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PLANT DERIVED POWDERS AS ALTERNATIVES FOR *SITOPHILUS ORYZAE* L. (COLEOPTERA: CURCULIONIDAE) CONTROL IN STORED MAIZE GRAINS

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ABSTRACT

Maize is the major staple food in Africa. In developing countries, post-harvest losses due to insect pests are up to 40% of total production. Inert powders can be alternative tools in integrated pest management programs of stored cereals. The aim of this study was to assess the efficacy of ashes from cashew (*Anacardium occidentale*) branches, charcoal powder, dried and milled *Eucalyptus citriodora* leaves, and diatomaceous earth (standard check), for *Sitophilus oryzae* control on stored maize grains. The experimental design was completely randomized with 11 treatments and 4 replications. Insect introduction was carried out immediately after treatment application and at 30 and 60 days after. The mortality assessment was performed by counting the dead insects at 10 and 20 days after each infestation. The emergence of *S. oryzae* adults was also assessed at three different times. The germination of treated grains was also evaluated. Data analysis was performed on the SISVAR statistical package. Diatomaceous earth showed maximum control efficiency (73.75%) at 10-day evaluation, reaching total control at a 20-day evaluation. The remaining treatment control efficiency was below 50% in all concentrations and evaluations. The lowest insect emergence rates were observed in grains treated with diatomaceous earth or charcoal powder, which were more efficient than cashew ashes, *Eucalyptus citriodora,* and untreated control. There was no significant effect of the tested products on seed germination so, they can be used in smallholder farmers' grain storage. The results allow us to affirm that *Eucalyptus citriodora* charcoal powder, and cashew ashes can be used to reduce *Sitophilus oryzae* damage in traditional agricultural systems. Considering the reduction in the *Sitophilus oryzae* progeny, the potential use of charcoal powder should be considered, given the efficiency compared to that of the diatomaceous earth. Thus, the plant-derived products tested in this study has the potential to be used in the integrated management of *Sitophilus oryzae* in stored products.

Key words: Ash, Charcoal, Diatomaceous earth, inert, dust, maize, storage, pest

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INTRODUCTION

The weevils *Sitophilus oryzae* (L.) and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), are considered the most important and cosmopolitan pests of stored grains, especially maize (*Zea mays* L.) in tropical and subtropical regions [1, 2]. This distribution is due to characteristics such as high biotic potential, cross-infestation, and ability to survive at great depths in the grain mass [2].

The main damage caused by *S. oryzae* is a reduction in grains mass, germination, and in the nutritional value, resulting in lower value and profitability [3]. The control methods mostly used include the correct management of storage facilities and the application of chemical insecticides [4, 5]. However, incorrect use of pesticides contributes to environmental, food, and human contamination. Chemical control also increases the possibility of the selection of resistant pest populations and the reduction of natural enemies [6, 7]. There is a need for alternative tools for pest control in stored products, mainly by small farmers who need to preserve their grain and seeds on the property and do not have access to chemicals because these products are costly or cannot access them. This is particularly more evident in remote areas in which farmers have no access to technology. Also, in these areas, the structures for grain storage are inappropriate [8, 9]. Therefore, pest control practices that are environmentally and economically viable for small-scale farmers become increasingly necessary [10]. Considering the available alternatives to control pests of stored grains, inert dust [11, 12] and plant extracts [13] are among the most promising. The potential control of these products is reflected in mortality, repellency, and offspring reducing, without affecting seed germination [11, 12, 13].

Inert dust can promote direct effect on the insect, causing abrasion in their cuticle, and indirect ones, when the silica present in their composition, adsorbs the cuticle lipids, causing death by dehydration [14, 15]. Apart from mortality caused by the adhesion of the powder to the insect body, mating capacity and mobility are impaired, causing a reduction in oviposition and the number of offspring [16, 17].

Considering the need for methods to control *S. oryzae* that are economically viable, and that are safe to the environment and the farmer, this study aimed to evaluate the effectiveness of inert powders for *S. oryzae* control in maize stored grains.

