Efficacy of diatomaceous earth and botanical powders against the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) on maize

Nukenine, E.K.*#[1], Goudoungou, J.W.[1], Adler, C.[2], Reichmuth, C.[2]
[1] Department of Biological Sciences, University of Ngaoundere, PO Box 454, Ngaoundere, Cameroon.
Email: ennukenine@fulbrightmail.org
[2] Federal Research Centre for Cultivated Plants – Julius Kühn - Institut, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Königin-Luise-Str. 19, 14195 Berlin, Germany

* Corresponding author
# Presenting author

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Abstract

The effectiveness of the diatomaceous earth SilicoSec, neem seed powder and *Plectranthus glandulosus* leaf powder, applied at four different rates with four exposure intervals (1, 3, 7 and 14 d) for the control of maize weevil, *Sitophilus zeamais* Motschulsky, on maize in the laboratory was determined. Treatment with SilicoSec was the most effective followed by neem seed powder and *P. glandulosus* powder. The highest tested content (2 g/kg) of SilicoSec caused 81.1% and 100% mortality of *S. zeamais* within 3 and 14 days of exposure, respectively. The application of the highest content (40 g/kg) for neem seed powder and *P. glandulosus* powder resulted in 86.8% and 59.5% mortality, respectively 14 days after exposure. Seven-day LC_{50}-values were 0.56 g/kg for SilicoSec, 19.7 g/kg for neem seed powder and 45.24 g/kg for *P. glandulosus* powder. The treatments reduced progeny emergence, percentage of grain damage, percentage of weight loss and percentage of germination loss, although *P. glandulosus* powder was less active for these parameters. Results suggest that SilicoSec can be considered as a potential component of an integrated pest management strategy against the maize weevil. However, in the poor tropical countries were the plant powders are widely available and food production dominated by subsistence agriculture, neem seed powder and *P. glandulosus* powder could be adopted also for the protection of stored maize against the infestation of *S. zeamais*.

Keywords: Diatomaceous earth, Botanical powders, Maize, Integrated weevil management, *Sitophilus zeamais*

1. Introduction

The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is one of the most serious cosmopolitan pests of stored cereal grain, especially of maize (*Zea mays* L.), in tropical and sub-tropical regions (Throne, 1994). The weevil has been reported to cause up to 80% grain damage in Cameroon during storage, where maize constitutes the most important food crop (Nukenine et al., 2002). Damaged grain has reduced nutritional value, low percentage germination, reduced weight and lowered market value (Demissie et al., 2008). Cheap and effective methods for reducing *S. zeamais* damage are needed in Africa to reduce food insecurity.

Control of *S. zeamais* populations around the world is primarily dependent upon continued applications of synthetic insecticides, which are often the most effective treatments for the disinfestations of stored food, feedstuffs and other agricultural commodities from insect infestation. Although effective, their repeated use for decades has disrupted biological control by natural enemies and led to outbreaks of other insect species and sometimes resulted in the development of resistance (Park et al., 2003). There are also serious concerns about environmental degradation and human health. Furthermore, the majority of farmers in Africa are resource-poor and have neither the means nor the skills to obtain and handle pesticides appropriately. Therefore, an environmentally safe and economically feasible weevil control practice needs to be available. The use of chemically inert materials, such as diatomaceous earths or plant products in large quantities to fill up the interstitial space in grain bulks and provide a barrier to insect movement, is quite widespread (FAO, 1999). Diatomaceous earths have proven to be very effective in smaller quantities. There is limited published work on the efficacy of diatomaceous earths in Africa (Stathers et al., 2002; Dimessie et al., 2008) In Africa, much research has focused on botanical pesticides (plant powders and extracts) and the mixing of grains with plant materials is an age-old practice among rural framers in the continent (Poswal et al., 1991; Shaaya et al. 1997; Tapondjou et al., 2000).

