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Effectiveness of two diatomaceous earths (FossilShield® and SilicoSec®) against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) infesting two maize varieties (CLH103 and SHABA) at storage

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Abstract

Influence of maize varieties CLH103 and SHABA on the effectiveness of FossilShield® and SilicoSec® against *Sitophilus zeamais* was evaluated in laboratory. Each product was used at 0, 0.5, 1, 1.5 and 2 g/kg and four exposure periods (1, 3, 7 and 14 days) for adult mortality and F_1 progeny production. Damaged and germinated seeds were also assessed. Malathion (positive control) was used at the recommended dosage (0.5 g/kg). Probit analysis showed that FossilShield® and SilicoSec® were more toxic to weevils on CLH103 than SHABA from 3 days post-infestation, while within 1 day post-infestation, the two products were rather more toxic on SHABA than CLH103. Student *t*-test showed significant difference between the two maize varieties treated with FossilShield® in the reduction of adult emergence at 1 g/kg while with SilicoSec® this difference was observed for all concentrations. Malathion as positive control completely inhibited the development of insects on the two maize varieties. Like Malathion at the single dosage (0.5 g/kg), the number of emerged insects was completely reduced by each diatomaceous earth (DE) at 2 g/kg. FossilShield® (from 1.5 g/kg) reduced completely grain damages in the CLH103 variety, while FossilShield® (2 g/kg) almost reduced grain damages (1.0%). SilicoSec® achieved complete reduction on CLH103 variety, while, on SHABA variety, the reduction of grain damage was significantly different (7.4%). Seed germination was not affect by the DEs. In this study, the results showed that utilization of FossilShield® and SilicoSec® could be favorably considered as alternative solution to Malathion against *Sitophilus zeamais*.

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Introduction

Maize (*Zea mays* L.) is an important food crop in Cameroon and many countries in African (Nukenine *et al.*, 2007) where it is widely cultivated and consumed. It is a major source of dietary proteins for both the humans) and animals (Mebarkia *et al.*, 2010. Post-harvest losses due to the storage pests have been recognized as an increasingly important problem in most parts of the tropics (Teshome and Tefera, 2011). In Cameroon, maize grain losses during storage are caused mostly by insets, especially *Sitophilus zeamais* Motschulsky (Nukenine *et al.*, 2007). The weevil has been reported to cause up to 80% grain damages in this country during storage (Nukenine *et al.*, 2002). As results, the nutritional value, low percentage germination, weight and lowered economical value are reduced (Demissie *et al.*, 2008).

Currently, there are a number of control methods against the post-harvest insect pests, but each comes with its inconvenient. Chemical control is the most commonly used and most effective at farm level. Although, they cause adverse effects on environment, development of resistant strains, hazardous effects to non-target species and beneficial organisms and residues in food crops, despite their satisfactory pest control (Odeyemi *et al.*, 2006; Sadeghi *et al.*, 2006). This motivated the search for safer alternative methods (Parsaeyan *et al.*, 2012). Diatomaceous earth (DE) obtained from the fossil remains of single cells algae is known as one of the most promising alternatives to traditional residual insecticides (Athanassiou *et al.*, 2008; Vayias and Stephou, 2009). DE acts as a desiccant; when DE particles are picked up by the insect cuticle, the epicuticular layer is destroyed, resulting in death through water loss (Korunic, 1998; Subramanyam and Roesli, 2000). Their benefits are their low toxicity to mammalians and the nearly negligence resistance development to insects (Parsaeyan *et al.*, 2012). In the last decade, several improved DE formulations have been successfully evaluated against several stored-product pest species *Tribolium confusum*, *Sitophilus oryzae* and larger grain borer (Athanassiou *et al.*, 2004, 2006, 2007), *Tribolium confusum* (Kavallieratos *et al.*, 2007), *Rhyzopertha dominica* and *Sitophilus oryzae* (Vassilakos *et al.*, 2006), *Tyrophagus putrescentiae* (Iatrou *et al.*, 2010), *Sitophilus zeamais* (Stathers *et al.,* 2004; Nukenine *et al.*, 2010; Khakame *et al.*, 2012), *Plodia interpunctella, Ephestia kuehniella* and *Ephestia cautella* (Sabbour *et al.*, 2012). The efficacy of DE formulations mined varies remarkably adherence to kernels (Korunic, 1997), particle size (Vayias *et al.*, 2009a) and temperature (Vayias *et al.*, 2009b). Since the efficacy of commercial DE formulations has been documented for a number of insect species on various stored grains, this often varies with the DE formulations (Subramanyam and Roesli, 2000), and among different commodities treated with a specific formulation (Athanassiou *et al.*, 2003, 2004; Vayias *et al.*, 2009b). Therefore, results on DE efficacy obtained on a particular commodity may not be transferable to other commodities (Athanassiou and Kavallieratos, 2005; Kavallieratos *et al.*, 2005). Additionally, grain moisture content is one of the most important factors influencing efficacy in pest control products (Khakame *et al.*, 2012). In Africa, the published works on DE formulations are lacking (Demissie *et al.*, 2008). However, some commercially DEs have been tested in some African countries (Wakil and Shabbir 2005; Wakil *et al.*, 2005; Wakil *et al.,* 2006; Mohale *et al.*, 2010; Wakil *et al.*, 2011; Khakame *et al.*, 2012). In Cameroon, SilicoSec® have been tested on the maize variety SHABA against *S. zeamais* and given the good results in four months of storage (Nukenine *et al.*, 2010), but not FossilShield® and SilicoSec® at the same time. In addition, several maize varieties are produced in different agro-ecological zones in Cameroon and their susceptibility to weevil attack varies according to grains variety.

