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Research Paper

Screening of Some Botanical Insecticides against Maize Weevil, *Sitophilus Zeamais* Motschlsky (Coleoptera: Curculionidae), on Maize

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Abstract

The effects of three, four, and five grams powders of seeds of *Azadrichta indica*, *Millettia ferruginea* and *Jatropha curcas*, leaves of *Croton macrostachyus* and *Euphorbia schimperiana* were evaluated separately on 26 unsexed maize weevils treated with 100 g of the botanicals in each 250 cm^3 glass jar arranged in RCD with three replications. A standard insecticide, Pirimiphos-methyl 2% dust and untreated check were included for comparison. Weevil mortality was recorded at 3, 7, 14, 21 and 28 days after treatment. During the last counting, both dead and alive weevils were counted and removed and the grains were kept under the same conditions for the emergence of F_1 generation. The numbers of emerging F_1 were recorded every other day for 33 days. Seed germination was tested using 15 randomly picked seeds from undamaged grains from each jar. All the treatments showed significant difference ($P<0.05$) mortality from the control seven days after treatment. The cumulative mortality of all the treatments was very high (97.43–100%) while that of the control was 11.53, 28 days after treatment. The cumulative number of emerged F_1 from the untreated control after 28 days was higher and significantly different from all the other treatments. Seed germination test revealed over 77% seed viability with no significant difference among all the treatments. The study revealed that the botanicals caused high weevil mortality, very low fecundity and insignificant effect on seed viability. Evaluation of the botanical powders against naturally infesting maize weevils should be conducted under farmers' storage conditions.

1. Introduction

Maize (*Zea mays* L.) is a cereal grass related to wheat, rice, oat and barley; ranking second after wheat and is followed by third-ranking rice in order of world grain production. This plant is regarded as versatile with many uses and is cultivated in a wider range of environments than wheat and rice and any other crop because of its greater adaptability (Asiedue, 1989; Parugrug and Roxas, 2008; Onuh et al., 2008). Maize is high yielding, easy to process, readily digested, and cheaper than other cereal (Onuh et al., 2008).

Maize is a very important cereal grain in Africa where it is widely cultivated and consumed. It serves as a source of dietary carbohydrate for humans (Asawalam et al., 2007). It is cultivated in Ethiopia in diverse ecological conditions and constitutes about 17% of the total cultivated area and 24% of the grain produced in the country (CSA, 1996). In Ethiopia, maize is one of the major cereal crops grown for its food and feed values. It is one of the most important staple foods and cash crops providing calories for the consumers and

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income for the traders (Waktole Sori and Amsalu Ayana, 2012).

Although production of corn has increased to meet the global demand, several biotic and abiotic factors play an important role in limiting the productivity. Among biotic factors contributing for storage losses, insect pests play a major role inflicting 20–30% damage of corn grain in tropical regions (Haque et al., 2000) due to favorable conditions for their development and poor storage conditions (Abraham Tadesse, 1997). Insects are most often considered as the principal cause of maize grain losses. The most important insect pests that cause damage to maize in the field and storage are lepidopterous stalk borers and coleopterous weevils, respectively. More than 37 species of arthropod pests are associated with maize grain in storage (Waktole Sori and Amsalu ayana, 2012).

Initial infestations of maize grain occur in the field just before harvest and the insects are carried into the store where the population builds up rapidly. The huge post-harvest losses and quality deterioration caused by this insect pest is a major obstacle to achieving food security in developing countries (Asawalam et al., 2007). Insect pests which commonly attack stored grains like maize weevil cause severe damage to the commodity resulting in losses in weight, seed viability, and nutritive quality of foodstuffs (Parugrug and Roxas, 2008). *Sitophilus zeamais* is one of the most destructive stored product pests of grains, cereals, and other processed and unprocessed stored products in sub-Saharan Africa. It causes qualitative and quantitative damage to stored products, with grain weight loss ranging between 20 to 90% for untreated stored maize, and the severity of damage depends on factors which include storage structures and physical and chemical properties of the produce. Heavy infestation of adults and larvae of maize weevil which cause postharvest losses have become increasingly important constraints to storage entomology and food security in the tropics (Ojo and Omoloye, 2016).

