase in the larvae mortality. The highest mortality (59.25%) was related to the concentration 20% of the diatomaceous earth after 72 h and the lowest mortality (18.12%) was observed at a concentration of 5% of kaolin after 24 h.

Regression equations and dose-response lines of diatomaceous earth and kaolin with mortality of *S. exigua* larvae are given in Fig. 1. Considering the values of R^2 for both compounds at different times, a positive correlation was recorded between the increases in the concentration and the mortality (Fig. 1).

DISCUSSION

The findings of present study displayed that *S. exigua* larvae were susceptible to the diatomaceous earth (Sayan®) and kaolin (kaolin®) and the mean mortality, in general, was increased with increases of exposure time. Promising toxic effects of the SilicoSec® formulation of diatomaceous earth were approved against *T. castaneum*, *T. confosum*[5] and *R. dominica*[22]. In the other work, the toxicity of Dryacide UF® formulation of diatomaceous earth was determined on the adults of *Sitophilus granaries* L. and *S. oryzae*[23]. In all mentioned studies the susceptibility of tested insects was increased with exposure time increases. Further, the insecticidal effects of kaolin against boll weevil *Anthonomus grandis* Bohe man were assessed by Silva and Ramalho[19] and it was found that these effects were augmented by increases in exposure time and applied concentrations. Finding of these researches, regard to toxic effects of diatomaceous earth and

Table 1: Variance analysis of the mortality data of *Spodoptera exigua* larvae treated by different concentrations of diatomaceous earth and kaolin at 24-72 exposure times

Insecticides	Source	df	Sum of square	Mean square	F	p-value	ω^2
Diatomaceous earth	Concentration	4	7167.345	1791.836	95.214	<0.0001*	6.631
	Time	2	1215.686	607.843	32.299	<0.0001*	1.101
	Concentration× Time	8	569.218	71.152	3.781	0.0018*	0.391
	Error	30	846.855	18.819			
	Total	44	9799.104				
Kaolin	Concentration	4	10988.727	2747.182	621.075	<0.0001*	21.553
	Time	2	1698.961	849.481	192.048	<0.0001*	3.320
	Concentration*×Time	8	752.351	94.044	21.261	<0.0001*	1.409
	Error	30	199.047	4.423			
	Total	44	13639.086				

Significant at $\alpha = 1\%$ according to Tukey's test

Table 2: Mortality (±SE) of *Spodoptera exigua* larvae affected by different concentrations of diatomaceous earth and kaolin at 24-72 exposure times

Concentrations (%)	Diatomaceous earth 24 h	Diatomaceous earth 48 h	Diatomaceous earth 72 h	Kaolin 24 h	Kaolin 48 h	Kaolin 72 h
0 = control	6.419 ± 0.000^{H}	6.419 ± 0.000^{H}	6.419 ± 00.000^{H}	6.419 ± 0.000^{G}	6.419 ± 0.000^{G}	6.419 ± 0.000^{G}
5	18.144 ± 2.020^{G}	25.620 ± 0.944^{F}	29.943 ± 1.354^{E}	18.124 ± 2.027^{F}	22.786 ± 0.00^{E}	27.423 ± 0.885^{D}
10	25.620 ± 0.945^{F}	30.802 ± 0.802^{E}	37.751 ± 0.854^{D}	22.319 ± 7.671^{EF}	28.282 ± 0.991^{D}	32.408 ± 0.802^{C}
15	30.000 ± 0.000^{EF}	37.011 ± 0.740^{D}	44.282 ± 1.373^{C}	23.731 ± 0.942^{E}	31.604 ± 0.924^{CD}	40.684 ± 0.832^{B}
20	32.404 ± 0.802^{E}	48.602 ± 1.382^{B}	59.253 ± 1.582^{A}	29.142 ± 0.858^{D}	40.685 ± 0.832^{B}	47.877 ± 1.171^{A}

Means in with the same letters are not significantly different at the 5% level according to the Tukey's test

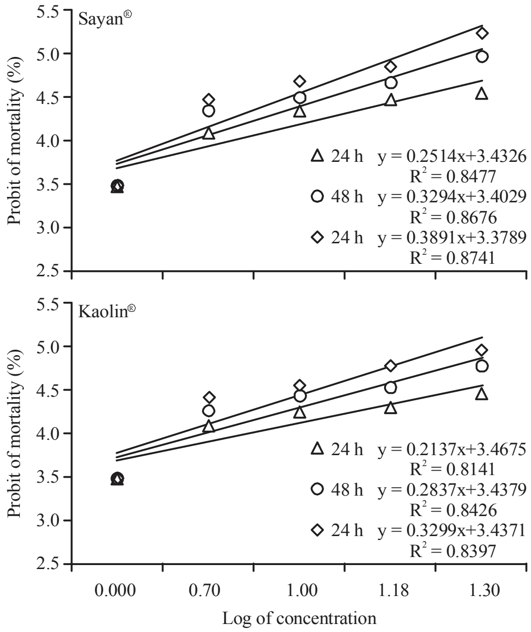

Fig. 1: Dose-response lines of Spodoptera exigua larvae affected by different concentrations of diatomaceous earth (Sayan®) and Kaolin at 24, 48 and 72 exposure times

its direct dependence with exposure time increase are consistent with the results of this research. However, the insecticidal of Sayan® formulation of diatomaceous earth and kaolin against *S. exigua* larvae assessed for the first time in the present study.

Recent studies have also been shown that it is also possible to combine these materials with other pest control agents. For example, Kavallieratos *et al.*[10] indicated that susceptibility of *R. dominica*, *S. oryzae* and *T. confusum* to the diatomaceous earth was increased by the utilization of an entomopathogenic fungi *Metarhizium anisopliae* (Metschinkoff). In the other study, the efficacy of diatomaceous earth formulations was intensified with natural pyrethrum and spinosad against *T. confusum*[24,25]. Keita *et al.*[26] showed the insecticidal effects of kaolin were improved with the application of some plant extracts against *C. maculatus*. Further, these materials are considered due to the very low cost and high availability. Hence, diatomaceous earth and kaolin have great potential in the integrated pest management strategies.

CONCLUSION

According to the results of present study, Sayan® as a diatomaceous earth produced in Iran has caused significant mortality on *S. exigua* larvae and it has the ability to compete with the well-known formulation of diatomaceous earth SilicoSec®. Further, kaolin has also a promising potential in the management of *S. exigua*.

SIGNIFICANCE STATEMENT

Application of chemical insecticides caused different negative side-effects such as residues on the environment, the threat for humans and insect pest resistance. So, the utilization of naturally safe and sound agents may be considered. Based on the results of previous studies for high potential of diatomaceous earth and kaolin in the management of insect pests, present laboratory study assessed the effectiveness of diatomaceous earth and kaolin against the larvae of beet armyworm *S. exigua*. It was found that Sayan® formulation of diatomaceous earth and Kaolin had significant toxicity on the *S. exigua* larvae and can be considered in the management of this key pest.

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