J. Crop Prot. 2022, 11 (2): 211-227

Research Article

Effects of processed kaolin on *Aphis fabae* and *Hippodamia variegata* on broad bean: A lab and field case study

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Abstract: The black bean aphid, *Aphis fabae* Scopoli, is a critical pest feeding on various host plants. This study was conducted to investigate the effect of kaolin on *A. fabae* and one of its natural enemies, *Hippodamia variegata* (Goeze). The investigation was carried out in Barkat broad bean cultivar during 2017-2019 in field and greenhouse conditions in Karaj, Iran. The experiment was conducted as a factorial randomized complete block design with four replications. Kaolin (Sepidan® WP %95) was tested at 3, 6, and 9%, and the sampling was carried out one day before application and three, six, nine, twelve, and fifteen days after application. As soon as the insects settled, kaolin foliar application began. The laboratory tests on black bean aphid showed the highest efficiency of kaolin 9% at 12 days after application, while the least was three days after application with kaolin 3%. Field trials indicated the highest efficiency of kaolin 9%, 9 days after application. Kaolin 9% caused the highest detrimental impact on *H. variegata* 15 days after foliar application.

Keywords: *Aphis fabae*, broad bean, *Hippodamia variegata*, kaolin, pest management

Introduction

The black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae), is one of the 14 aphid species of several cultivated crops worldwide (Völkl and Stechmann, 1998; Blackman and Eastop, 2007). It occurs in Europe, Western Asia, and Arab countries, particularly Jordan (Mustafa and Qasem, 1984), Africa, and South America. *A. fabae* also has a wide variety of hosts (Béji *et al*., 2015). More than 200 host plant species have been reported worldwide, and around 50 plant species are susceptible to attack by this aphid in Iran (Blackman and Eastop, 2007; Azami-Sardooei *et al*., 2018). Aphids cause direct damage to the host plant by extracting plant sap, which provides essential food materials that promote aphids and plant growth. Since phloem sap is richer in sugars than the amino acids that aphids need for growth, most of the sap is excreted as honeydew. This sugar-rich honeydew will cover the leaf surface when aphid populations are extremely high, providing an ideal substrate for the growth of sooty mold fungi that affect the quality of produced pods. Moreover, these fungi, along with honeydew, decrease the efficiency of respiration and photosynthesis, hence the final yields. In addition

Handling Editor: Khalil Talebi-Jahromi

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Received: 12 May 2021, Accepted: 16 July 2022
Published online: 14 August 2022

to the direct feeding activity, black bean aphid can transmit over 42 non-persistent and persistent plant viruses of beans and peas, beets, crucifers, cucurbits, Dahlia, potatoes, tomatoes, and tulips, such as beet yellow net, and potato leaf roll viruses (McKinlay, 1992; Blackman and Eastop, 2007).

Currently, *A. fabae* is primarily treated with broad-spectrum insecticides. The extensive use of chemical insecticides will result in a resurgence of the pest, secondary pest outbreaks, the accumulation of pesticide residues in the environment, the destruction of the ecosystems because of the death of non-target organisms, and the development of insecticide resistance in target pests (Hardin *et al*., 1995; Longley *et al*., 1997; Ogendo *et al*., 2003; Mihale *et al*., 2009; Kataria and Kumar, 2012).

To reduce pesticide use, other approaches that do not represent a risk to human health have been developed by scientists. The innovative development of insect control is the use of aqueous particle films formulations based on kaolin, a white, non-porous, non-swelling, non-abrasive aluminosilicate mineral ($Al_4Si_4O_{10}[OH]_8$) that is easily dispersed in water and is chemically inert over a wide pH range (Glenn *et al*., 1999). Kaolin particles can be coated with organo-silicone oil, stearic acid, chrome complexes, or plant and mineral materials to become hydrophobic (Puterka *et al*., 2000). Kaolin clay (Surround) was used in apple orchards in the USA for the first time (Alavo and Abagli, 2011). Kaolin has also been implemented as a novel way to suppress various arthropod pests and diseases of food crops (Glenn *et al*., 1999; Unruh *et al*., 2000; Glenn and Puterka, 2005; Showler and Setamou, 2005; Karagounis *et al*., 2006; Hall *et al*., 2007). When plants are sprayed with kaolin, the powdery film sticks to the plant and fruit as the water evaporates and protects by acting as a physical barrier. If the insect land on the plant, the clay particles of the coating may stick to the insects and act as a repellent. It may also serve as a deterrent to insect settlement, oviposition, and feeding. Kaolin is used to eradicate diseases, reduce the negative impacts of environmental stresses on crop plants, and protect crops from pests (Glenn and Puterka, 2005). The side effects of kaolin on non-target insects and spiders are usually minimal due to its mode of action (Glenn and Puterka, 2005; Showler and Setamou, 2005).