MATERIALS AND METHODS

The insects used in the bioassay were obtained from stock cultures reared from Embrapa Soybean, Brazil, and multiplied in the Entomology Laboratory of the State University of Londrina, Brazil. For insect rearing, plastic containers (500 mL) were used and set up as follows: In each container, 200 grams of wheat grains were added and 250 unsexed *S. oryzae* adults were released. The containers were kept in a climate chamber (25 ± 2 ° C and 12h light) for 15 days; thereafter all insects were removed from the containers and the grains were kept for a further 45 days under the same controlled conditions. Emerging, adults were used in the experiments. Maize grains of IAPAR 51 variety (Agronomic Institute of Parana – IAPAR, Brazil), produced without pesticides, were

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used as the substrate for treatments. Intact and undamaged maize grains were manually selected and kept in a freezer (-18°C) for a week in order to eliminate possible insect infestations. The grain moisture content was measured on a humidity meter (GEHAKA AGRI G600) and dried to 12% humidity at 60°C. Batches of 1 kg dried maize grains were treated individually with the treatments and doses listed in table 1. The cashew ashes were produced by burning the cashew tree. The leaves of *Eucalyptus Citriodora* were dried for 2 hours on air circulation at 60ºC to reduce water content, and ground with a mill (Marconi MR 340), which was also used for grinding *Eucalyptus Citriodora* charcoal. The grains were mixed with treatments by manual shaking for two minutes, in transparent plastic bags.

From each batch, 600g of treated maize was divided into 50g portions and distributed in 12 containers with a volume of 250 ml and were grouped into three sets of four containers. Maize in the first set of containers was treated (day 0) and the second and third set, at 30 and 60 days after treatment (DAT), respectively. Because of the small insect size and to prevent damage and stress, sex determination was not done. Each container was infested with 20 unsexed adults and sealed with tulle fabric, secured with latex elastic and kept in a climatic chamber (25±2°C and 12h light), until the evaluation. The experimental design was completely randomized with 11 treatments and four replications. The treatment distribution can be seen in table 1.

Mortality was assessed by counting dead insects at ten and 20 days after each infestation. In the second evaluation at 20 days after infestation, live insects were also removed and the grains remained at the same conditions for an additional 40 days, after that the score on emerging adults was performed. This evaluation was performed for the three dates of infestation 0, 30, and 60 days after treatment. The data were analyzed by means using the Abbott formula (1925) (equation 1) [18], to correct the mortality and, for adult emergence, the Henderson and Tilton formula (equation 2) [19]. Data were analyzed using the Variance analysis system (SISVAR) statistical package [20] and corrected by the square root of x+0.5 and subjected to analysis of variance and comparison of means by Tukey test at 5% significance.

$$\text{Corrected (\%)} = [1 - (PTa \div PCa)]*100$$

Equation 1: Abbott´s formula

$$\text{Corrected (\%)} = [1 - (PCb*PTa) \div (PCa*PTb]*100$$

Equation 2: Henderson and Tilton formula

The abbreviations used refers to insect population: before grain treatment (PTb), after grain treatment (PTa), in control before treatment application (PCb) and after treatment application (PCa).

The seed germination test was carried out at 90 days after treatment. Treatments consisted of seeds treated with 5% charcoal powder, 5% cashew ashes, 8% *Eucalyptus Citriodora*, 0.1% diatomaceous earth, and untreated control. Twenty seeds of each treatment were arranged in boxes of polystyrene crystal (11 x 11 x 3,5 cm) (Gearbox) with moistened paper in the germinator (De Leo) and evaluated after 8 days, at room temperature.

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Diatomaceous earth was chemically analyzed and quantified at the laboratory of Applied Nuclear Physics of the State University of Londrina, using the X-Ray Fluorescence Spectrometer, EDX-720 model from Shimadzu Co.

RESULTS AND DISCUSSION

Mortality

The most effective treatment to control adults of *S. oryzae*, at 10 days after infestation was diatomaceous earth in infestation performed at 0 and 30 days after treatment with 73.75% and 69.23% of mortality, differing significantly (P<0.05) from all other treatments. However, a significant reduction in mortality was observed in the grains treated with Diatomaceous earth, 60 Days After Treatment that produced 26.25% of mortality at 10 days after infestation but, did not differ significantly (P<0.05) from cashew ashes (5%) which caused 23.75% mortality. There were no significant differences among the other treatments in all concentrations assessed and each did not differ from the control (Table 2).

Considering the assessment of mortality at 20 days after infestation, the maximum efficiency (100%) was observed on maize treated with diatomaceous earth, in infestations at 0 and 30 days after treatment. Diatomaceous earth treatment caused 96.15% of insect mortality at 60 days after treatment, significantly superior to all other treatments (P<0.05).

No significant insect control was observed for cashew ashes (5%) for the three evaluated periods. Within charcoal powder treatment, no statistical difference among concentrations and times of infestation were observed. However, for *Eucalyptus Citriodora* treatments, at 8 and 4%, there were differences within the three evaluation periods, as an increase in mortality which was observed at time 0 when compared with 30 and 60 days after treatment.

