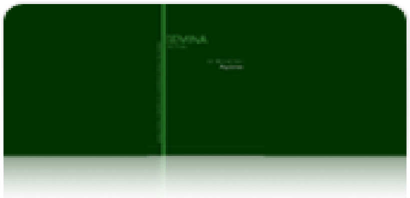

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Inert powders alone or in combination with neem oil for controlling *Spodoptera eridania* and *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae

Pós inertes sozinhos ou em combinação com óleo de neem no controle de lagartas de *Spodoptera eridania* e *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

Kelly Christiane Constanski[1*]; Janaína Zorzetti[1]; Patrícia Helena Santoro[2]; Adriano Thibes Hoshino[3]; Pedro Manuel Oliveira Janeiro Neves[4]

Abstract

Laboratory studies was carry out to evaluated the potential of inert powders: bentonite, kaolin and diatomaceous earth (DE) applied as dust and aqueous suspension alone and associated with neem oil to the control *Spodoptera eridania* and *Spodoptera frugiperda* second instar larvae. In dust applications, 100% mortality of *S. eridania*, at the concentrations of 1.5 and 2.0 grams of bentonite, and 93.3% of *S. frugiperda*, at the concentration of 2.0 g were observed. DE at the height concentration (2.0 g) caused 76.2% mortality of *S. eridania* and 46.6% of *S. frugiperda*. Kaolin, in all concentrations, was the only treatment that did not differed from the control for *S. eridania* but was different, in the high concentration, for *S. frugiperda*. When the plants were treated, larvae of *S. eridania* fed in the treatment DE, had an increase of two days in larval period. For the sex ratio, the percentage of males was greater in the treatments with kaolin, for both species. When powders was applied suspended in water separately or combined with neem oil, the best results were observed in combined of the bentonite (10%) + neem, with 100% mortality for both species, and kaolin (10%) + neem with 78.5% and 95.6% mortality for *S. frugiperda* and *S. eridania*, respectively. These treatments were classified as non-additive synergistic.
Key words: Bentonite. Diatomaceous earth. Kaolin. Sustainable agriculture.

Resumo

Estudos em laboratório foram realizados para avaliar o potencial dos pós inertes: bentonita, caulim e da terra de diatomáceas (TD), na forma sólida e em suspensão aquosa isoladamente e associados ao óleo de nim no controle das lagartas de segundo instar de *Spodoptera eridania e Spodoptera frugiperda.* Nas aplicações como pó, observou-se 100% de mortalidade para *S. eridania* nas concentrações de 1,5 e 2,0 g de bentonita e de 93,3% para *S. frugiperda* na concentração de 2,0 g. A TD na maior concentração (2,0 g) causou 76,2% de mortalidade em *S. eridania* e 46,6% de *S. frugiperda*. O caulim, em todas as concentrações, foi o único tratamento que não diferiu da testemunha para *S. eridania*, mas foi diferente, na maior concentração para *S. frugiperda*. Quando as plantas foram tratadas, lagartas de *S. eridania* alimentadas no tratamento TD, tiveram um aumento de dois dias no período larval. Para a razão sexual, a porcentagem de machos foi maior nos tratamentos com caulim para ambas as espécies.

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Quando os pós foram aplicados em suspensão em água separadamente ou em combinação com óleo de neem, os melhores resultados foram observados na combinação bentonita (10%) + neem, com 100% de mortalidade para ambas as espécies e caulim (10%) + neem com 78,5% e 95,6% de mortalidade para *S. frugiperda* e *S. eridania*, respectivamente. Estes tratamentos foram classificados como não aditivos sinérgicos.
Palavras-chave: Agricultura sustentável. Bentonita. Caulim. Terra de diatomáceas.