Additionally, the kaolin coat can minimize solar damage and heat stress on the plants by reflecting UV and heat radiation. Moreover, kaolin can increase yield by raising carbon assimilation (Thomas *et al*., 2004; Glenn and Puterka, 2005; Lapointe *et al*., 2006). It has no detrimental effect on human health or the environment; these characteristics and its mode of action, which are not vulnerable to the development of resistance (Glenn and Puterka, 2005), have resulted in the authorization of its use in organic agriculture (Regulation, 1991). Processed kaolin may be less expensive than conventional insecticide treatments from an economic standpoint, which is a significant factor for low-input crops (Hall *et al*., 2007). The most important mechanisms of action against arthropod pests are: (i) deterrence (orienting insects away from the particle film after contact); (ii) decreased mating success; (iii) increased developmental time and mortality and decreased body mass;(iv) decreased ability to recognize kaolin-coated plants as host; (v) impeded movement and host-finding ability; and (vi) impeded ability of insects to grasp the plant (Puterka *et al*., 2000; Wyss and Daniel, 2004; Glenn and Puterka, 2005; Puterka *et al*., 2005; Sackett *et al*., 2005; Barker *et al*., 2006). Kaolin can also increase germination and enhance aphid infection of a fungal aphid pathogen, *Pandora neoaphidis* (Remaudière and Hennebert) Humber, as an indirect effect (Eigenbrode *et al*., 2006). Although the technology of kaolin particle film has contributed to suppressing some diseases (Glenn *et al*., 2001), the emphasis has now shifted to arthropod pest control. Furthermore, using kaolin in greenhouses will not have problems like rain or wind washout, which is why this compound is suitable for use in greenhouse conditions (Namvar *et al*., 2017). Despite these suppressing effects on various pest species, some research indicated that kaolin could increase the rate of insect infestation (Showler and Armstrong, 2007; Marko *et al*., 2008).

The kaolin particle film technology has been carried out on different crops and is effective in suppressing many pests such as psyllids (Puterka *et al*., 2000; Liu and Trumble, 2005; Puterka *et*

al., 2005; Hall *et al*., 2007), leafhoppers (Glenn *et al*., 1999; Knight *et al*., 2001; Glenn and Puterka, 2005; Marko *et al*., 2008), aphids (Wyss and Daniel, 2004; Showler and Setamou, 2005; Eigenbrode *et al*., 2006; Karagounis *et al*., 2006), and heteropteran (Knight *et al*., 2001; Lalancette *et al*., 2005), coleopteran (Thomas *et al*., 2004; Lalancette *et al*., 2005; Lapointe *et al*., 2006), lepidopteran (Knight *et al*., 2000; Unruh *et al*., 2000; Knight *et al*., 2001; Sisterson *et al*., 2003; Thomas *et al*., 2004; Lalancette *et al*., 2005; Sackett *et al*., 2005; Barker *et al*., 2006) and dipteran pests (Mazor and Erez, 2004; Saour and Makee, 2004).

The application of kaolin to orchard crops resulted in the suppression of damage caused by *Bemisia argentifolii* Bellows and Perring (Homoptera: Aleyrodidae) on melon (Liang and Liu, 2002); *Aphis spiraecola* Patch (Homoptera: Aphididae), *Cacopsylla pyricola* Foerster (Hemiptera: Psyllidae), *Tetranychus urticae* Koch (Acarina: Tetranychidae) and *Empoasca fabae* (Harris) (Homoptera: Cicadellidae) in pear and apple (Glenn *et al*., 1999); *Circulifer tenellus* (Baker) (Homoptera: Cicadellidae) on chili pepper (Creamer *et al*., 2005); *E. fabae*; *Cydia pomonella* (L.); *Choristoneura rosaceana* (Harris); *Conotrachelus nenuphar* (Herbst), and *Diaprepes abbreviatus* (L.) (Glenn *et al*., 1999; Knight *et al*., 2000; Lapointe, 2000; Puterka *et al*., 2000; Unruh *et al*., 2000; Pasqualini *et al*., 2002; Delate and Friedrich, 2004). It has been found that the abundance of certain pests such as *Dysaphis plantaginea* (Passerini), *Quadraspidiotus perniciosus* (Comstock), *Phyllonorycter elmaella* Doganlar & Mutuura, and *Panonychus ulmi* (Koch) decreased in orchards treated with kaolin, particularly in the years with high population numbers in all orchards (Knight *et al*., 2001; Lalancette *et al*., 2005; Arbabi *et al.*, 2020). The results obtained from Izadmehr *et al*. (2015) showed that 5% processed kaolin reduced the population of *Bemisia tabaci* Gennadius pupae and had a better effect than the chemical insecticide Proteus. Therefore, using 5% processed kaolin to control whitefly and prevent contamination in cotton fields by this pest is recommended as an integrated cotton pest management program. Keyhanian and Abbasi Mojdehi (2018) revealed that 5% kaolin with volk oil and water could be used to control olive psyllid nymphs, *Euphyllura straminea* Loginova, as soon as the first white cotton threads were observed. No risk of phytotoxicity, positive effects in reducing evapotranspiration, increasing photosynthesis, and improving fatty acids quality in olive oil are some of the factors justifying the promotion of this healthy mineral.

It has been reported that kaolin is effective against *Bactrocera oleae* (Rossi) (Saour and Makee, 2004), *Agonoscena targionii* (Lichtenstein) (Saour, 2005), and *Ceratitis capitata* (Wiedemann) on peach, apple, and Date plum (Mazor and Erez, 2004). On peach, kaolin provided control of *Grapholita molesta* (Busck); *Conotrachelus nenuphar* (Herbst), and *Popillia japonica* Newman. It was also effective against late-season tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois); stink bugs *Acrosternum hilare* (Say), *Euschistus servus* (Say), and *Euschistus tristigmus* (Say) (Lalancette *et al*., 2005); and pistachio psyllids, *Agonoscena pistaciae* Burckharat & Lauterer (Farazmand *et al.*, 2014); and *Diaphorina citri* Kuwayama on citrus (Mohammadipour and Naseri, 2018). Kaolin has no inhibitory effect on the feeding of tomato *Tuta absoluta* (Meyrick) larvae, so using kaolin alone will not be effective for its damage control. This efficacy may be due to the feeding behavior of the larvae. Suppose the larvae do not leave the leaf surface of the host plant. In that case, they will first endure the critical conditions caused by the kaolin coating on the leaves, feed on some parts of the epidermis, and enter the middle layer, continuing to feed in a safer environment. 2.5% kaolin is also recommended to repel the larvae of this pest. (Abdollahi *et al.*, 2016).