The maize weevil is a major insect pest of stored maize in Ethiopia. It causes substantial quantitative and qualitative loses manifested by seed perforation, and reduction in weight, market value and seed germination. Losses ranging from 20 to 30% are common in some localities in the country. Under farm-storage conditions in the Bako area, up to 100% damage to maize stored for 6-8 months have been reported (Girma Demisie et al., 2008). In an assessment of insect store pests of maize in Jimma Zone of Oromia Regional State, Waktole Sori and Amsalu Ayana (2012) recorded maize weevil as the dominant species in all areas surveyed followed by Angoumois grain moth (*Sitotroga cereallela),* rice weevil (*Sitophilus oryzae)* and flour beetle (*Tribolium confusum*) and the pests are widespread, abundant and cause damage and loss to maize grain among the different arthropods.

Besides storage insect pests, a number of microorganisms attack maize in the field as well as in the storage and of these fungi are among the principal causes of deterioration and loss of maize grain (Ominski et al., 1994). Insects play an important role in infection of maize by *Fusarium* spp. They can act as wounding agents or as vectors spreading the fungus from origin of inoculum to plants (Dowd, 1998). Borers and insects of the family Nitidulidae are most often cited as favoring maize infection by *Fusarium* species.

The common control methods for this pest are the use of chemical insecticides, biological control, and botanical insecticides among others (Ojo and Omoloye, 2016). However, synthetic pesticides have been used for many years to control agricultural pests including those that damage durable food crops in storage like *S. zeamais* (Girma Demisie et al., 2008; Tilahun Fromssa and Daniel Hagos, 2016). They are effective in controlling pests yet expensive, dangerous to human health and may create other problems in post-harvest industry (Parugrug and Roxas, 2008). Considerable problems may arise from the continued application of these insecticides which include high persistence, poor knowledge of application, pest resurgence, genetic resistance by the insect, lethal effects on non-target organisms in addition to direct toxicity to users, and increasing costs of application that subsistence farmers cannot afford (Asawalam *et al.*, 2007; Girma Demisie et al., 2008; Tilahun Firomsa and Daniel Hagos, 2016).

These problems associated with the use of synthetic insecticides have necessitated research on the use of alternative eco-friendly cheaper insect pest control methods amongst which are the use of powdered plant parts and their extracts (Asawalam et al., 2007). The current concerns of synthetic insecticides and the desire for residue-free grains by consumers demand for searching and developing alternative management options to integrate with other control measures such as varietal resistance and botanicals.

The use of botanical pesticides to protect plants from pests is very promising because of several distinct advantages. Pesticidal plants are generally much safer than conventionally used synthetic pesticides. Pesticidal plants have been in nature as its component for millions of years without any ill or adverse effect on the ecosystem. In addition, plant-based pesticides are renewable in nature and cheaper. Also, some plants have more than one chemical as an active principle responsible for their biological properties. These may be either for one particular biological effect or may have diverse ecological effects. The chances of developing quick resistance to different chemicals are highly unlikely (Parugrug and Roxas, 2008).

Plant-derived pesticides can be transferred into practical applications in natural crop protection, which can help the small-scale farmers (Parugrug and Roxas, 2008). The use of natural and easily biodegradable crop protection inputs like azadirachtin can be a useful component of an IPM strategy since the compound is known for its low toxicity against beneficial insects (Koona and Njoya, 2004).

Therefore, the current study was undertaken to evaluate the insecticidal effect of powders of seeds of *Azadrichta indica* and *Millettia ferruginea*, leaves of *Croton macrostachyus*, *Euphorbia schimperiana*, and *Jatropha curcas* as grain protectant against *S. zeamais* under laboratory condition.

2. Material and Methods

2.1. Description of the Study Area

This experiment was conducted at Melkassa Agricultural Research Center, entomology laboratory from February to May, 2018. The Center is located at the Central Rift Valley of Ethiopia at 8°24’N latitude and 39°21’E longitude and at an elevation of 1550 m above sea level and the mean relative humidity is 80%, and it receives annually 763 mm rain falls. The maximum and minimum annual mean temperatures are 28.4°C and 14°C respectively (MARC, 1996). According to the recent agro-ecological zones classification of Ethiopia (MoA, 2000), the Melkassa Hypo Calcic Regosol ecotope falls in the zone termed hot to warm semi-arid lowlands. Loam and clay loam soil textures are the dominant textural classes (MARC, 1995; Tsion et al., 2009).