In the current study, we examined effectiveness of two diatomaceous earths (FossilShield® and SilicoSec®) against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) infecting two maize varieties (CLH 103 and SHABA) at storage for different exposures and dose rates.

Material and methods

Sitophilus zeamais culture

The test insects used in our study, *S. zeamais* was reared on maize grains SHABA under ambient laboratory conditions (t ≈ 23.73-26.26°C; r.h. ≈ 46.26-77.22%). Parent adults were obtained from a colony kept since 2005 in the Applied Chemistry laboratory at the University of Ngaoundere. The cultures of *S. zeamais* continuously reared in the ambient laboratory conditions since 2009. Mixed-sex adults used in these experiments were 2 weeks old.

Maize varieties

The Cameroonian maize varieties used were CLH103 and SHABA (Table 1). Both maize varieties were obtained from the Institute of Agrocultural Research for Development (IARD) of Yaoundé-Cameroun. These varieties were cleaned and disinfested by keeping them in a room freezer at -14°C for 21 days prior to setting up the experiment. Before the beginning of the experiments the grains were left under ambient laboratory conditions (t ≈ 21.7-25.6°C; r.h. ≈ 76.1-79%) at least 10 days to equilibrate with the relative humidity level. The moisture content of the two varieties has been determined according to AFNOR method (1982). They were 11.30% and 13.20% for CLH103 and SHABA respectively.

Table 1. Characteristics of maize varieties used.

Varieties	Grain texture	Grain color
CLH103	Dent-cornea	White
SHABA	Dent	White

Diatomaceous earth formulations

SilicoSec® is a DE formulation of freshwater origin containing 92% SiO_2, 3% Al_2O_3, 1% Fe_2O_3, and 1% Na_2O. The average particle size is between 8-12µm. FossilShield® has a particle size of 5-30µm and is composed of 73% amorphous SiO2, 3% aerosol and other mineral compounds. The two diatomaceous earth formulations were obtained from Agrinosa Company-Biofa (Münsingen, Germany).

Bioassays

Assessment of mortality of Sitophilus zeamais

Disinfested maize grains of each variety (50 g) were placed in 1 L volume glass jars and treated with 0.025, 0.05, 0.075 and 0.1 g of each tested diatomaceous earth corresponding to 0.5, 1, 1.5, 2 g/kg respectively (Nukenine *et al.,* 2010). Malathion 5% at the single recommended dosage of 0.5g/kg dust was used as positive control. Each jar was shaken manually for 2 min to distribute DE in whole grain mass. Additionally, untreated grains of each maize variety were included as a negative control. After treatment application, 20 of 2 weeks old *S. zeamais* of mixed sexes were introduced in the treated and untreated grains of each glass jar. Then, each jar was then covered with cotton clothe to prevent insects from escaping and closed with a perforated metal lid for sufficient ventilation. All treatments were maintained in the laboratory under ambient conditions (t ≈ 21.7-25.6°C; r.h. ≈ 76.1-79%). The temperature and relative humidity (r.h.) of laboratory were recorded with thermohygrometer EL-USB-2+ (China). Each treatment was repeated three times. The number of live and dead insects in each jar was sieved and counted after 1, 3, 7 and 14 days of treatment and the percentage insect mortality was calculated using Abbott's formula (Abbott, 1925).