Introduction

Spodoptera spp. (Lepidoptera: Noctuidae) are very important pests, because they are polyphagous and can cause great losses to several crops (POGUE, 2002; SANTOS, 2007). These insects are frequently controlled with chemical pesticides, which are applied as soon as the insects appeared in the crop. Nevertheless, wrong spray equipment's adjustment, incorrect choice of pesticides and inadequate crop management have increased the number of pesticides applications without an efficient pest control. The pesticides, when used improperly, can cause death, poisoning, production losses and increase insect's resistance, in addition to environmental and human contamination (GALLO et al., 2002). So, alternative control methods are being studied and used, including biological control, repellants or insecticidal substances which are naturally produced by some plants (KUMAR; POEHLING, 2006; LOVATTO et al., 2004), as well as some products of mineral origin, like inert powders (LORINI et al., 2001; SANTORO et al., 2010). Regarding plants with insecticidal activity, neem (*Azadirachta indica* A. Juss) has been cited to be very efficient to control some insects species. Neem contains azadirachtin, a triterpenoid with strong anti-feeding, repellent, and oviposition reduction action. Also, acts as a growth regulator, and may cause significant mortality, particularly at the immature stages (VIANA et al., 2006).

Inert powders are rich in silicon and can prevent the attack of pests and plant diseases. Prolonged activity, along with the difficulty of insects to develop resistance to this type of product, make these important tools in pest control (COSTA; MORAES, 2006; KORUNIC, 1998; LORINI, 2001). Rock powders act by contact as well as, like diatomaceous earth, by the adherence of powder particles to the body of the insects by contact. The inert powder acts removing the epicuticular wax's, favoring water loss and causing dehydration in insects (LORINI, 2001). Also, according to Ebeling (1971) insects spend a lot of energy in the reconstruction process of the cuticle, replacement of lipids and cleaning the powders, attached to the body and to the mouthparts. Oliveira et al. (2009) suggest the importance of performing preliminary studies to check the potential of this powder combined with other control agents.

Therefore, the aim of this study was to study the efficiency to control *Spodoptera eridania* and *Spodoptera frugiperda* by inert powders in solid form (dust) and aqueous suspension, alone and in combination with neem oil.

Material and Methods

Laboratory assays were performed with *Spodoptera eridania* and *Spodoptera frugiperda,* 2nd instar larvae, provided by the insect-rearing laboratory of EMBRAPA Soja. The larvae were maintained on artificial diet until reached 2nd instar, when they were used to test to efficiency of diatomaceous earth (KeepDry®) (DE) (composed of 86% amorphous silicon dioxide, with particles of approximately 15 µm), Bentonite (Rocksil) (composed of mineral soil, with 20.6% Al_2O_3, 17.4% SiO_2, 9.8% S, 1.3% CaO, 0.34% TiO_2, 0.18% MgO, 0.16% Fe_2O_3, and 0.1% P_2O_5 – Lia Agro Ltd Brazil, and Kaolin (Protesyl) [composed of kaolinite mineral clay ($Al_2Si_2O_5(OH)_4$), with 46.5% SiO_2, 39.5% $A_{l2}O_3$, and 13.9% H_2O]; and neem oil (Neemazal, Trifolio-M GmbH, Lahnau, Germany, NZ, Trademark of Sustain-Ability Ltd, Motueka).

All of the tests used tomato leaflets and maize leaves from 40 day old plants grown in a greenhouse without the application of pesticides. The tomato leaflets and the maize leaves were first washed with sodium hypochlorite (1%) and dried with paper towels. The tomato leaflets were cut into 15 cm^2 pieces, and the maize leaves into 36 cm^2 pieces, hereafter referred to as leaflet pieces and leaf pieces, respectively.

Mortality by Inert powders

In polypropylene bags (30 × 40 × 0.05 cm), leaflet pieces (32) and leaf pieces (32) were placed separately with which powder at concentration 1.0, 1.5 or 2.0 g and were manually shaken for 30 s. Then, four treated tomato leaflets were placed on a 9 cm diameter polystyrene crystal dish containing ten *S. eridania* larvae (2nd instar). For *S. frugiperda*, one larvae were placed in each plate containing one piece of the maize leaf, due to cannibalism. After two days, the treated leaves were replaced by untreated ones. For the control groups, only untreated leaflet or leaf pieces were offered. The experiment was performed in a climate controlled chamber (25 ± 1 °C, 12 h photoperiod and 70% RH) and mortality was evaluated on the 3rd and 6th days. A completely randomized experimental design was used with eight replications for *S. eridania* and 30 replications for *S. frugiperda*, where each dish was considered an experimental unit.

The mortality data obtained were analyzed by the chi-square test and mortality was corrected by the Schneider and Orelli formula.