2.2. Plant material collection and preparation

Five locally available plants including seed of *Azadrichta indica* A. Juss (collected from Dire Dawa city), seed of *Millettia ferruginea* (Hochst) Baker (collected from Addis Ababa city), leaf of *Croton macrostachyus* Hochst (from Adama district), leaf of *Euphorbia schimperiana* Scheele (from Adama district), and leaf of *Jatropha curcas* L. (collected from compound of Melkassa Agricultural Research Center) were evaluated to determine insecticidal effect as grain protectant against *Sitophilus zeamais*. These plant materials were air-dried under shade, pulverized separately into a fine powder using micro plant grinding machine and sieved through a 0.25 mm (250 μm) pore size mesh sieve to obtain uniform fine dust particles. Each plant material was kept in a plastic bag in a cool place until treatment.

2.3. Insect rearing

Parent adult maize weevils were obtained from Melkassa Agricultural Research Center. Following the methods used by Girma Demisie et al. (2008) and Bekele Jembere et al. (1995), maize weevils were cultured under laboratory conditions at 27±2°C, 60-65% R.H. and 12:12 h light:dark regimes on whole clean, undamaged and uninfested maize grain variety Melkassa 2 provided by the Research Center. The grains were washed from impurities using distilled water, dried and disinfested by keeping in a deep freezer at -20 ± 2°C for 96 h. After disinfestations, the seeds were cleaned and kept for two weeks at the experimental conditions for acclimatization and adjusted to moisture contents of 12 to 13% before use. Two kilograms of the disinfected seeds were placed in four-liter capacity plastic containers covered with perforated lids and replicated 10 times. About 800 unsexed adult maize weevils were introduced onto the grain in each plastic container and kept to oviposit for two weeks, after which they were removed and discarded. The grains were kept for progeny emergence to be used for the experiment.

2.4. Treatment application

Three different rates 3, 4 and 5 g, equivalent to 3, 4 and 5%, respectively, of each of the five botanicals were weighed separately and added to each 100 g of clean maize put in each 250 cm^3 glass jar and shook well manually for uniform mixing. A standard insecticide, Pirimiphos-methyl 2% dust (2 g) and untreated check were included for comparison. The experiment was laid out in a complete randomized design (CRD) with three replications. Twenty-six newly unsexed emerged adult weevils (one day old) were placed in each jar and covered with muslin cloth held in place with rubber bands. The jars were kept in the laboratory at room temperature (25 ± 2 °C and 60-65% R.H. and 12:12 h light: dark regimes).

2.5. Data collection

2.5.1. Weevil mortality

Weevil mortality was recorded 3, 7, 14, 21, and 28 days after initial infestation (DAI) based on Girma Demisie et al. (2008) and dead weevils were counted and discarded each time. During the last counting, both dead and alive weevils were counted and removed and the grains were kept under the same conditions for the emergence of F_1 generation.

2.5.2. F_1 progeny emergence

After removing dead and alive weevils, the seeds were kept under the same conditions for the F_1 progenies to emerge. The numbers of F_1 progeny weevils emerged were recorded every other day and were stopped after 33 days (60 days, DAI) to avoid overlapping of generations.

2.5.3. Seed germination test

Seed germination test was performed at the end of the F_1 progeny for all the treatments to check the effect of the botanicals on germinating power (seed viability) of the seeds. Accordingly, 15 undamaged grains (seeds without visible holes) were randomly picked from each treatment and placed on a moistened filter paper in Petri dishes replicated three times and the numbers of germinated seeds were recorded after seven days and the percentage germination was computed.

2.6. Data analysis

Adult weevil mortality data in each replication was expressed as a percentage of the total number of adult weevils introduced, F_1 progeny emergence of each replication was counted and computed as means, and seed germination was computed as percentage. Prior to statistical analysis, data on percentage mortality were arcsine-transformed, while percentage of seed germination and the number of F_1 progeny emergence were square root-transformed to reduce variance heterogeneity (Gomez and Gomez, 1984). All data were analyzed using one-way Analysis of Variance (ANOVA) model by SAS software, version 9.2 package (SAS, 2008). Treatment mean separations were conducted using Student-Newman-Keuls Test (SNK) at 5% level of significance.