Assessment of F_1 progeny production

In each treatment, after the last (14-d) mortality count, all *S. zeamais* adults (dead and live) were removed and DE discarded from the glass jars, and maize were left in the ambient conditions (t ≈ 22.6-25.6°C; r.h. ≈ 72.5-80%) for an additional period of 5 weeks. At the end of this interval, the emerged *S. zeamais* adults in each glass jar were counted every week for four weeks. The mean number of F_1 adult progeny and the percentage reduction in adult emergence or inhibition rate (%IR) was calculated using %IR = (Cn-Tn) x100/Cn; where Cn is the number of newly emerged insects in the untreated (control) jar and Tn is the number of insects in the treated jar.

Assessment of population increase and seed damage

Concentration (0.5, 1, 1.5 and 2 g/kg) of FossilShield® or SilicoSec® for 200 g grain of each maize variety were admixed as described above in the mortality assessment. Total of 30 of 2 weeks old insects of mixed sexes were introduced into each jar containing treated or untreated maize grains. Each treatment with the same concentration was repeated four times. After four months, the number of live insects was determined for each jar. Seed damage assessment was performed by counting the damaged and undamaged grains for each variety. It percentage was calculated using the following formula:

$$\text{Seed damage (\%)} = \frac{\text{No of seeds damaged} \times 100}{\text{Total no of seeds}}$$

Assessment of germination test

Thirty undamaged grains of each maize variety were picked randomly from each treatment glass jar after separation of damaged grains. The grains were placed on moistened sand in perforated plastic plates. Each treatment was replicated four times. Germination was evaluated and recorded after 10 days (Rao *et al.,* 2005; Demissie *et al.,* 2008). Data were recorded and calculated for the percentage of seed germination. This was calculated using the following formula: Viability index (%) = (NG x 100)/TG where NG = number of seeds germinated and TG = total number of seeds tested in each perforated plastic plate.

Statistical analysis

Data on % cumulative mortality, % reduction of F_1 progeny, % damaged grains, % of weight loss and % of germination were tested for normality and heterogeneity of variance and then subjected to the analysis of variance (ANOVA) procedure of the Statistical Analysis System (SAS 2003). Arcsine [(square root(x/100)], square root (x+0.5) and log-transformed (x+1) values were used for ANOVA with data on mortality and % reduction in F_1 progeny, and live insects, respectively. Turkey test (P=0.05) was applied for mean separation. Student *t*-test of was applied for means separation of maize varieties treated with each DE. A logarithmic transformation [log10(x+1), where x = content in %] was performed before regression analysis. Probit analysis (Finney, 1971; SAS 2003) was applied to determine lethal contents causing 50% (LC_{50}) mortality of *S. zeamais* after 1, 3, 7 and 14 days exposure.

Results

Mortality of Sitophilus zeamais on the two maize varieties treated with each diatomaceous formulation