Development of S. eridania and S. frugiperda fed with plants treated with inert powders

Pieces of tomato leaflets (32) or maize leaves (32) were manually shaken for 30s in polypropylene bags containing 0.25 g of inert powder which were later offered to 2nd instar larvae of *S. eridania*, kept in covered polystyrene crystal boxes (11 × 11 × 3.5 cm) containing 2 g of autoclaved vermiculite covered with filter paper, and to individual larvae of *S. frugiperda* in 9 cm diameter polystyrene crystal plates. The experiment was kept under controlled conditions (25 ± 1 °C and 12 h photoperiod and 70% RH). The effects of the inert powders were evaluated for the (I) larval development period, (II) average pupae weight, (III) mortality and (IV) sex ratio. A completely randomized design was used with ten replications of 10 larvae/each, for *S. eridania* and 30 replications with one larva/each for *S. frugiperda*.

Mean values of the larval development period and average pupal weight were analyzed using the Kruskal Wallis test, while mortality data and sex ratio were analyzed with a chi-square test.

Mortality of S. eridania and S. frugiperda fed on plants treated with bentonite

For this test bentonite was chosen since it caused high mortality (over 80%) when applied in the solid form. Thirty days old plants of tomato and maize, maintained in a greenhouse, were dusted with bentonite using a Guarany® duster (1 g/plant). After two hours, tomato leaflets and maize leaves were cut as described early and left in polystyrene crystal boxes (*S. eridania*) and plates (*S. frugiperda*) as also previous described. Then, 2nd instars larvae of *S. eridania* and *S. frugiperda* were distributed and left under controlled conditions (25 ± 1 °C and 12 h photoperiod and 70% RH). The treated leaflets/leaves were replaced by untreated ones after two days and larval mortality was evaluated on the 3rd and 6th days after the replacement of the leaflets/leaves. A completely randomized experimental design was used with eight replicates of 10 larvae/each for *S. eridania* and 30 replicates with one larva/each for *S. frugiperda*. Data for *S. eridania* were compared by using the Mann Whitney test, and those for *S. frugiperda* by the chi-square test.

Inert powders suspended in water and combined with neem oil for controlling S. eridania and S. frugiperda

In this study, the inert powders were suspended in distilled water at concentrations of 5%, 7.5%, and 10% w/v, separately and combined with 3% neem oil. Tomato leaflets of approximately 12 cm^2 and pieces of maize leaves of 12 cm2 were individually sprayed using an airbrush sprayer connected to a compressor vacuum pump (Fanem-Diapump; 1 kgf cm-1), using 0.2 mL of the solution/suspension, applying about 0.1 mL per witch leaflets/leave surface. Control leaflets/leaves pieces were sprayed only with distilled water. After one hour, 2nd instars *S. eridania* and *S. frugiperda* larvae were placed in nine *cm* polyethylene crystal plates containing one leaflet of tomato (*S. eridania*) or one piece of maize leaf (*S. frugiperda*). After 48 h, the leaflets/ leaf pieces were replaced with untreated ones. The experiment was kept under controlled conditions (25 ± 1 °C, 12 h photoperiod and 70% RH) and mortality was evaluated on the 3rd and 6th day after the replacement of the leaflets/leaf pieces. A completely randomized experimental design with eight replications with 10 *S. eridania* larvae/each and 30 replications with one *S. frugiperda* larva/ each, was used.

Data were subjected to chi-square test and analyzed as proposed by Koppenhofer et al. (2000). This method evaluates the effects of the interactions among the agents, where the sum of the mortalities caused by the agents must be less than 116% so that it is possible to observe non-additive synergistic or antagonistic effects. If the sum is equal to or greater than 116%, the calculated χ2 values will always be less than those tabulated, characterizing additive interaction. Considering the equation χ2 = (MPN − ME)2/ME, in which MPN is the mortality in the treatment, with the agents being applied in combination; and ME is the expected mortality, indicated as ME = MP + MN × (1 – MF/100), in which MP is the mortality caused by the powder, and MN is the mortality caused by the neem oil alone. For significant χ2 values (3.84; df = 1; $p < 0.05$), the effect is considered to be non-additive synergistic when MPN – ME > 0, or non-additive antagonistic when MPN – ME < 0. If χ2 is not significant, the effect is considered to be additive.