3. Results and Discussion

3.1. Effect of botanicals on parent adult weevil mortality

The results of the effects of the botanical treatments on weevil mortality at different days after treatment application are depicted in Table 1. *Azadirachta indica*, *M. ferruginea* and *J. curcas* relatively inflicted higher weevil mortality than *C. macrostachyus*, *E. schimperiana,* and the untreated control three days after infestation. Only the chemical insecticide, Pirimiphos-methyl 2% dust, caused 100% weevil mortality within three days of treatment application, whereas, the efficacy of the botanical powders increased with exposure time and the cumulative mortality was found to be very high and no significant difference ($P<0.05$) was found among the botanicals and the chemical insecticide 28 days after treatment application. Seven days after treatment application, all the treatments showed significant difference ($P<0.05$) from the untreated control at all rates (3, 4, and 5 g w/w, i.e., 3, 4 and 5 %, respectively).

At all rates of application, there was no significant difference ($P>0.05$) among *C. macrostachyus, E. schimperiana* and *M. ferruginea*, and the untreated control three days after application. However, significant different ($P<0.05$) mortality was observed between these botanicals and the control with exposure of time.

Except *C. macrostachyus*, *E. schimperiana* and *M. ferruginea*, the rest of the botanical treatments and the chemical caused 100 % weevil mortality 14 days after treatment. However, 28 days after treatment application, high percentage of mean cumulative mortality (97.43 – 100 %) was recorded by all the botanical treatments at all rates and there was no significant difference ($P>0.05$)

among all the botanical treatments and the standard chemical, whereas a significant difference (P<0.05) was recorded between all these treatments and the untreated control (11.53%). There was almost no significant difference (P>0.05) among the different rates of each of the same botanical treatment in their mortality effect and this indicated that the lowest rate was as effective as the highest rate.

The results showed that the mean cumulative percentage mortality inflicted by all the botanical powders at all rates was almost as effective as the synthetic chemical insecticide, Pirimiphos-methyl 2% dust at 2 g, differed only in their speed of action and this revealed that the botanicals had profound effect comparable with the standard insecticide. Thus, the results of this showed that the botanicals can be used by resource poor farmers for the management of maize weevil.

In a similar study, Asmare Dejen (2002) recorded a percentage mean cumulative mortality of 84.40% with seed powder of *A. indica*, 48.90% of *C. macrostachyus*, 100 % with leaf powder of *Jatropha curcas* and 34.5% with leaf powder of *M. ferruginea* 28 days after infestation at 50 g/100 kg (5%) of each botanical product on sorghum. Except leaf powder of *J. curcas*, the mortality effect of rest of the products is lower than that of the current study. In the current study the seed powder of *J. curcas* (at 3, 4, and 5%) inflicted 100 % weevil mortality 14 days after infestation which may show that the seed powder is more toxic than the leaf powder. In a different similar work, Kifle Gebreziher et al. (2016) reported that neem seed powder both at 5 and 10% rate caused 100 % maize weevil mortality on sorghum seven days after treatment and the effect of 2.5% increased with exposure time and achieved 100% mortality after 21 days after treatment application.

Botanical extracts kill and repel pests, affect insect growth and development, have antifeedant and arrestant effects, and have antifungal, antiviral and antibacterial properties against pathogens. Botanical insecticides have long been touted as attractive alternatives to synthetic chemical insecticides for pest management because botanicals pose little threat to the environment or to human health (Said and Pashte, 2015). The use of plant products as protectants could offer a solution for the problems of availability, health risks, costs and resistance over synthetic pesticides (Said and Pashte, 2015).

Table 1: Percentage mean mortality of maize weevil on maize grains treated by powders of different botanicals at different rates after different days of initial treatment application