Plectranthus glandulosus Hook f. (syn. *Coleus laxiflorus* (Benth.) Roberty) (Lamiaceae) is an annual, glandular and strongly aromatic herb, used in folk medicine for the treatment of colds and sore throat in the Adamawa region of Cameroon, (Ngassoum et al., 2001). The few studies on the insecticidal effect of the plant reported high efficacies of the leaf powder (Nukenine et al., 2007; 2010a) and essential oils against *S. zeamais* (Nukenine et al., 2010b). Insecticidal activity from products of the neem tree, *Azadirachta indica* A. Juss (Meliaceae) is widely reported in the literature (Schmutterer, 1990; Isman, 2006). However, in northern Cameroon the medicinal use of the plant seems to shield its employment in storage protection.

The objective of this study was, therefore, to evaluate the insecticidal and reproduction inhibitory effects of SilicoSec (a diatomaceous earth of fresh water origin), neem seed powder and *P. glandulosus* leaf powder against the maize weevil in the laboratory.

2. Materials and methods

2.1. Test insects

Maize weevil was reared on maize grains under fluctuating laboratory conditions. Adult weevils were obtained from a colony kept since 2005 in the Applied Chemistry laboratory at the University of Ngaoundere.

2.2. Insecticide materials

The insecticide materials used were SilicoSec (Biofa AG, Münsingen, Germany), neem seed powder and *P. glandulosus* leaf powder. Neem fruits were collected on the ground below the trees in Maroua (10°33, 16’ N, longitude 14°15, 04’ E and altitude 356 masl), Far-North region, Cameroon in November 2008 (dry season). The seeds were removed from the fruits, sundried, and cracked to remove the husks. The kernels were crushed in a mortar until the powder passed through a 1-mm sieve mesh. The powder was stored in opaque containers inside a refrigerator at 4°C until needed for bioassay.

The leaves of *P. glandulosus* were collected in October (end of wet season) of 2008 around Ngaoundere (latitude 7° 22' North and longitude 13° 34' East, altitude of 1100 masl), located in the Adamawa region (plateau) of Cameroon. The plants were less than one-year old and only the green leaves were harvested. The leaves were dried at room temperature for seven days, and then crushed in a mortar until the powder passed through a 0.4-mm mesh sieve. The powder was stored in opaque containers inside a refrigerator at 4°C.

2.3. Toxicity tests and F1 progeny production

The application rates of the powders were 0.5, 1, 1.5 and 2 g/kg for SilicoSec (Demissie et al., 2008), 5, 10, 20 and 40 g/kg for neem seed powder (Chouka, 2007) and *P. glandulosus* powder (Nukenine et al., 2007). These rates were obtained by adding 0.025, 0.05, 0.075 and 0.1 g powder for SilicoSec and 0.25, 0.5, 1 and 2 g powder for neem seed powder and *P. glandulosus* powder to 50 g maize in a glass jar and shaken well to get uniform coating. Twenty 7 to 14-d old adult weevils of mixed sex were introduced into each jar. Untreated controls were included. The experiments were laid out in a completely randomized design on shelves. The treatments had four replications. All treatments were maintained in the laboratory under ambient conditions, and the daily temperature and r.h. in the laboratory ranged from 17.3 – 28.8°C and 56.3 – 97.8%, respectively. Mortality was recorded 1, 3, 7 and 14 days after infestation. On the 14th day post-infestation, all insects were removed and the different jars containing grains were kept under the same experimental conditions. The counting of F1 adults was done once a week for five weeks commencing 6 weeks post-infestation. The insect rearing had shown that emergence started only after the 5th week post-infestation

2.4. Population increase and damage

Three rates of the insecticidal materials (1, 1.5 and 2 g/kg for SilicoSec and 10, 20 and 40 g/kg for the plant powders), for 200 g seed were admixed as described above. A lot of 30 seven to 14-days-old insects of mixed sexes were introduced into each jar containing treated or untreated seed. Each treatment with the same dosage was repeated three times. After four months, the number of live and dead insects was determined for each jar. Damage assessment was performed by measuring the weight of the sieved powder and that of the grains without powder (final weight). The amount of grain powder (frass plus faeces) was expressed as the total powder minus the weight of plant powder used. Percent weight loss was determined as follows: (initial weight-final weight/100) x 100.