Statistical analyses

The data were submitted to normality and homoscedasticity analyses. As they did not meet the requirements for parametric analysis, non-parametric tests were performed using the program BioEstat 5.0 (AYRES et al., 2007).

Results and Discussion

Mortality by inert powders

The highest mortality of *S. frugiperda* and *S. eridania* were observed in the treatment bentonite, with 100% total mortality for *S. eridania* at concentrations of 1.5 g and 2.0 g, and 93.3% total mortality for *S. frugiperda* at a concentration of 2.0 g (Table 1). However, for *S. frugiperda* no differences in the mortality, between the tree concentrations, were observed (Table 1). The insecticide activity of bentonite is not reported in the literature. So, high mortality may be related to the presence sulfur in its composition. Sulfur is cited to have acaricidal/ insecticidal activity as observed for *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) where sulfur applied on wheat grains negatively affected the insect population (GONÇALVES et al., 2007). Similar results were found for *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) in field and laboratory studies that indicated that sulfur is harmful to adults and immature of *Trichogramma carverae* and *Trichogramma funiculatum* increasing mortality (THOMSON et al., 2000). These results were also observed for *Podisus* sp. (Hemiptera: Pentatomidae) (TORRES et al., 2002).

Diatomaceous earth in the concentrations of 1.5 and 2.0%, promoted mortality of 65 and 76.2 for *S. eridania*, respectively and 46.6% in the two concentrations, for *S. frugiperda*, results that are different from the control. A relationship between increased in DE concentration and insect mortality was observed for *Plodia interpunctella* (Lepidoptera: Pyralidae) (MARSARO JUNIOR et al., 2008). According to Marsaro Junior et al. (2006) and Mewis and Ulrichs (2001), DE in low dosages require a longer exposure period of the insects to cause higher mortality. DE promote desiccation and abrasiveness by adsorption to the insect cuticle breaking the wax layer of the epicuticle causing water losses. The damage is more severe when height dosages are used than with lower ones, resulting in a fast insect death (ATHANASSIOU et al., 2005).

Mortality in the treatment with kaolin did not differ from the control in all concentrations for *S. eridania* and at the two smaller concentrations to *S. frugiperda* larvae.

Table 1. Total and corrected mortality (%) for *Spodoptera eridania* (n = 80) and *S. frugiperda* (n = 30) 6 days after feeding on tomato plant leaflets and maize leaves treated with inert powders applied in solid form at different quantities.

Treatments	*Spodoptera eridania*		*Spodoptera frugiperda*	
	[a]TM (%)	[b]CM (%)	TM (%)	CM (%)
DE 1.0	23.7 bc	17.5	23.3 cde	14.7
DE 1.5	65.0 ab	62.1	46.6 bcd	40.6
DE 2.0	76.2 ab	74.3	46.6 bcd	40.6
Kaolin 1.0	7.5 c	0.0	0.0 f	0.0
Kaolin 1.5	25.0 bc	18.9	16.6 def	7.3
Kaolin 2.0	41.2 abc	36.4	26.6 cde	18.4
Bentonite 1.0	32.5 bc	27.3	60.0 abc	55.5
Bentonite 1.5	100.0 a	100.0	70.0 ab	66.6
Bentonite 2.0	100.0 a	100.0	93.3 a	92.5
Control	7.5 c		10.0 ef	

[a]Mean values followed by the same letter do not differ among themselves according to the Chi-square test results for various proportions, $p < 0.01$; TM Total mortality; [b]CM Mortality corrected by using Schneider and Orelli formula; DE diatomaceous earth.

Development of larvae of S. frugiperda and S. eridania fed on plants treated with inert powders

The larval development period of *S. frugiperda* was not affected by feeding on leaves treated with inert powders, but kaolin and DE did affect the larval development of *S. eridania* (Table 2). In spite of the low doses used, larvae of *S. eridania* fed on bentonite-treated leaflets died after 4th day after treatment, limiting the evaluation of the larval feeding activity.

Pupal weight attained by *S. frugiperda* was not affect by leaf treatment. Larvae of *S. eridania* fed on DE treated leaflets yielded lighter pupae, while the kaolin treat leaflets produced heavier pupae (Table 2). Santos and Boiça Júnior (2001) discuss, in their studies, that the lower weight of the pupae observed on a given food source is probably related to the lack of preference of the insect for the food or to the ingestion of substances that can harm its development.