Mortality of *S. zeamais* caused by the tested DE formulations was significantly affected by concentrations ($F_{(4,\ 239)}$ = 126.88; P<0.0001) and exposure periods ($F_{(3,\ 593)}$ = 161.36; P<0.0001). The classification of insecticides in term of efficacy is FossilShield® > SilicoSec® and the susceptibility of two maize varieties was CLH103 > SHABA. After 1 day of exposure, mortality ranged from 0.0% to 10.0% for CLH103 and 0.0% to 6.7% for SHABA both treated with FossilShield® from 0.5 to 2 g/kg of concentration. For the same exposure period, mortality caused by SilicoSec® ranged from 0.0% to 5% for CLH103 and 0.0% to 1.7% for SHABA. With the increase of concentration and exposure periods, this mortality reached 100% at the higher concentration of 2 g/kg for the two maize varieties treated with FossilShield® from 7 days of exposure. In the case of SilicoSec®, complete mortality was achieved at the same concentration within 14 days of exposure. The *t-test* values showed no significant difference between the two maize varieties in term of efficacy of FossilShield® and SilicoSec® (Table 2). Positive control (Malathion) at the recommended concentration (0.5 g/kg) caused total mortality of *S. zeamais* within 1 day of exposure (Table 2). Additional to mortality, the LC_{50} values decreased with the increased exposure periods (Table 2). SilicoSec® was more toxic to *S. zeamais* adults of on SHABA (LC_{50} = 0.11 g/kg) than CLH 103 (LC_{50} = 0.23 g/kg) (Table 2). Similar results were observed with FossilShield® (SHABA: LC_{50} = 0.18 g/kg; CLH103: LC_{50} = 0.33 g/kg) within 1 day of exposure. For the other exposure periods (3, 7 and 14 days), FossilShield® and SilicoSec® were more toxics on the maize variety CLH103 than SHABA. The lowest LC_{50} (0.02 g/kg) was obtained with the two DE formulations on CLH103 within 14 days of exposure.

Table 2. Corrected cumulative mortality of *Sitophilus zeamais* exposed to FossilShield® and SilicoSec® under ambient laboratory conditions (t ≈ 21.7-25.6°C; r.h. ≈ 76.1-79%).

Exposure periods (days)	Contents (g/kg)	Percentage of mortality (mean ± standard error)					
		Products – Maize varieties					
		FossilShield®			SilicoSec®		
		CLH 103	SHABA	*t*-value	CLH 103	SHABA	*t*-value
1	0	0.0±0.00b	0.0±0.00c		0.0±0.00b	0.0±0.00b	
	0.5	0.0±0.00b	0.0±0.00c	/	0.0±0.00b	0.0±0.00b	/
	1	3.3±3.33b	0.0±0.00c	1.00ns	1.7±1.67b	0.0±0.00b	1.00ns
	1.5	6.7±4.41b	1.7±1.67c	1.06ns	1.7±1.67b	0.0±0.00b	1.00ns
	2	10.0±5.00b	6.7±1.67b	0.63ns	5.0±2.89b	1.7±1.67b	1.00ns
	Malathion 5%	100.0±0.00a	100.0±0.00a		100.0±0.00a	100.0±0.00a	
	F value	51.62***	347.70***		112.28***	429.30***	
	LC_{50} (g/kg)	0.33	0.18		0.23	0.11	
3	0	0.0±0.00d	0.0±0.00d		0.0±0.00b	0.0±0.00c	
	0.5	8.3±4.41d	3.3±3.33cd	0.90ns	8.3±6.01b	0.0±0.00c	1.39ns
	1	50.0±10.41c	30.0±10.00bc	1.39ns	41.7±22.42ab	8.3±3.33bc	1.47ns
	1.5	73.3±4.41bc	45.0±15.28b	1.78ns	51.7±25.22ab	35.0±18.03bc	0.54ns
	2	85.0±5.00b	50.0±15.00b	2.21ns	81.7±11.67a	55.0±22.55b	1.05ns
	Malathion 5%	100.0±0.00a	100.0±0.00a		100.0±0.00a	100.0±0.00a	
	F value	60.09***	26.09***		8.5***	20.30***	
	LC_{50} (g/kg)	0.05	0.09		0.06	0.09	
7	0	0.0 ± 0.00b	0.0 ± 0.00c		0.0 ± 0.00c	0.0 ± 0.00c	
	0.5	35.0±17.56b	30.0±18.93bc	0.19ns	41.7±15.90bc	16.7±9.28bc	1.36ns
	1	81.6±7.26a	68.3±9.28ab	1.13ns	63.3±17.64ab	33.3±16.41bc	1.24ns
	1.5	85.0±5.77a	90.0±5.00a	-0.65ns	76.7±14.53ab	55.0±18.03b	0.94ns
	2	100.0±0.00a	100.0±0.00a	/	91.7±4.41ab	71.7±18.56ab	1.05ns
	Malathion 5%	100.0±0.00a	100.0±0.00a		100.0±0.00a	100.0±0.00a	
	F value	25.18***	23.86***		13.79***	13.59***	
	LC_{50} (g/kg)	0.03	0.04		0.03	0.06	
14	0	0.0±0.00b	0.0±0.00d		0.0±0.00c	0.0±0.00c	
	0.5	65.0±27.84a	48.7±8.14c	0.56ns	65.0±11.55b	23.6±20.75c	1.74ns
	1	88.3±9.28a	81.1±3.85b	0.72ns	78.3±16.67ab	40.6±18.46bc	1.52ns
	1.5	98.3±1.67a	93.3±3.33ab	1.34ns	91.7±6.01ab	79.1±7.40ab	1.32ns
	2	100.0±0.00a	100.0±0.00a	/	100.0±0.00a	100.0±0.00a	/
	Malathion 5%	100.0±0.00a	100.0±0.00a		100.0±0.00a	100.0±0.00a	
	F value	12.97***	104.55***		27.68***	19.27***	
	LC_{50} (g/kg)	0.02	0.03		0.02	0.04	