		Percent mean weevil mortality days after treatment application			
Treatments	Rate/g	3	7	14	28 (Cumulative)
Azadirachta indica	3	57.69b	42.30b	100.00a	100.00a
	4	30.77cd	69.23ab	100.00a	100.00a
	5	44.87bc	53.84b	67.95ab	97.43a
Croton macrostachyus	3	2.57d	35.90b	32.07b	100.00a
	4	0.00d	78.20ab	16.67b	94.86a
	5	3.84d	74.36ab	16.67b	94.86a
Millettia ferruginea	3	28.20cd	44.87b	21.07b	97.43a
	4	28.20cd	52.56b	28.85b	100.00a
	5	21.79cd	58.97b	52.56ab	100.00a
Euphorbia schimperiana	3	3.84d	57.69b	23.07b	97.43a
	4	0.00d	53.84b	21.79b	100.00a
	5	0.00d	55.13b	30.77b	100.00a
Jatropha curcas	3	38.46bc	61.34b	100.00a	100.00a
	4	38.46bc	61.34b	100.00a	100.00a
	5	34.61bc	65.38ab	100.00a	100.00a
Pirimiphos-methyl 2 % dust	2	100.00a	100.00a	100.00a	100.00a
Untreated control		0.00d	1.28c	8.97b	**11.53b**
CV		41.68	26.33	37.88	2.76

Means with the same letter in the same column are not significantly different at $\alpha = 0.05$

Tloba and Ekrakene (2006) reported that the mortality caused by different plants could be attributed to several mechanisms. The use of plant powders could have resulted to death as a result of physical barriers effect of the plant materials. This is because the powder has the tendency of blocking the spiracles of insects, thus impairing respiration leading to the death of insects. While feeding on whole grains by the weevil (*S. zeamais*) or the larvae might pick up a lethal dose of the treatment thus resulting in stomach poisoning. Schmutterer (1990) stated that *azadirachtin* has deterrent, antifeedant, growth disrupting, anti-ovipositional and fecundity reducing properties on a range of insects.

Bekele Jembere (2002) evaluated the toxicity of Millettia seed against *S. zeamais* and reported higher mortality of the weevil within 48 hours after treatment. Rotenone is one of the dominant compounds found in the seed and stem bark of Millettia, and is a well-known botanical insecticide through contact and stomach poisoning (Saxena 1983; Bekele Jembere, 2002). It is also highly toxic to fish and soluble in polar solvents (Bekele Jembere, 2002). Tebkew Damte and Mekasha Chichaybelu (2002) also tested the toxicity of Millettia seed against Adzuki bean beetle (*Callsobruchus chinunesis*) and found that it gave complete protection of stored chickpea for six months in the laboratory, even though it was not effective in controlling this storage pest when used by farmers. It deterred egg-laying. Bayeh Mulatu and Tadesse Gebremedhin (2000) reported from their laboratory study that the oils of *M. ferruginea* and *A. indica* were able to effectively control Adzuki bean beetle infestation of faba bean by partially or completely preventing egg-laying, and no bruchids emerged from the few eggs laid.

In a laboratory test of *J. curcas* seed powder at 1.00, 2.00 and 3.00 g/100 g (1, 2, and 3 %) against maize weevil, Ojiako et al. (2014) reported that the seed powder at the highest rate (3.00 g) inflicted adult mortality 6.67, 40.00, 70.0 and 100 % after two, three and seven days of infestation, respectively, with lowest adult emergence (0.67 - 2.67). The mortality effect of the other lower rates of the seed powders (1 and 2 g) increased with exposure time, i.e., the 1 g seed powder caused 0.00, 16.67, 53.33 and 83.33 after 1, 23, and 7 days, respectively, while the 2 g caused 3.33, 23.33, 66.67 and 90.00 % after one, two, three and seven days, respectively.

3.2. Effect of botanicals on emergence of F_1 progeny

The mean number of F_1 progeny (emerged adults) from the maize grains treated with three rates of different plant powders and a standard insecticide is presented in Table 2. On the first day of observation, no progeny was emerged from all the treatments except in the untreated control. In *A. indica***,** *J. curcas* and chemical treated grains, no progeny were emerged after 28 days. The mean number of F_1 progeny throughout the observation period from all the botanical treatments at different rates was very low and there was a significant difference between all the other treatments and the untreated control.