The inert powders not only affected the larval developmental period and weight of the pupae, but also led to different mortality rates, with the strongest effect observed for the larvae of *S. eridania* fed on plants treated with bentonite and DE (Table 2). The treatment of leaves with DE caused significantly higher mortality in *S. frugiperda* than bentonite, but the effects of bentonite and kaolin were not significantly different (Table 2).

Table 2. Development of *Spodoptera eridania* (n = 80) and *Spodoptera frugiperda* (n = 30) 2nd instars larvae fed with plants treated with inert powders (0.25 g), applied as dust, under laboratory conditions.

	Treatment	Larval development period (days) [a]	Mortality (%)[a]	Pupae weight (g)[a]	Sex ratio[a]	
					Females	Males
Spodoptera eridania	Control	10.8 a	19.0 d	0.19 ab	64.2 a	35.8 b
	Kaolin	11.2 b	27.0 c	0.21 a	26.0 b	73.9 a
	Bentonite	ND*	100.0 a	ND	ND	ND
	DE	12.6 c	85.0 ab	0.17 b	50.0 ab	50.0 ab
	p-value[b]	<0.01 [KW]	<0.01 [CH]	<0.01 [KW]	<0.01 [CH]	<0.01 [CH]
Spodoptera frugiperda	Control	10.4	16.6 c	0.14	54.1 a	45.8 b
	Kaolin	10.5	56.5 ab	0.14	15.3 b	84.6 a
	Bentonite	9.5	76.6 a	0.12	28.5 ab	71.4 ab
	DE	10.6	36.6 bc	0.14	57.8 a	42.1 b
	p-value[b]	0.64 [KW]	<0.01 [CH]	0.18 [CH]	<0.05 [CH]	<0.05 [CH]

[a]Values followed by the same letters in the column do not differ among themselves; [b]p-value with "KW" indicates significance in the Kruskal Wallis test and with the letters "CH" indicate significance in the Chi-square test; *(ND) it was not possible to evaluate due to the mortality of the caterpillars (100%); DE diatomaceous earth.

Mortality of S. frugiperda and S. eridania fed on plants treated with bentonite

High mortality was observed for the two *Spodoptera* species at the sixth day of evaluation (Table 3). As we could not detect any signs of feeding on the treated surfaces, we believe that the mortality observed was due to starvation or the action of components available in the powder.

Bentonite is used as fertilizer and have been cited in the literature as a promising product in controlling plant pathogens BRANCAGLIONE et al., 2009). However, we are not aware of any information on the activity of bentonite as an insecticide.

Table 3. Mortality (%) of *Spodoptera frugiperda* (n = 30) and *Spodoptera eridania* (n = 80) 2nd instars larvae, fed on plants treated with bentonite in the greenhouse.

Treatments	Mortality (%)[a]			
	3 days		6 days	
	S. frugiperda	*S. eridania*	*S. frugiperda*	*S. eridania*
Bentonite	40.0 a	8.8 a	86.7 a	83.3 a
Control	6.7 b	0.0 b	6.7 b	2.5 b
p-value [2]	<0.01 [CH]	<0.01 [MW]	<0.01[CH]	<0.01[MW]

[a]Mean values (± standard error) followed by the same letter in the column do not differ among themselves; [2]p-values with "CH" indicate significance in the Chi-square test and "MW" indicate significance in the Mann Whitney test.

Inert powders suspended in water and combined with neem oil

S. frugiperda larval mortality was observed in all treatments. The associated use of powders and neem oil increased the mortality rate of *S. frugiperda*, but not of *S. eridania* (Table 4). Without neem oil, bentonite caused less mortality, but even at the lowest concentration, it was superior to the control. Moreover, for *S. frugiperda*, no difference was observed between kaolin and the control group in the three concentrations. However, when neem was associated with kaolin at 10%, mortality was 80% for *S. frugiperda*. Similar results were observed where the combination of kaolin and neem oil led to 100% mortality of *Zabrotes subfasciatus* (Bohemann) (Coleoptera: Chrysomelidae: Bruchinae) (MIKAMI et al., 2010).

For *S. frugiperda*, mortality by DE was greater than that in the control at all concentrations; however, there was no mortality difference between DE concentrations used. Nevertheless, when this powder was used in combination with neem no significant increases in mortality were observed.