Kaolin can be an effective substitute for diazinon in the *Ommatissus lybicus* (de Bergevin) control program (Pezhman *et al*., 2017). Moreover, foliar application of 5% processed kaolin on vineyards or its combined application with wettable sulfur can successfully control the number of *Arboridia kermanshah* Dlabola (Abedini *et al*., 2017).

The effects of kaolin have been investigated against aphid species like *Aphis craccivora* Koch (Alavo, 2010) and *Aphis gossypii* (Glover) (Alavo *et al*., 2011). Research conducted by Cottrell *et al*. (2002) revealed that the accumulation of *Tinocallis caryaefoliae* (Davis) on pecan seedlings decreased by kaolin spraying. Consequently, the production and longevity of nymphs on seedlings decreased. The effect of kaolin on *D. plantaginea* was examined in apple trees and showed a significant reduction in the number of aphids (Wyss and Daniel, 2004). Application of various kaolin concentrations on cotton in West Africa showed that 5% kaolin significantly reduced the population of *A. gossypii* (Alavo *et al*., 2011). Weekly applications of 5% kaolin significantly decreased *A. craccivora* populations in cowpea, *Vigna unguiculata* (L.) (Alavo, 2010). Numbers of cotton aphid predators, such as ladybird beetles (Coleoptera: Coccinellidae), minute pirate bugs (Heteroptera: Anthocoridae), and green lacewings (Neuroptera: Chrysopidae), were not affected by kaolin application to cotton (Showler and Setamou, 2005).

The purpose of this study was (a) to evaluate the efficacy of processed kaolin in controlling the major bean aphid, the black bean aphid *Aphis fabae* Scopoli, and (b) to evaluate the side effects of processed kaolin on non-target arthropods, paying particular attention to the natural enemy of aphid, *Hippodamia variegata* (Goeze).

Materials and Methods

The research was conducted on Barkat broad beans during 2017-2019 under greenhouse and field conditions in Karaj, Iran. Broad bean seeds were sown at 3-4 cm and spaced 15 cm apart on March 6. Regular irrigation was performed weekly.

The soil fertility was improved by applying N fertilizer (as urea) at the rate of 100 kg ha^{-1} (in three stages: one-third planting time / one third before Stem formation / one third before flowering), P fertilizer (as triple superphosphate) at the rate of 50 kg ha^{-1} (at planting time) and K fertilizer (as potassium sulfate) at the rate of 150 kg ha^{-1} (at planting time). Weed control was manual, and no chemical herbicides were used.

Field trial

The experiment was factorial in the Randomized Complete Block Design (RCBD). Experimental factors included foliar application with various concentrations of kaolin (3%, 6%, and 9%; Sepidan® WP; Kimia Sabzavar Co., Tehran, Iran) and sampling times (one day before application and 3, 6, 9, 12, and 15 days after application). The powder and two liters of water were poured separately into the back sprayer and mixed well. A sprayer with a stirrer was used during the spraying. The water-treated plants served as a control. The experiment was performed in four replications, and a margin of 5 and 3 m was considered between different experimental replications and experimental treatments, respectively. Treatments were divided into five rows of 4m with a distance of 40 cm from each other. The experiment consisted of 64 experimental units.

Different concentrations of kaolin powder were mixed well before application. Foliar kaolin spray was applied using a Backpack sprayer equipped with a continuous agitator to keep the material suspended. Ten plants were selected and sampled for each treatment. Foliar application began when plants were naturally infested with aphids, and their natural enemies were also observed. The records were conducted one day before and then 3, 6, 9, 12, and 15 days after application.

The effectiveness of different treatments was estimated as the percentage reduction in the adult population according to the Henderson-Tilton formula (Henderson and Tilton, 1955):

$$\text{Efficacy\%} = 1 - (T_a/T_b \times C_b/C_a) \times 100,$$

Where: T_b and C_b are pre-treatment densities, and T_a and C_a are post-treatment densities of insects in the treated (T) and control (C) plots, respectively.

Greenhouse experiment

The experiments were carried out in the laboratory. Barkat variety of broad bean seeds was planted in 10 separate pots. Potting soil was prepared from the field and fertilized according to the field conditions. Pots were transferred to a growth chamber at 25 ± 1 °C, 70% RH, and a photoperiod of 16: 8 (L: D) h. Plants were free of contamination when they were transferred to

the greenhouse. Each pot, considered a treatment, was placed in a separate cage covered by a net. Insects were collected from broad beans and reared in the laboratory on the potted plants to have same-age insects. *H. variegata* was fed approximately 35 aphids daily. Then pots were infected manually so that 30 insects of the same age were allocated to each cage. Sampling was conducted one day before application and then 3, 6, 9, 12, and 15 days after application, and the results were recorded. The effectiveness of different treatments was estimated as the percentage reduction in population according to Abbott's formula (Abbott, 1987):

$$\text{Efficacy\%} = (1- T_a / C_a) \times 100$$

where: T_a and C_a are post-treatment densities of insects in the treated (T) and control (C) plots, respectively.

Statistical data analysis

Design Expert 12 software (StatSoft, Tulsa, OK, USA) was used for the experimental design, data analysis, and linear model creation. Contour and 3D surface plots were developed to understand the interaction of different factors.

Results

The population of *A. fabae* and adults of *H. variegata* were recorded before and after the kaolin application. Sampling proceeded until 15 days after the application for each treatment.