The very low/and absence of F_1 progeny emergence from the botanically treated grains may be associated to the very high mortality of the treated weevils. The mortality effect of all the treatments was very high (97.43-100%) as compared to the untreated control (11.53) (Table 1). Adult weevils did not emerge from treated maize with *A. indica* and *J. curcas* at all rates unlike the other botanical treatments (Table 2) and this may indicate that besides causing high weevil mortality, the botanicals have significant effect on insect fertility. In line with the present findings, Dekeba Moges et al. (2016) reported a reduction in F_1 progeny emergence in botanically treated grains which might be due to increased adult mortality, ovicidal, and larvicidal properties of the tested botanical powders. The absence of adult weevil or F_1 emergence indicated the efficacy of the botanicals for the control of maize weevil. The botanicals caused high mortality of *S. zeamais* on one hand and completely hindered or significantly reduced progeny emergence on the other hand, indicating its potential use in the management of maize weevil. These findings coincide with the work of Dagna et al. (2015) who reported that botanicals such as neem seed powder completely hinders progeny emergence, percentage seed damage and seed weight losses caused to maize grains by maize weevil, probably due to the huge array of *azandirachtin* activities on the insect’s hormone system. In a similar work by Kifle et al. (2016), no progeny was also emerged from sorghum treated by 5 and 10 % neem seed powder.

Table 2: Mean number of F1 progeny of maize weevil emerged from treated maize by different botanicals at different rates and percentage mean germination of treated seeds

Treatments	F_1 progeny emergence				Germinated seeds (%)
	Rate/g	1 day	2 days	Cumulative after 28 days	
Azadirachta indica	3	0.00b	0.00c	0.00c	97.78a
	4	0.00b	0.00c	0.00c	88.89a
	5	0.00b	0.00c	0.00c	91.11a
Croton macrostachyus	3	0.00b	1.33c	1.33c	95.55a
	4	0.00b	1.00c	1.00c	82.22a
	5	0.00b	0.33c	0.33c	75.55a
Millettia ferruginea	3	0.00b	0.00c	0.00bc	84.44a
	4	0.00b	1.00c	1.00bc	100.00a
	5	0.00b	1.33bc	1.33bc	97.78a
Euphorbia schimperiana	3	0.00b	0.67b	0.67b	95.56a
	4	0.00b	1.67bc	1.67bc	93.33a
	5	0.00b	1.00c	1.00c	91.11a
Jatropha curcas	3	0.00b	0.00c	0.00c	88.89a
	4	0.00b	0.00c	0.00c	77.78a
	5	0.00b	0.00c	0.00c	82.22a
Pirimiphos-methyl 2 % dust	2	0.00b	0.00c	0.00c	97.78a
Untreated control		1.33**a**	**4.67a**	**6.00a**	91.11a
CV		178.69	**77.69**	**74.27**	13.67

Means with the same letter in the same column are not significantly different at $\alpha = 0.05$

3.3. Effect of botanicals on germination of seeds

There was no significant difference (P>0.05) percentage mean seed germination among all the treatments. However, relatively lower rate of germination was recorded in *Croton macrostachyus* (75.55 % at 5 % rate) followed by *J. curcas* (77.78 % at 4 % rate) treated seeds (Table 2). The higher percentage mean mortality of the botanically treated maize grains and higher seed germination in all the treatments indicated that the botanicals can control the maize weevils and none of the botanicals significantly affected the germination (seed viability) of the maize grains. This indicated that resource-poor farmers can use the botanicals for the control of maize weevil and also use the treated grains for planting the grains. In a similar work, Asmare Dejen (2002) also reported that powders of *Datura stramonium*, *J. curcas*, *Phytoloca dodecondra* and *A. indica* did not show any visible adverse effect on germination capacity of the grains. This is also in agreement with the findings of Ojiako et al. (2014) who reported that *J. curcas* seed powder does not have negative effects on the viability of treated seeds and found that percentage mean germination of treated seeds was 93.00, 90.00 and 96.67 % treated with 1.00, 2.00 and 3.00 ml of seed powder, respectively.

4. Conclusion and Recommendation

The current study revealed that all the botanical powders tested against maize weevil caused very high adult weevil mortality and also highly affected their progeny production (fertility) which revealed their insecticidal activity. Besides, the botanicals did not affect significantly the germination capacity of the treated seeds. Therefore, all these qualities of the botanicals can be considered for potential use of their powders in the management of *S. zeamais* under subsistence farmer’s storage conditions. However, evaluation of the botanical powders against naturally infesting maize weevils under farmers’ storage conditions should be conducted.

The results of the current study showed that generally there was no significant difference among the different rates (3, 4, and 5%) of each botanical powder on the weevil mortality and progeny emergence. Therefore, further study is necessary to identify the minimum effective concentration of the botanical insecticides which can cause maximum weevil mortality.

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