Means in the same column followed by the same lower case letter do not differ significantly at P=0.05 (Tukey's HDS test and *t*-test of Student). Each datum represents the mean of three replicates of 20 insects for each dosage. ns P >0.05 ; *** P< 0.001.

F_1 Progeny production

Sitophilus zeamais F_1 progeny emerged in maize grains were significantly (P<0.001) affected by each of the DE formulation, concentration and maize variety (Table 3). The *t*-test values showed significant difference (P<0.05) between the two maize varieties treated separately with each DE formulation on reduction in adult emergence relative to control (Table 3). FossilShield®, from 1.5 g/kg was more effective on CLH103 with complete reduction of adult emergence of *S. zeamais*, than on SHABA (88.6% in reduction). For both maize varieties treated with SilicoSec®, the significant difference was observed from 0.5 g/kg of product. As the result, SilicoSec was also more effective on CLH103 than on SHABA. Like Malathion 5% (0.5 g/kg), FossilShield® (from 1.5 g/kg) achieved complete reduction in adult emergence on CLH103 than on SHABA (88.6%). With the same concentration, SilicoSec® also recorded total reduction in adult emergence on CLH103 than on SHABA (86.7%).

Table 3. Progeny production of *Sitophilus zeamais* in grains treated with four concentrations diatomaceous earths (FossilShield® and SilicoSec®) under ambient laboratory conditions (t ≈ 22.6-25.6°C; r.h. ≈ 72.5-80%).

Reproduction inhibition (Means ± standard error)						
	FossilShield®			SilicoSec®		
Contents g/kg	CLH103	SHABA	*t*-value	CLH103	SHABA	*t*-value
	Mean number of F_1 adult progeny					
0	81.7±21.15a	70.3±13.86a	0.45ns	81.7±21.15a	70.3±13.86a	0.45ns
0.5	14.0±9.71b	19.3±4.10b	-0.51ns	14.0±6.43b	52.3±8.17a	-3.67*
1	3.7±2.03b	15.3±3.48b	-2.70*	3.0±1.15b	15.0±4.51b	-2.56*
1.5	0.0±0.00b	7.3±2.40bc	-3.05*	1.3±0.88b	10.3±4.18bc	-2.11ns
2	0.0±0.00b	4.7±167bc	-2.8*	0.3±0.33b	5.7±2.03bc	-2.6*
Malathion 5%	0.0±0.00b	0.0±0.00c	/	0.0±0.00b	0.0±0.00c	/
M ± MSE	16.7±7.88A	19.5±6.11A	-0.30ns	16.7±7.78A	25.6±6.80A	-0.87ns
F value	17.54***	28.88***		24.62***	22.96***	
	% reduction in adult emergence relative to control					
0	0.0±0.00b	0.0±0.00e	/	0.0±0.00c	0.0±0.00d	/
0.5	73.9±22.12a	72.5±1.04d	0.06ns	82.6±6.26b	24.1±5.78c	6.86*
1	93.9±4.52a	78.5±0.94dc	3.34*	95.2±2.92b	79.6±4.01b	3.14*
1.5	100.0±0.00a	88.6±3.72bc	3.06*	98.7±0.72a	86.7±4.64b	2.57*
2	100.0±0.00a	93.0±2.35b	2.98*	99.7±0.27a	92.2±2.52b	2.97*
Malathion 5%	100.0±0.00a	100.0±0.00a	/	100.0 ± 0.00a	100.0±0.00a	/
M ± MSE	78.0±9.30A	72.1±8.15A	0.47ns	79.4 ± 8.79A	63.8±9.23B	1.22ns
F value	26.09***	267.33***		132.23***	134.01***	

Mean ± S.E. in the same column for same category of insecticide, followed by the same capital or minuscule letter do not differ at *P*=0.05 (Turkey's test and *T* test of Student). Each datum represents the mean of three replications of 20 insects for each dosage. ns *P* >0.05; * *P* <0.05; *** *P* <0.001.