2.5. Seed germination

In order to assess the viability of seeds, seed germination was tested using 30 randomly picked seeds from undamaged grains after separation of damaged and undamaged grains in each jar. The seeds were placed on moistened sand in perforated plastic trays and the number of germinated seeds was recorded after 10 days (Rao et al., 2005).

2.6. Statistical analysis

Data on % cumulative mortality and % reduction in F_1 progeny were arcsine-transformed [(square root(x/100)] and the number of F_1 progeny produced was log-transformed (x + 1). The transformed data were subjected to the ANOVA procedure using the Statistical Analysis System (Zar, 1999; SAS Institute, 2003). HSD test (P = 0.05) was applied for mean separation. Probit analysis (Finney, 1971; SAS institute, 2003) was applied to determine lethal dosages causing 50% (LC_{50}) and 95% (LC_{95}) mortality of *S. zeamais* at 3, 7 and 14 d after treatment application. Abbott's formula (Abbott, 1925) was used to correct for control mortality before probit analysis and ANOVA.

3. Results

The results of the toxicity tests showed that the insecticidal materials caused significant mortality to *S. zeamais* (Table 1). Mortality increased with powder content level and time post-exposure for all the insecticidal materials. In general, SilicoSec was more toxic to the weevil than the plant powders and neem seed powder caused higher mortality to *S. zeamais* than *P. glandulosus* powder. At the highest tested powder content, SilicoSec (2 g/kg), neem seed powder (40 g/kg) and *P. glandulosus* powder (40 g/kg) caused 100, 86, and 59% mortality to *S. zeamais*, respectively, within 14 days of exposure.

Table 1 Corrected cumulative mortality of *Sitophilus zeamais* exposed to SilicoSec and two plant powders with LC_{50} values under fluctuating laboratory conditions (t = 17.3 – 28.8°C, r.h. 56.3 – 97.8%).

Insecticide	**Content (g /kg)**	**%Mortality (mean ± SE) - Exposure period (days)**			
		1	**3**	**7**	**14**
SilicoSec	0	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
	0.5	5.00 ± 2.04^{ab}	20.86 ± 12.74^{bc}	44.47 ± 8.61^{b}	44.36 ± 12.44^{b}
	1	5.00 ± 2.04^{ab}	48.75 ± 9.12^{ab}	71.19 ± 8.20^{ab}	75.70 ± 14.31^{ab}
	1.5	10.00 ± 4.08^{ab}	59,21 ± 15,25^{ab}	85.00 ± 10.21^{a}	93.75 ± 4.73^{a}
	2	13.75 ± 4.27^{a}	81.12 ± 10.84^{a}	86.12 ± 3.71^{a}	100.00 ± 00.00^{a}
	F	4.02*	8.57***	25.25***	22.24***
	LC_{50}	–	1.06	0.56	0.58
A. indica	0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
	5	2.5 ± 1.44^{a}	7.83 ± 6.27^{a}	15.60 ± 7.44^{bc}	20.33 ± 13.12b^{c}
	10	2.5 ± 1.44^{a}	10.23 ± 10.53^{a}	36.72 ± 6.53^{ab}	50.46 ± 13.06^{ab}
	20	2.5 ± 1.44^{a}	14.28 ± 7.61^{a}	50.42 ± 18.03^{ab}	54.34 ± 14.67^{ab}
	40	3.75 ± 2.39^{a}	23.16 ± 8.79^{a}	66.61 ± 15.97a	86.12 ± 7.44^{a}
	F	0.76ns	1.27*	5.23**	8.98***
	LC_{50}	–	–	19.74	12.72
P. glandulosus	0	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}
	5	0.00 ± 0.00^{a}	1.25 ± 1.25^{b}	8.82 ± 4.25^{bc}	11.38 ± 8.25^{ab}
	10	1.25 ± 2.50^{a}	6.38 ± 1.21^{ab}	17.83 ± 5.87^{abc}	22.84 ± 15.78^{ab}
	20	2.5 ± 1.44^{a}	9.01 ± 5.35^{ab}	30.46 ± 8,84^{ab}	40.27 ± 18.34^{a}
	40	2.5 ± 1.25^{a}	11.52 ± 1.17^{a}	47.17 ± 17.65^{a}	59.48 ± 10.42^{a}
	F	0.48 ^{ns}	4.36*	3.88*	3.64*
	LC_{50}	–	–	45.24 $^{\#}$	28.48