Aphid in the laboratory

The statistical analysis based on Table 1 showed each factor effect and its interactions with the kaolin efficiency on *A. fabae* in the laboratory during three years of the experiment. The R^2 value, R^2adjusted, and Predicted R^2 of kaolin efficiency on aphid was 0.957, 0.943 and 0.923 (2017), 0.986, 0.981, and 0.975 (2018), respectively, while they were 0.983, 0.978, and 0.97 in 2019, respectively. Based on the ANOVA analysis, kaolin and the sampling day had significant effects ($P < 0.05$) on kaolin efficiency. Similarly, the interactions between kaolin and the sampling day significantly affected kaolin efficiency (Table 1).

Table 1 Analysis of variance of kaolin efficiency on *Aphis fabae* population in the laboratory during three years of the experiment.

Year of the experiment	Source	df.	Mean Square	F-value	P-value	Fit statistics
2017	Model	14	953.97	70.45	< 0.0001	Std. Dev. = 3.68
	A-Kaolin	2	2885.38	213.08	< 0.0001	Mean = 60.72
	B-Day	4	1742.87	128.71	< 0.0001	CV% = 6.06
	AB	8	72.50	5.35	0.0001	R^2 = 0.9573
	Pure Error	44	13.54			Adjusted R^2 = 0.9437
	Cor total	58				Predicted R^2 = 0.9239
						Adeq Precision = 26.2832
2018	Model	14	978.70	229.48	< 0.0001	Std. Dev. = 2.07
	A-Kaolin	2	3230.80	757.54	< 0.0001	Mean = 58.67
	B- Day	4	1639.15	384.34	< 0.0001	CV% = 3.52
	AB	8	85.45	20.04	< 0.0001	R^2= 0.9862
	Pure Error	45	4.26			Adjusted R^2 = 0.9819
	Cor total	59				Predicted R^2 = 0.9754
						Adeq Precision = 51.2697
2019	Model	14	1091.79	190.48	< 0.0001	Std. Dev.= 2.39
	A-Kaolin	2	3552.45	619.77	< 0.0001	Mean = 55.72
	B-Day	4	1802.11	314.40	< 0.0001	CV% = 4.30
	AB	8	121.47	21.19	< 0.0001	R^2 = 0.9834
	Pure Error	45	5.73			Adjusted R^2 = 0.9782
	Cor total	59				Predicted R^2 = 0.9705
						Adeq Precision = 43.1659

Fig. 1a shows the percentage of kaolin efficiency on the aphid's population on different days in 2017. The laboratory study results of *A. fabae* showed that in the first year of the experiment, three, six, and nine days after kaolin application, a significant difference was observed among kaolin concentrations.

The lowest kaolin efficiency on *A. fabae* was observed three days after application by kaolin 3%, while the highest was observed 12 days after application by kaolin 9%. Kaolin 3% showed no significant difference among 9, 12, and 15 days of the application, and there was no significant difference between kaolin 6% and 9% at 12 and 15 days after application. Kaolin 6% had no significant difference between 12 and 15 days post-treatment. The highest kaolin efficiency was observed 12 and 9 days after application by kaolin 9%. Surprisingly, the kaolin efficiency increased through the sampling days up to 12 days. Then the kaolin efficiency decreased so that in kaolin 9%, the efficiency on the 15^{th} day after the application was significantly less than on the 12^{th} day (Fig. 1a).

Fig.1b-c represents the percentage of kaolin efficiency in the aphid population in 2018 and 2019. All the treatments showed significant differences in these years, except 9 and 15 days after sampling, where kaolin 9% was used in the third year. In the second year of the experiment, all the treatments were significantly different from each other. The highest kaolin efficiency was observed 12 days after the application of kaolin at 9%. Moreover, kaolin efficiency 15 days after the application was less than nine days in any kaolin concentrations (Fig. 1b).

Field experiment

The analysis of kaolin efficiency on the aphid population in the field showed that both factors, including kaolin and sampling day, had a significant effect. However, a significant interaction was not observed between factors (Table 2).

The R^2 value, R^2 adjusted, and predicted R^2 of kaolin efficiency on *A. fabae* population were 0.892, 0.88, and 0.861 (2017), 0.86, 0.844, and 0.82 (2018), while they were 0.909, 0.899, and 0.883 in 2019 respectively. The R-squared value represented that the model fits the data. Fig. 2a shows the percentage of kaolin efficiency in the aphid population in the field in 2017.

The least kaolin efficiency was observed three days after application by kaolin 3%. In contrast, the highest percentage of kaolin efficiency was observed 9 days post-treatment by kaolin 9%. Also, no significant difference was observed between 15 and 6 days and 12 and 6 days after applications. The results in 2018 were the same (Fig. 2b). But there was a difference in 2019, i. e. no significant difference was observed between 15 and 6 days after application in any of the concentrations (Fig. 2c).

Natural enemy in the laboratory

The ladybird population analysis showed that both the kaolin and the exposure time significantly impacted *H. variegata*. However, no significant interaction was observed between the two factors (Table 3).

The R^2 value, R^2 adjusted and Predicted R^2 were 0.81, 0.79, and 0.76 (2017); 0.85, 0.83, and 0.81 (2018), respectively. However, they were 0.74, 0.72, and 0.67 in 2019. The R-squared value suggested that the model corresponds to the data. Fig. 3a displays the percentage of kaolin efficiency on the population of ladybirds in the laboratory on various days after treatment in 2017. In this experiment, three days after kaolin 3% application, the lowest kaolin efficiency in *H. variegata* population was observed. At the same time, the highest kaolin efficiency was observed 15 days after kaolin 9% application and had no significant difference with the population after 12 days of application. No significant differences between 9 and 12 days and 12 and 15 days after treatment in kaolin concentrations were found. The experiment showed no significant difference between 12 and 15 days post-treatment in 2018 and 2019 (Fig. 3b-c).