Population increase and seed damage

Population increased of *S. zeamais* and seed damages were highly significant (*P*<0.001) between the two maize varieties in comparison with untreated control (Table 4). SHABA treated with FossilShield® recorded highest number of alive insects (381.3) at 0.5 g/kg, while on CLH103 no insect emerged. Furthermore, at this concentration, the Student *t*-test indicated that this DE protected better CLH103 than SHABA (*t* = -7.36; *P*<0.05). With SilicoSec®, complete inhibition of number of insects was observed on CLH103 variety than SHABA (59) at 2 g/kg. Also SilicoSec® protected better CLH103 variety than SHABA variety according to the *t*-test of Student (*t* = -3.53; *P*<0.05). No seed damage was recorded in CLH103 while in SHABA, 3.8% of seed damaged was observed from 1.5 g/kg of FossilShield®. In CLH103 treated with SilicoSec®, no seed damage was observed at 2 g/kg, while in SHABA, the mean percentage of seed damage ranged from 7.42-72.7% (Table 4). there was a significant difference between the two maize varieties treated with FossilShield® at the dosage of 0.5 g/kg while this difference was observed with SilicoSec® at all dosages according to *t*-test (*P*<0.05).

Seed germination

In general, seed viability was not affected significantly by the two DE formulations (*P*<0.001). In the maize grains treated with FossilShield®, mean germination percentage ranged from 70-88.9% for CLH103 while on SHABA it ranged from 46.7-56.7% (Table 5). Means percentage of germination in CLH103 treated with SilicoSec® ranged from 80-90%, while in SHABA it ranged from 34.4-63.3%.

Table 4. Number of live insects of *Sitophilus zeamais* and percentage of seed damage for maize admixed with four concentrations of FossilShield® and SilicoSec® and stored for four months under ambient laboratory conditions (t ≈ 22.6-25.6°C and r.h. ≈ 70.7-80%).

Contents g/kg	FossilShield®		*t*-value	SilicoSec®		*t*-value
	CLH103	SHABA		CLH103	SHABA	
	Number of live insects					
0	661.7±100.24a	797.0±79.70a	-1.06ns	661.7±100.24a	797.0±79.70a	-0.58ns
0.5	0.0±0.00b	381.3±51.84b	-7.36*	74.0±37.61b	582.7±195.54ab	-1.58ns
1	0.3±0.3b	171.3±95.88bc	-1.78ns	4.7±2.91bc	287.0±76.22bc	-3. 70*
1.5	0.0±0.00b	19.0±13.05cd	-1.46ns	4.3±4.33bc	121.0±47.50cd	-2.44ns
2	0.0±0.00b	0.3±0.33d	-1.14ns	0.0±0.00c	59.0±16.70cd	-3.53*
Malathion 5%	0.0±0.00b	0.0±0.00d	/	0.0±0.00c	0.0±0.00d	/
M ± SE	110.3±61.43B	228.2±72.41B	-1.24ns	124.1±53.48A	307.8±77.50A	-1.74ns
F value	150.50***	37.36***		45.21***	20.74***	
	Seed damage (%)					
0	99.9±0.00a	99.8±0.00a	/	99.9±0.01a	99.8±0.00a	2.00ns
0.5	0.3±0.17bc	66.6±4.67b	-14.18***	16.0±9.49b	72.7±10.41b	-4.03*
1	0.5±0.18b	16.4±8.11c	-1.96ns	0.9±0.34c	31.0±3.89c	-7.71**
1.5	0.0±0.00c	3.8±1.89d	-2.01ns	1.47±0.92c	15.1±4.52cd	-2.96*
2	0.0±0.05bc	1.0±0.74d	-1.24ns	0.0±0.00c	7.42±1.54de	-4.80**
Malathion 5%	0.0±0.00c	0.0±0.00d	/	0.0±0.00c	0.0±0.00e	/
M ± SE	16.8±9.01B	31.3±9.40B	-1.11ns	19.7±8.90A	37.7±9.01A	-1.42ns
F value	2640.96***	103.15***		111.96***	72.96***	