Means ± S.E. in the same column for the same category of insecticide, followed by the same letter do not differ significantly at *P* = 0.05 (Tukey's test)). Each datum represents the mean of four replicates of 20 insects each.
ns P > 0.05 * P < 0.05, *** P < 0.001. # LC value obtained by extrapolation.
- Estimated LC values are too large or estimation impossible due to inadequate mortality

For the same time-point, and in the same order, the lowest tested powder contents caused 44, 20 and 11% weevil mortality. Within 1 d of exposure 10, 4 and 3% mortality was recorded for SilicoSec, neem seed powder and *P. glandulosus* powder, respectively, for the highest tested powder contents. The 7-d LC_{50} values (Table 1) clearly demonstrate that SilicoSec (0.56 g/kg) was more toxic to *S. zeamais* than neem seed powder (17.74 g/kg) and *P. glandulosus* powder (45.24 g/kg)

All the three treatments generally caused significant reduction in progeny production relative to the control, which was dose dependent (Table 2). SilicoSec at 1 g/kg and neem seed powder at 5 g/kg caused >90% suppression of F1 progeny emergence. Higher concentration levels of these two powders roughly achieved complete suppression of progeny emergence. The highest and lowest tested concentration level of *P. glandulosus* powder reduced F1 progeny production by 69.6 and 18.5%, respectively.

Table 2 Progeny production of *Sitophilus zeamais* in grains treated with SilicoSec, Neem seed powder and *Plectranthus glandulosus* leaf powder under fluctuating laboratory conditions (temperature = 17.3 – 28.8°C and r.h. =56.3 – 97.8%).

Insectcide	**Content (g/kg)**	**Mean number of F_1 adult progeny**	**% reduction in adult emergence relative to control**
SilicoSec	0	91.50 ± 16.68^a	0.00 ± 0.00^c
	0.5	29.75 ± 8.01^b	63.95 ± 13.00^b
	1	8.50 ± 3.52^b	92.05 ± 2.53^a
	1.5	4.50 ± 2.33^b	95.85 ± 1.83^a
	2	2.00 ± 2.00^b	98.38 ± 1.63^a
	F	19.35 ***	47.55 ***
A. indica	0	91.50 ± 16.68^a	0.00 ± 0.00^c
	5	5.25 ± 1.25^b	94.125 ± 0.89^b
	10	0.50 ± 0.29^b	99.20 ± 0.47^a
	20	0.25 ± 0.25^b	99.55 ± 0.45^a
	40	0.00 ± 0.00^b	100.00 ± 0.00^a
	F	29.02 ***	8008.49 ***
P. glandulosus	0	91.50 ± 16.68^a	0.00 ± 0.00^c
	5	76.25 ± 19.52^a	18.85 ± 11.66^{bc}
	10	69.50 ± 16.45^a	20.85 ± 13.37^{bc}
	20	37.00 ± 15.13^a	57.00 ± 13.37^{ab}
	40	28.00 ± 13.95^a	69.60 ± 10.67^a
	F	2.68 ns	6.89 *

Means ± S.E. in the same column for the same category of insecticide, followed by the same letter do not differ significantly at $P = 0.05$ (Tukey's test)).
Each datum represents the mean of four replicates of 20 insects each.
ns $P > 0.05$ * $P < 0.05$, *** $P < 0.001$

Apart from percentage seed damage which did not differ among content levels for neem seed powder, there were significant differences in the number of live insects, percentage seed damage, percentage weight loss and percentage germination among powder content levels for all the three insecticide materials (Table 3). SilicoSec and neem seed powder were effective in reducing the rate of the weevil population increase, seed damage, seed weight loss and germination losses, while *P. glandulosus* powder was less effective for the four parameters.