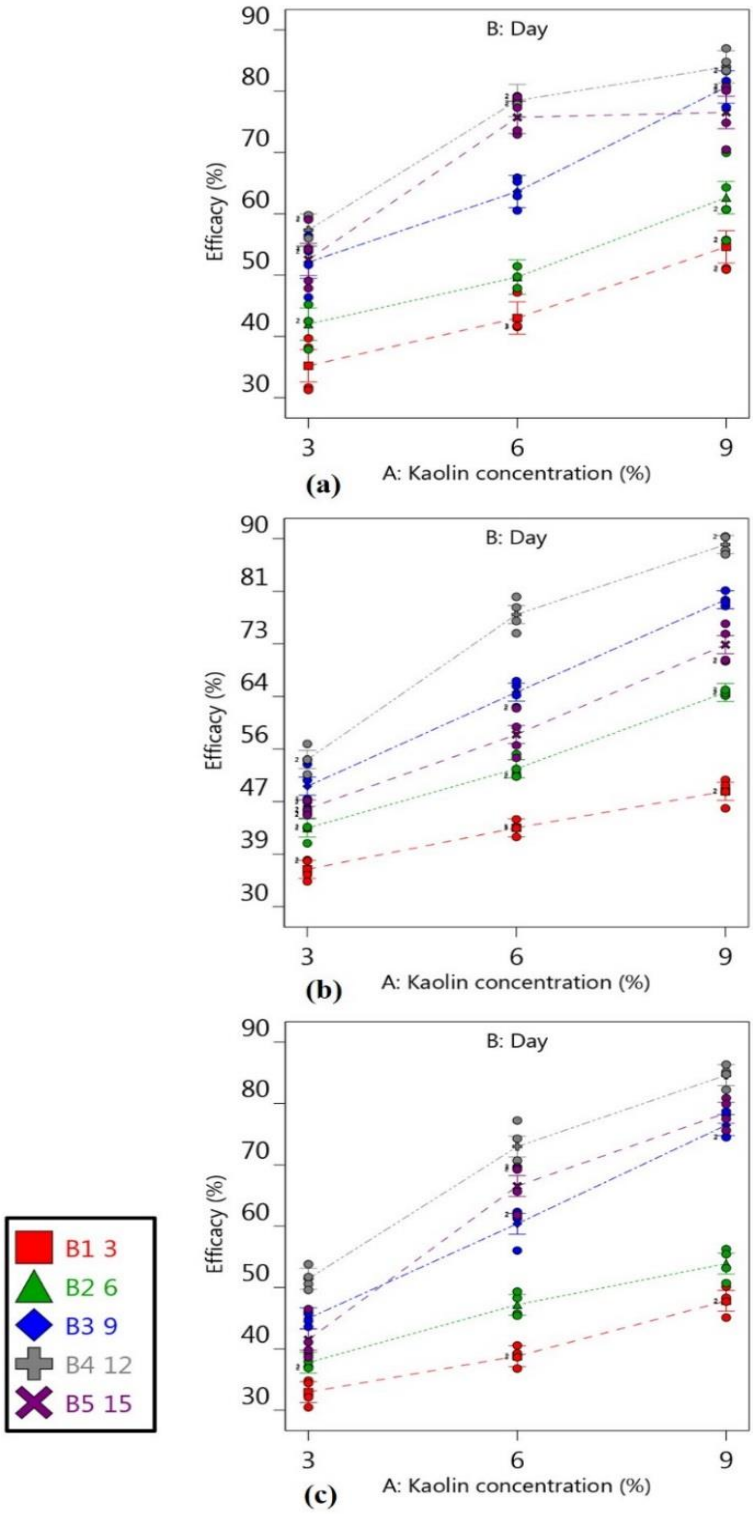

Figure 1 Efficiency of kaolin concentration and exposure time on *Aphis fabae* population in the laboratory in (a) 2017, (b) 2018, and (c) 2019.

Table 2 Analysis of variance of kaolin efficiency on *Aphis fabae* population in the field during three years of the experiment.

Year of the experiment	Source	df.	Mean Square	F-value	P-value	Fit statistics
2017	Model	6	1393.38	71.88	< 0.0001	Std. Dev. = 4.40
	A-Kaolin	2	2742.57	141.48	< 0.0001	Mean = 46.88
	B-Day	4	733.08	37.82	< 0.0001	CV% = 9.39
	Residual	52	19.38			R^2 = 0.8924
	Lack of Fit	8	22.66	1.21	0.3179	Adjusted R^2 = 0.8800
	Pure Error	44	18.79			Predicted R^2 = 0.8616
	Cor total	58				Adeq Precision = 29.8080
2018	Model	6	1026.00	53.35	< 0.0001	Std. Dev. = 4.39
	A-Kaolin	2	2080.51	108.19	< 0.0001	Mean = 44.50
	B-Day	4	514.02	26.73	< 0.0001	CV% = 9.85
	Residual	52	19.23			R^2 = 0.8603
	Lack of Fit	8	31.57	1.86	0.0914	Adjusted R^2 = 0.8441
	Pure Error	44	16.99			Predicted R^2 = 0.8204
	Cor total	58				Adeq Precision = 25.3244
2019	Model	6	1921.12	87.20	< 0.0001	Std. Dev. = 4.69
	A-Kaolin	2	3891.46	176.64	< 0.0001	Mean = 44.71
	B-Day	4	971.89	44.12	< 0.0001	CV% = 10.50
	Residual	52	22.03			R^2 = 0.9096
	Lack of Fit	8	39.69	2.11	0.0552	Adjusted R^2 = 0.8992
	Pure Error	44	18.82			Predicted R^2 = 0.8837
	Cor total	58				Adeq Precision = 32.1149

Discussion

Since the early 1970s, public concerns about human health and the environment have led entomologists to minimize pesticide use by developing integrated pest management approaches to pest control. Currently, using chemical pesticides is the only effective way for pest management, but alternative methods and control materials are needed to ensure food safety and environmental sustainability. (Peng et al., 2011).