For *Eucalyptus Citriodora* 30 days after treatment infestation, the efficacy did not differ from the control. For infestation at 60 days after treatment, the treatments diatomaceous earth, cashew ashes (5 and 2%), and charcoal powders (5%) were significantly greater than control (Table 2).

The best results for *S. oryzae* control were obtained in maize treated with diatomaceous earth. Adult mortality (73,75%) was observed as early as 10 days after infestation using diatomaceous earth and reaching 100% mortality after a longer contact time between the pest and the product (20 days). The efficacy of diatomaceous earth has been cited in several studies [11]. Results showed 100% mortality of *Plodia interpunctella* (Lepidoptera: Pyralidae) three days after exposure to 0.1% of diatomaceous earth [21]. The same percentage in pest mortality was observed nine days after contact in maize grains treated with 0.15% of diatomaceous earth in the control of *S. zeamais* [22].

Diatomaceous earth has long residual insecticidal action and generally does not lose its effect during the storage period [11]. The highest efficacy of diatomaceous earth can be

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related to its high concentration in silicon (Table 4). The silicon adsorbs the epicuticle lipids of the insect cuticle, promoting a greater loss of water, which is crucial for the survival of stored grain pests whose habitat is extremely dry [14, 15].

Adults emergence

A lower emergence of *S. oryzae* adults was found in maize grains treated with diatomaceous earth, which did not differ significantly from charcoal powder at the three concentrations at 60 and 90 days after treatment (Table 3). However, at 120 days after treatment evaluation, the treatments with the lowest concentration of charcoal powder (1%), resulted in a significantly higher ($P<0.05$) number of emerging adults in comparison to diatomaceous earth treatment (Table 3). Insect emergence from grains treated with highest concentrations of cashew ashes (5 and 2%) at 60, 90, and 120 days after treatments, did not differ from grains treated with charcoal powder (1%). Regarding adult insect emergence counts still, assessments at 90 days after treatment showed that treatment with cashew ashes (5 and 2%) did not differ from treatment with diatomaceous earth and charcoal powder (2 and 1%). Further, cashew ashes treated grains (5, 2 and 1%) at 120 days after treatment, did not differ from grains treated with charcoal powder (5, 2 and 1%), and, in its highest concentration, and grains treated with cashew ashes (5%), did not differ from diatomaceous earth treated grains. Treatment with *Eucalyptus citriodora* allowed the largest insect emergence for the study periods, differing from diatomaceous earth and charcoal powder in all concentrations, and cashew ashes (5 and 2%) (Table 3).

Considering days after treatment for each treatment, a lower emergence of insects was observed on the diatomaceous earth treated grains evaluated early (60 days), which differed from the emergence observed for grains evaluated at 90 and 120 days after treatment. For charcoal powders, there was no difference for the emergence of insects at 60 and 90 days after treatment, as well as for *Eucalyptus Citriodora*, where only the grain assessed at 120 days showed a higher percentage of adults emergence. There was no difference between the three treatment periods, at the highest and lowest concentrations for grains treated with cashew ashes, except between 60 and 120 days, at the concentration of 2% with higher emergence in grains that had been evaluated later (Table 3).

Within the three evaluation dates, all treatments had the lowest adult emergence when compared with the control.

Low mortality has been found using charcoal powder even at the highest concentration (5%) at different infestation times. However, the adults' emergence from treated grains was not different from the emergence observed in diatomaceous earth treated grains. This result is significant and the hypotheses for that decrease in emergence are probably because insects in contact with grains treated with charcoal powders were unable to mate or to lay eggs, reducing population growth and consequently the damage. In this study, the decrease in insect emergence was observed until the final evaluation period at 120 days. Based on these results, the use of charcoal powder in a concentration greater than 5% is recommended for *S. oryzae* populations control mainly in regions where synthetic insecticides are not available, such as in poor and subsistence agriculture.

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Seed germination

The germination of seeds treated and preserved for 90 days was not affected. Even without a statistical difference ($P<0.05$), the seed germination ranged from *Eucalyptus Citriodora* (78.5%) to control (90%).

Several studies show that in general, inert dusts, namely diatomaceous earth, rice husk ash, wood ash, fly ash, cow dung do not interfere in seed germination [23, 24, 25].