4. Discussion

Our experiments showed that the three insecticide powders possess some toxic components which could cause a noticeable weevil mortality. However, the action of the botanical powders was slower than that of SilicoSec. Between the botanical powders, *P. glandulosus* was less toxic than neem seed powder. This contention is supported by the lower 7-d LC_{50} values for SilicoSec (0.56 g/kg) relative to those of neem seed powder (19.74 g/kg) and *P. glandulosus* powder (45.24 g/kg). Although the results with SilicoSec are encouraging, other authors recorded higher mortalities of *S. zeamais* caused by the diatomaceous earth. Demissie et al. (2008) reported that SilicoSec caused 99 and 100% mortality of *S. zeamais* within 3 and 7 d exposure periods, respectively, at the rate of 2%, whereas within the same time-point and content rate, the present study recorded 81 and 86% mortality, respectively. The difference in mortality between the two studies could be linked at least in part to variation in environmental conditions and insect strains. Our laboratory temperature and r.h. varied respectively between 17.3 – 28.8°C and 56.3 – 97.8% and those of Demissie et al. (2008) between 20 – 30° C and 66.5 – 76.5 %. The efficacy of diatomaceous earth reduces with increasing relative humidity (Korunic, 1988; Vayias et Athanassiou, 2004). Within 14 d of exposure, and at a concentration rate of 40 g/kg, neem seed powder caused respectively 30% (Pereira and Wohlgemuth, 1982), 38% (Chouka, 2007) and 86% (present study) mortality to *S. zeamais*. With similar concentration level and time post-exposure, Nukenine et al. (2007), Chouka (2007) and the present study recorded respectively 100, 83 and 29% mortality of *S. zeamais* caused by *P. glandulosus* powder. These discrepancies in the toxicity of the plant powders are not surprising, since geographical location, time of harvest, r.h. among others, greatly influence the activity of botanicals against insects (Schmutterer, 1990; Korunic, 1988; Vayias and Athanassiou, 2004). However, these differences show that efficacy data with botanicals require the determination of contents of pure compounds

Table 3 Population increase (mean number of progeny for three jars ± SE) and damage parameters of *Sitophilus zeamais*, and percentage germination in maize admixed with different contents of SilicoSec, Neem seed powder and *Plectranhus glandulosus* leaf powder and stored for four months under fluctuating laboratory conditions (temperature = 17.3 – 28.8°C and r.h. = 56.3 – 97.8%).

Insecticide	Content (g/kg)	Number of live insects	Seed damage(%)	Weight loss (%)	Germination (%)
SilicoSec	0	463.7 ± 35.4^{a}	94.5 ± 0.2^{a}	26.0 ± 3.0^{a}	0.0 ± 0.0^{b}
	1	16.7 ± 4.4^{b}	4.4 ± 1.4^{b}	0.6 ± 0.2^{b}	70.0 ± 6.7^{a}
	1.5	6.7 ± 4.1^{b}	2.7 ± 1.5^{b}	0.4 ± 0.2^{b}	68.4 ± 2.9^{a}
	2	1.0 ± 1.0^{b}	0.6 ± 0.2^{b}	0.1 ± 0.0^{b}	66.7 ± 10.2^{a}
	F	160.9 ***	1906.9 ***	87.9 ***	29.9***
A. indica	0	463.67 ± 35.4^{a}	94.5 ± 0.2^{a}	94.5 ± 0.2^{a}	0.0 ± 0.0^{b}
	10	0.7 ± 0.7^{b}	4.1 ± 1.6^{b}	4.1 ± 1.6^{b}	17.8 ± 6.9^{a}
	20	0.0 ± 0.0^{b}	0.4 ± 0.4^{c}	0.4 ± 0.4^{c}	12.2 ± 5.5^{a}
	40	0.0 ± 0.0^{b}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	6.7 ± 1.9^{ab}
	F	171.3 ***	3127.3 ***	3127.3 ***	3.2 ns
P. glandulosus	0	463.7 ± 35.4^{a}	94.50 ± 0.2^{a}	94.5 ± 0.2^{a}	0.0 ± 0.0^{b}
	10	621.0 ±25.2^{a}	93.92 ± 0.3^{a}	93.9 ± 0.3^{a}	0.0 ± 0.0^{b}
	20	287.0 ±28.6^{ab}	64.04 ± 6.8^{b}	64.0 ± 6.8^{b}	0.0 ± 0.0^{b}
	40	218.3 ± 116.1^{b}	48.12 ± 7.6^{b}	48.1 ± 7.6^{b}	27.8 ± 6.7^{a}
	F	8.1 *	20.5 **	20.5 **	10.2*