Kaolin is an appropriate tool for integrated pest management programs and enables the control of many pests and diseases (Glenn and Puterka, 2005; Peng *et al*., 2011). This product has fewer environmental mal effects than chemical pesticides (Glenn and Puterka, 2005) and may have a better and longer-lasting impact on pests than some pesticides (Braham *et al*., 2007; Hassanzadeh *et al*., 2014). Although the mechanism of action of kaolin particles on insects and mites is not fully understood, it seems that the effects of the white color of the kaolin coating will prevent adult insects from landing o n the treated plants (Liang and Liu, 2002; Liu and Trumble, 2005). Kaolin particles create a physical barrier on plants, creating an unfamiliar environment for pests that make them not recognize the plant as a host, and ultimately prevent movement, feeding, mating, and oviposition (Glenn *et al*., 1999; Puterka *et al*., 2000; Cottrell *et al*., 2002).

Generally, based on the results of the laboratory study on the black bean aphid, *A. fabae*, the mean of the highest percentage of kaolin efficiency was 89.03% at the concentration of 9%, twelve days after foliar application, while the least was 26.52% three days after foliar application with kaolin 3% (Fig. 1b,c). Also, findings of the field conditions indicated that the mean of the highest efficiency percentage of kaolin was 71% at a concentration of 9% and 9 days after foliar application.

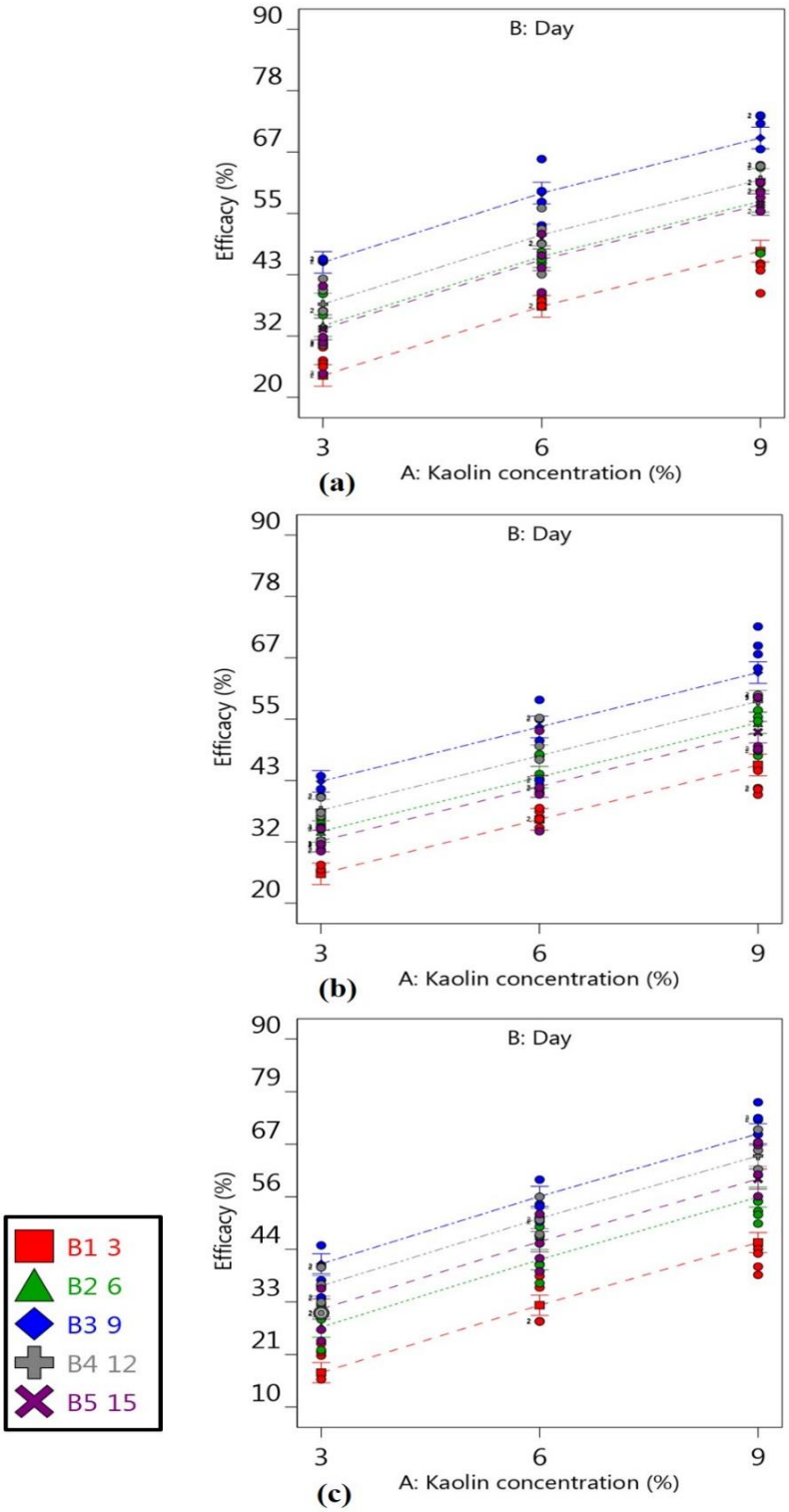

Figure 2 Efficiency of kaolin concentration and exposure time on *Aphis fabae* population in the field in (a) 2017, (b) 2018, and (c) 2019.