Considering the assessed parameters specifically, mortality, adult emergence, and seed germination, besides charcoal powder, there existed no useful contribution from the different treatments into adult mortality and to adult emergence reduction. These treatments performed significantly less than diatomaceous earth and charcoal powder, and better than control in adults' emergence inhibition.

Based on these results, the treatment of maize grains with charcoal powders is effective in *S. oryzae* adult emergence reduction and remained persistent at least for the period of evaluation (80 days). Also, the grain treatment with charcoal powder did not interfere with seed germination, which makes this product useful for protecting stored grains from damage by pests on small farms, especially in the poorest and family farming agriculture with limited.

The dose of cashew ashes and *Eucalyptus citriodora* treatments, studied in this research, showed little acceptable results. Since they are environmentally and economically sustainable products, one must find an efficient and effective way of using them in the integrated management of stored grain pests.

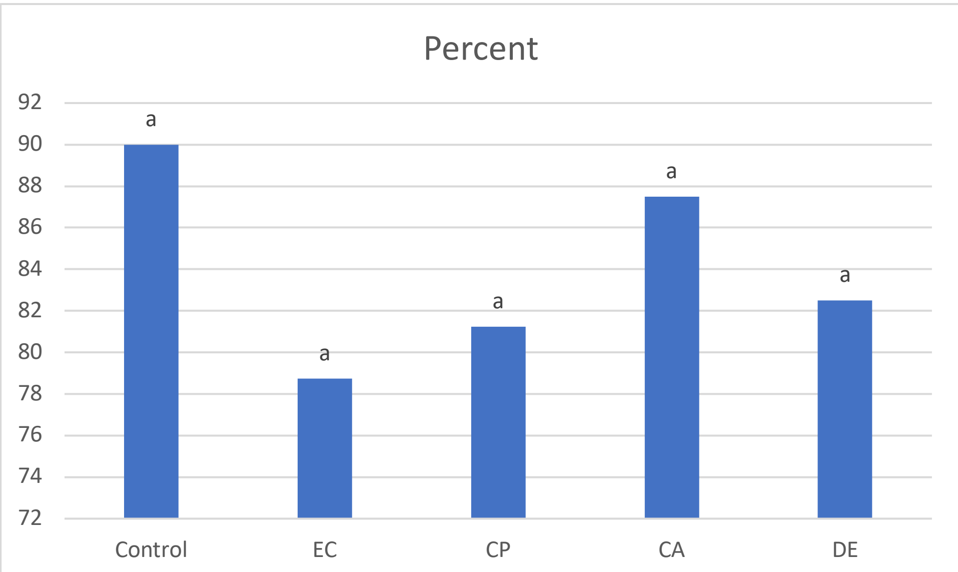

Figure 1: percentage of seed germination of maize treated with *Eucalyptus citriodora* (EC), charcoal powder (CV), diatomaceous earth (DE), cashew ashes (CA) and control

CONCLUSION

The results of this study show that plant derived material has the potential to be used as stored grain protectants. Despite the lower performance than diatomaceous earth commercial product, the charcoal dust remained effective in *Sitophilus oryzae* progeny reduction. Consequently, resource poor family farming regions, the product can be applied in stored grain pests' integrated management. Although, the performance of cashew ashes and *Eucalyptus citriodora* leaves remained low, the application of these environmental and economically sustainable products must be considered, looking for alternative ways of practical and efficient utility. In general, the tested treatments had no negative effect on seed germination, an important feature in stored grain pests' integrated management.

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Table 1: Dose (%) plant-derived powders tested against *Sitophilus oryzae* in stored maize

Treatment	Dose (%)
Cashew ashes (*Anacardium occidentale*)	1, 2 and 5
Charcoal powder (*Eucalyptus* sp) (Cacique®)	1, 2 and 5
Eucalyptus citriodora leaves	2, 4 and 8
Diatomaceous earth (Keepdry®)	0.1
Untreated control	0

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Table 2: Percentage mortality of *S. oryzae* adults at 10 and 20 days after each infestation (0, 30, and 60 days after treatment)