Means ± S.E. in the same column for the same category of insecticide, followed by the same letter do not differ significantly at $P < 0.05$ (Tukey's test)). Each datum represents the mean of four replicates of 30 initial insects each. ns $P > 0.05$ * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Concerning the mode of action, diatomaceous earth absorbs the lipids of the insect's epicuticle and dead ensues from loss of water and desiccation (Vayias et al., 2008). Neem powder is rich in alkaloids (azadiracthtin) and other molecules like salanine and melandriol, which after ingestion, cause digestive disorders and loss of appetite (antifeedant activity) (Schmutterer, 1990). *Plectranthus glandulosus* powder contains several monoterpenes (Ngassoum et al., 2001; Nukenine et al., 2007) which could be toxic to the weevil by reversible competitive inhibition of acetyl cholinesterase by occupation of the hydrophobic site of the enzyme's active centre (Ryan and Byrne, 1988). These mechanisms are all possible in the present study apart from the antifeedant mechanism of neem seed powder which was less apparent, since up to 23 and 67% mortality was recorded within the relatively short exposure periods of 3 and 7 days, respectively.

In addition to causing adult mortality, the insecticide powders either completely hindered or significantly reduced progeny emergence, indicating their potential for use in the management of the maize weevil. Kavallieratos et al. (2005) reported that SilicoSec was effective against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) in maize, wheat, barley, oats and rice. In previous studies, *P. glandulosus* powder and neem seed powder also greatly inhibited progeny production of *S. zeamais* (Nukenine et al., 2007; 2010b; Chouka, 2007). Reductions in insect population growth rate, percentage damaged grains, percentage weight loss and percentage germination losses were observed in all the treatments although *P.*

glandulosus powder did not affect insect population growth and the rate of seed germination. Stathers et al. (2000; 2002) reported that diatomaceous earth products did not have negative effects on seed germination. Diatomaceous earths are very promising alternatives to traditional pesticides, as they have low mammalian toxicity, low or zero residual effects in food and are effective against the target pests (Korunic, 1988). However, at present their cost and lack of availability is preventing their widespread use in developing countries (Stathers et al., 2000; 2002). In some communities in Africa, grains with holes were unacceptable for either planting or food and were discarded by consumers (Dunkel et al., 1986). For the neem and *P. glandulosus* powders, the dosage of 20 g/kg reduced grain damage by almost 100 and 30%, respectively. At that powder application rate, F1 progeny emergence was reduced by roughly 100% and over 50% respectively for neem seed powder and *P. glandulosus* powder. This indicates that at the practicable powder content of 20 g/kg, there will be few or no holes on grains, from feeding or emergence, and that the net losses in stored maize caused by *S. zeamais* to subsistence farmers may be greatly reduced by using neem seed powder. The neem plant is widely available in the northern parts of Cameroon, but the use of neem products in stored product protection is insignificant in the country. Therefore, the presented data encourage the production and promotion of insecticidal products from this plant in the country.

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