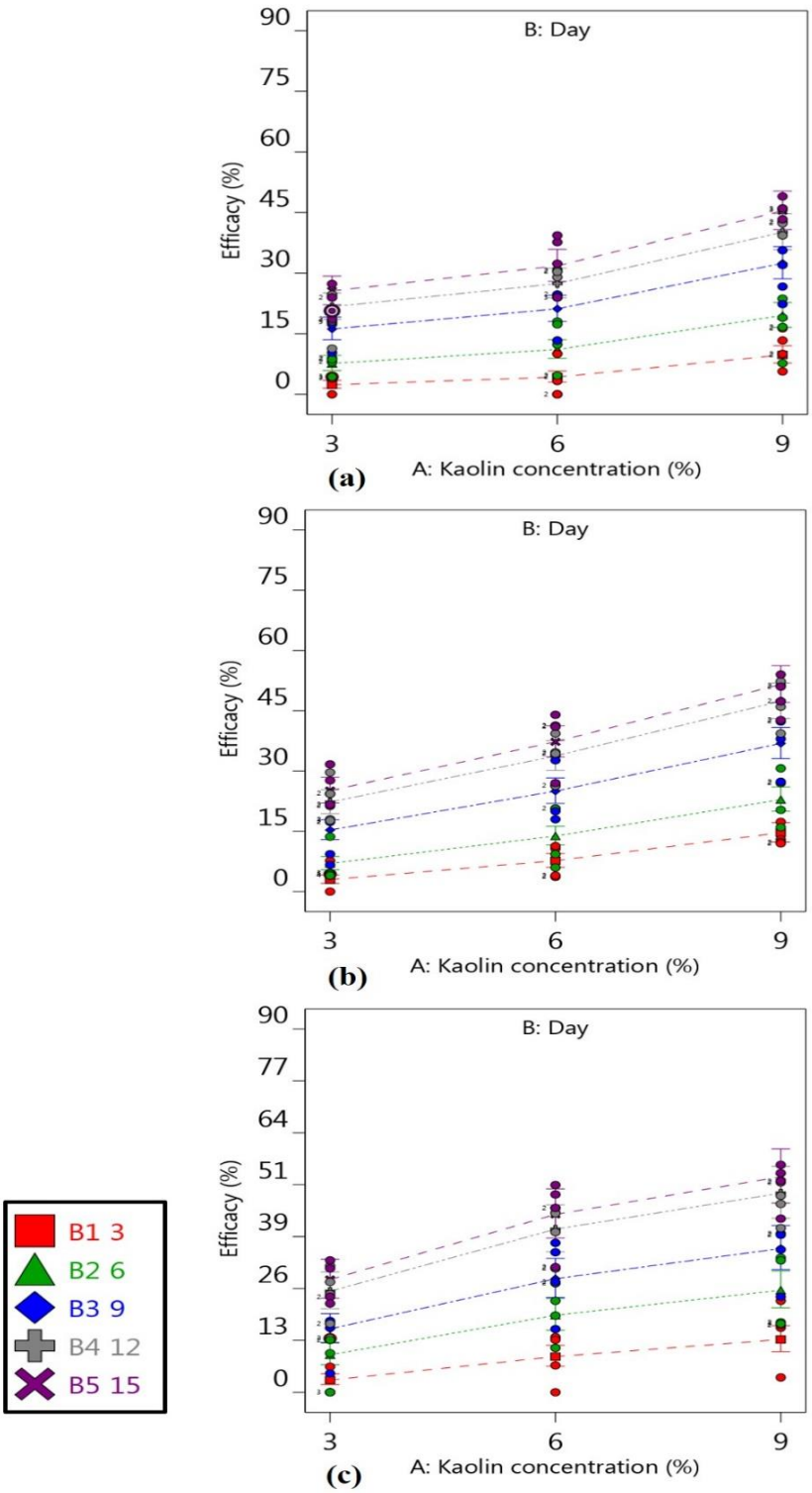

Figure 3 Efficiency of kaolin concentration and exposure time on *Hippodamia variegata* adult population in the laboratory in (a) 2017, (b) 2018 and (c) 2019.

In contrast, the lowest was 20.72% at a concentration of 3%, three days after foliar application. *H. variegata* (Coleoptera: Coccinellidae) is a crucial predator of aphids and other insect pests (Abdolahi Mesbah *et al*., 2015). In the current experiment, kaolin efficiency was evaluated on *A. fabae* and its natural enemy, *H. variegata*. The efficiency of kaolin decreased in the laboratory and field conditions after 15 and 9 days, respectively, leading to an increase in the pest population on the plant. The results of this study also indicated that kaolin is not fast-acting and takes time to show its control effects. Consequently, population monitoring would make predicting aphid's population possible, and foliar application should be performed about 9-12 days before population peak. Research on various pests showed that kaolin could affect life cycle, reproductive potential, population, and ultimately pest damage on plants (Nateghi *et al*., 2013; Pease *et al*., 2016; Guedes *et al*., 2020; Labbé *et al*., 2020; Abbasi Mojdehi *et al*., 2021). Additionally, the results of some reports indicated that the use of kaolin could increase the population of *A. gossypii* on cotton, *Eriosoma lanigerum* Hausmann, and *D. plantaginea* in apple orchards (Showler and Armstrong, 2007; Marko *et al*., 2008; Alavo and Abagli, 2011) or possibly lead to secondary pest outbreak (Peng *et al*., 2011).