Treatments*	Concentration (%)	Percentage mortality 10 days after infestation		
		(0)[a] 10[b]	(30)[a] 40[b]	(60)[a] 70[b]
DE	0.1	73.75 ± 0.63 Aa	69.23±0.91 Aa	26.25±0.85 Ba
CA	5	10.00 ± 0.71 Bb	7.69 ±0.41 Bb	23.75±1.18 Aa
	2	8.75±0.48 Ab	10.26 ±0.65 Ab	8.75±0.48 Ab
	1	11.25±0.75 Ab	2.56 ±0.58 Ab	3.75±0.48 Ab
CP	5	10.00±0.41 Ab	2.56 ±0.41 Ab	7.50±0.65 Ab
	2	5.00±0.41 Ab	1.28 ±0.48 Ab	3.75±0.48 Ab
	1	2.50±0.29 Ab	0.00 ±0.25 Ab	2.50±0.29 Ab
EC	8	7.50±0.29 Ab	3.84 ±0.95 Ab	7.50±0.65 Ab
	4	6.25±0.48 Ab	2.56 ±0.71 Ab	3.75±0.25 Ab
	2	2.50±0.29 Ab	1.28 ±0.48 Ab	5.00±0.41 Ab
Control	0	0.00±0.00 Ab	0.00 ±0.29 Ab	0.00±0.00 Ab
CV (%)			26.75	
		Percentage mortality 20 days after infestation		
		(0)[a] 20[b]	(30)[a] 50[b]	(60)[a] 80[b]
DE	0.1	100.00±0.00 Aa	100.00±0.00 Aa	96.15±0.25 Aa
CA	5	19.23±0.48 Abcd	26.92±0.48 Ab	30.77±0.87 Ab
	2	44.87±3.59 Ab	32.05±0.48 ABb	21.79±1.25 Bbc
	1	21.79±0.75 ABbcd	30.77±0.65 Ab	11.54±0.48 Bcd
CP	5	25.64±0.50 Abc	26.92±0.48 Ab	21.79±0.85 Abc
	2	11.54±0.48 Acde	12.82±0.41 Abc	11.54±0.85 Acd
	1	12.82±0.41 Acd	7.69±0.41 Acd	7.69±0.41 Acd
EC	8	29.49±0.85 Abc	7.69±0.82 Bcd	10.26±0.29 Bcd
	4	25.64±0.29 Abc	3.85±0.63 Bcd	6.41±0.48 Bcd
	2	7.69±0.41 Ade	3.85±0.75 Acd	6.41±0.48 Acd
Control	0	0.00±0.29 Ae	0.00±0.29 Ad	0.00±0.29 Ad
CV (%)		19.99		

Means followed by the same capital letter in the row and lowercase letters in the columns do not differ statically between each other by Tukey 5%. Statistical analysis performed separately for assessments at 10 and 20 days after infestation

*DE – Diatomaceous earth, CA – Cashew branches ashes, CP – Charcoal powder, EC – *Eucalyptus citriodora* leaves dried and milled, CV – Coefficient of variation.

[a] Days after treatment – infestation, [b] Days after treatment – mortality evaluation

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Table 3: Percentage of *S. oryzae* adult's emergence at 60, 90 and 120 days after grains treatment

		Days after grains treatment					
Treatments	Concentration (%)	60		90		120	
DE*	0.1	10.58±1.31	Aa	23.97±1.71	Babc	20.71±1.04	Ba
CP	5	11.31±2.02	Aa	14.88±1.08	Aa	22.50±1.11	Bab
	2	10.58±0.85	Aa	17.77±1.18	Aab	26.79±1.38	Babc
	1	18.61±1.03	Aab	23.97±0.87	Aabc	33.57±2.10	Bbc
CA	5	21.53±1.80	Ab	28.93±1.84	Abcd	26.43±1.55	Aabc
	2	25.18±1.03	Abc	33.06±2.61	Abcd	34.25±1.78	Bbc
	1	34.31±2.22	Acd	38.02±1.47	Ade	39.29±3.59	Acd
EC	8	40.88±2.12	Ad	51.24±1.58	Aef	57.14±1.96	Be
	4	42.70±1.89	Ad	53.72±1.71	Abef	51.07±4.21	Bd
	2	45.26±1.96	Ad	61.16±1.87	Abf	56.07±1.38	Be
Control		100.00±1.79	Ae	100.00±1.76	Ag	100.00±3.03	Af
CV (%)				8.14			

Means followed by the same capital letter in the row and lowercase letters in the columns do not differ statically between each other by Tukey 5%
*DE – Diatomaceous earth, CA – Cashew branches ashes, CP – Charcoal powder, EC – *Eucalyptus citriodora* leaves dried and milled, CV – Coefficient of variation

Table 4: Chemistry composition of diatomaceous earth (Keepdry®)

Components	Diatomaceous earth (%)
SiO_2	88.893
Al_2O_3	9.045
Fe_2O_3	0.811
CaO	0.298

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