The highest mortality rate from the treatments using DE was for DE and neem at 10% that promote 93.75% mortality. This association was cited to be efficient in controlling *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) when applied on artichokes, and proved to be safe for predators such as Coccinellids, Chrysopids, and Anthocoridae important biological control agents in integrated pest management programs (EL-WAKEIL; SALEH, 2007)

The non-additive synergistic effects observed in this study (Table 4) and the mortality levels caused by bentonite and by kaolin at high concentrations, combined with neem show that these agents have potential for controlling the two *Spodoptera* spp. The combinations of neem oil and DE at 5% and 7.5% were considered non-additive antagonistic for *S. frugiperda*. This effect may be related to the action of azadirachtin on the chemoreceptors of the insects, repelling them and suppressing feeding (BANKEN; STARK, 1997) which would impede the action of the DE.

The contact of DE with the insect is necessary to cause a damage in the cuticle through adsorption to the epicuticle wax and through abrasion, making them permeable to water and promoting death through desiccation (KORUNIC, 1998). However, 10% DE and neem had an additive effect for *S. frugiperda*, which may be related to the small increase in mortality compared to lower concentrations of DE combined with neem, which, although not statistically significant, was sufficient to prevent antagonistic effects.

For *S. eridania* larvae, all the combinations of powders with neem oil were classified as non-additive synergistic (Table 4), indicating the potential for using them jointly for management of this pest.

These results suggest that the use of inert powders in solid form or suspended in water with neem oil may provide protection to maize and tomato plants, negatively affecting larvae of *S. eridania* and *S. furgiperda*. However, more study's in the field are need to confirm the results and define strategy's and the possible interactions of this management tools with other control methods for these species.

Table 4. Mortality (%) of *Spodoptera frugiperda* (n = 30) and *Spodoptera eridania* (n = 80) 2nd instars larvae, six days after feeding on plants treated with inert powders suspended in water and combined with neem oil, and the interaction between these products.

	S. frugiperda		*S. eridania*	
Treatments	Total Mortality[a]	Interaction[b]	Total Mortaliy[c]	Interaction[b]
DE 5,0	43.3 cde	-	8.7 df	-
DE 7,5	53.0 bcd	-	41.2 bc	-
DE 10	53.3 bcd	-	40.0 bc	-
Kaolin 5,0	10.0 ef	-	0.0 f	-
Kaolin 7,5	10.0 ef	-	15.0 df	-
Kaolin 10	26.6 def	-	12.5 df	-
Bentonite 5,0	30.0 de	-	0.0 f	-
Bentonite 7,5	50.0 bcd	-	21.2 bcd	-
Bentonite 10	53.3 bcd	-	18.7 bcd	-
Neem	46.6 cde	-	35.0 bc	-
DE 5,0 + Neem	33.3 de	Non-additive synergistic	63.0 abc	Non-additive synergistic
DE 7,5 + Neem	50.0 bcde	Non-additive synergistic	77.5 ab	Non-additive synergistic
DE 10 + Neem	66.6 bcd	Additive	93.7 a	Non-additive synergistic
Kaolin 5,0 + Neem	36.6 cdef	Non-additive synergistic	72.5 abc	Non-additive synergistic
Kaolin 7,5 + Neem	66.6 bcd	Additive	97.5 a	Non-additive synergistic
Kaolin 10 + Neem	80.0 abc	Non-additive synergistic	96.2 a	Non-additive synergistic
Bentonite 5,0 + Neem	43.3 cde	Non-additive synergistic	100.0 a	Non-additive synergistic
Bentonite 7,5 + Neem	86.6 ab	Additive	100.0 a	Non-additive synergistic
Bentonite 10 + Neem	100.0 a	Non-additive synergistic	100.0 a	Non-additive synergistic
Control	6.6 f		13.7 df	

[a]Mean values followed by the same small letter in the column do not differ among themselves by the Chi-square test for various proportions, $p < 0.01$; [b]Chi-square values calculated; Tabulated $\chi^2 = 3.84$ for 1 degree of freedom, $p < 0.05$; DE diatomaceous earth.
[c]Mean values followed by the same small letter in the column do not differ among themselves by the Kruskal-Wallis.

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