So, the side effects of crop protection methods must be evaluated when implementing new methods. In this context, it is particularly essential to assess the impact on non-target organisms like parasitoids and predators, which are the basis of natural biological controls (Pascual *et al*., 2010). In some agricultural ecosystems, processed kaolin has been reported to have no adverse effects on predators (Karagounis *et al*., 2006) and parasitoids (Sackett *et al*., 2007). In other cases, the relative abundances of certain generalized predators were reduced by processed kaolin, whereas the relative abundances of others were not affected (Sackett *et al*., 2007). Based on the obtained results in this study, kaolin harmed *H. variegata*, so the natural enemy population decreased fifteen days after application. No significant difference between kaolin 6% and 9% were identified in the first year of application (Fig. 3a). Some days after kaolin application *H. variegata* made up its population again. A current laboratory study on *H. variegata* showed that the highest efficiency of kaolin was 15 days after foliar application by kaolin 9% with 51.49% while the lowest was 4% three days after foliar application by kaolin 3%. Kaolin directly or indirectly affects the Coccinellidae population, and this population decline may be due to prey reduction (Sackett *et al*., 2007; Marko *et al*., 2008; Pascual *et al*., 2010). In addition, Chrysopidae, Scelionidae, Pteromalidae, Aphelinidae, Salticidae, Philodromidae, Reduviidae, Formicidae, and Anthocoridae populations would be reduced by kaolin treatments (Showler and Setamou, 2005; Pascual *et al*., 2010). Due to its mechanism of action, kaolin should theoretically not be toxic to natural enemies. However, it has minor effects on the *Chrysoperla carnea* (Stephens) and the parasitoid bees *Scutellista cyanea* Motschulsky and *Chelonus inanitus* (Bengochea *et al*., 2010; Porcel *et al*., 2011). Moarefi *et al*. (2021) showed that increasing the concentration of kaolin at different stages of plant growth reduces the population of *H. varegata*, *Coccinella septempunctata* L. and *C. carnea*. However, the results of some studies described that kaolin has no impact on *C. septempunctata* and *Trichogramma cacoeciae* Marchal (Panagiotis *et al*., 2019). Kaolin can also be used with *C. septempunctata* and *H. variegata* in pest management (Panagiotis *et al*., 2019). Generally, high concentrations and high kaolin coverage negatively affect natural enemies' life cycles. The reduction in the population of natural enemies could be due to the direct effects of kaolin on the natural enemy, as well as the feeding of the natural enemy by the infected host. Kaolin can also reduce insects' mobility by adhering to the host body and limiting access to the food source and mate (Moarefi *et al*., 2021).

In conclusion, kaolin can influence black bean aphids and reasonably control the pest population at a concentration of 9%. It should

also be noted that the effect of kaolin decreases over time (as mentioned above). In addition, high concentrations have adverse effects on the natural enemy populations, so they cannot be used continuously at high concentrations. These two issues indicated that using kaolin could not wholly control the pest, but it can be considered an appropriate control method in the integrated pest management program. The results of this survey proposed that kaolin offers some nonchemical pest management opportunities. Nonetheless, the efficiency of kaolin is often species-specific and must be studied for each pest in its environment. Further research is also suggested to examine kaolin's physiological effects on beneficial insects such as natural enemies of pests and pollinators.

Conflict of interests

The authors declare that they have no conflict of interest.

Authors' contributions

Author A is a member of the scientific board of Institute A and the project supervisor. Author B is a member of the scientific committee of institute B and designed the experiment and analyzed the data. Author C was responsible for managing field and greenhouse experiments.

Acknowledgments

We thank the Karaj Branch of Islamic Azad University for sponsoring this project.

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اثرات کائولن فرآوری شده روی شته سیاه باقلا *Aphis fabae* و *Hippodamia variegata* در گیاه باقلا: مطالعه آزمایشگاهی و مزرعه ای

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چکیده: شته سیاه باقلا، *Aphis fabae* Scopoli، یکی از آفات کلیدی است که از گیاهان مختلف تغذیه می‌کند. این مطالعه به‌منظور بررسی اثرات کائولن روی شته سیاه باقلا و یکی از دشمنان طبیعی آن، *Hippodamia variegata* (Goeze) در آزمایشگاه و مزرعه انجام شد. پژوهش‌ها روي بذرهای باقلای برکت طی سال‌های ۱۳۹۶ تا ۱۳۹۸ در کرج انجام شد. این آزمایش به‌صورت فاکتوریل در قالب طرح بلوک‌های کامل تصادفی و با چهار تکرار انجام شد. فاکتورهای آزمایش شامل غلظت کائولن (۳، ۶ و ۹ درصد) و زمان نمونه‌گیری (یک روز قبل از استفاده و سه، شش، نه، دوازده و پانزده روز پس از استفاده) بودند. برای هر تیمار، از ده گیاه نمونه‌برداری انجام شد. به‌محض استقرار حشرات روی گیاهان محلول‌پاشی کائولین (سپیدان®، WP%95) آغاز شد. اثر تیمارهای مختلف بر کاهش جمعیت در آزمایش‌های مزرعه‌ای و گلخانه‌ای به‌ترتیب با فرمول هندرسون-تیلتون و ابوت برآورد شد. تجزیه‌وتحلیل داده‌ها با استفاده از نرم-افزار Design Expert 12 انجام شد. در مطالعات آزمایشگاهی روی شته سیاه باقلا، بیشترین کارایی کائولین در غلظت ۹ درصد و ۱۲ روز پس از محلول‌پاشی در سال دوم مشاهده شد درحالی‌که کم‌ترین آن سه روز پس از محلول‌پاشی در غلظت ۳ درصد در سال سوم بود. آزمایشات مزرعه‌اي نشان داد که بیشترین کارایی کائولن ۹ روز پس از محلول‌پاشی در غلظت ۹ درصد در سال اول بود. مطالعه روي *H. variegata* نشان داد که بالاترین کارایی کائولن ۱۵ روز پس از محلول‌پاشی با غلظت ۹ درصد در سال سوم بود.

واژگان کلیدی: *Aphis fabae*، باقلا، *Hippodamia variegata*، کائولن، مدیریت آفات