Means ± S.E. in the same column within the same group of treatments, followed by the same capital or minuscule letter do not differ significantly at *P* = 0.05 (Tukey's test and *t*-test of Student). Each datum represents the mean of three replicates of 30 insects for each dosage. ns *P*>0.05; * *P*<0.05; ** *P*<0.01; *** *P*<0.001.

Table 5. Percentage (%±S.E.) of seed germination from two maize varieties treated with FossilShield® and SilicoSec® and untreated control after four month of storage under ambient laboratory conditions (t ≈ 24.1-25.6°C and r.h. ≈ 70.7-74.5%).

Contents g/kg	**Seed germination (% ± S.E.)**		***t*-value**
	CLH103	**SHABA**	
	FossilShield®		
0	0.0±0.00c	0.0±0.00b	/
0.5	88.9±4.02ab	46.7±1.93a	9.46***
1	80.0±5.10ab	52.2±4.85a	3.95*
1.5	70.0±5.10b	46.7±10.0a	2.07ns
2	85.6±2.94ab	56.7±3.84a	5.97**
Malathion 5%	91.1±2.94a	42.2±2.94a	11.77***
M ± SE	69.3±7.81A	40.7±4.87A	3.10**
F value	77.76***	39.71***	

Contents g/kg	Seed germination (% ± S.E.)		t-value
	CLH103	SHABA	
	SilicoSec®		
0	0.0±0.00b	0.0±0.00b	/
0.5	88.9±2.20a	42.2±4.00a	10.22**
1	80.0±3.33a	34.4±6.78a	6.03**
1.5	90.0±1.91a	43.3±0.00a	24.51***
2	90.0±5.10a	53.3±8.37a	3.74*
Malathion 5%	91.1±2.94a	41.1±2.94a	12.02***
M ± SE	73.3±8.07A	35.7±4.43A	4.08***
F value	62.14***	35.76***	

Percentage ± S.E. in the same column within the same group of treatments, followed by the same capital or minuscule letter do not differ significantly at P=0.05 (Tukey's test and *t*-test of Student). Each datum represents the mean of four replicates of 20 insects for each dosage. ns P > 0.05; * P <0.05; ** P <0.01; *** P <0.001.

Discussion

In this study, the mortality of *Sitophilus zeamais* caused individually by FossilShield® and SilicoSec® on the two maize varieties was significantly high compared to the untreated control. This mortality increased with concentrations and days of exposure. Similar results were obtained by a number of previous studies on different beetles exposed to different DE formulations (Ceruti *et al.*, 2008; Matti and Awaknavar, 2009; Nukenine *et al.*, 2010; Athanassiou *et al.*, 2011; Shams *et al.*, 2011; Khakame *et al.*, 2012; Chiriloaie *et al.*, 2014; Shafighi *et al.*, 2014). The work done by these authors showed the increased mortality of beetles with increased days of exposure. For each DE formulation used in the present study, mortality of *S. zeamais* did not exceed 10% with the two maize varieties within 1 day of exposure. But with increased concentration and exposure periods, this mortality was total at higher for the two maize varieties. The insecticidal efficacy of DE is determined by its degree of adherence to the kernel, a physical characteristic of each type of grain (Korunic, 1997, 1998). Athanassiou *et al.* (2005) and Nukenine *et al.* (2010) reported similar results with SilicoSec® on stored wheat against of *S. oryzae* (L.) and on maize against *S. zeamais* respectively. Since DE acts as a desiccant; when DE particles are picked up by the insect cuticle, the epicuticular layer is destroyed, resulting in death through water loss (Korunic, 1998; Subramanyam and Roesli, 2000). This might explain the highest mortality of weevil at the highest concentration of each DE in this study because active compound increase with the increased concentration so the insect picks up more DE particles in the highest concentration.

In our study, the difference on the percentage of reduction in F_1 emergence were observed on both maize varieties treated with each DE formulation according to the *t*-test of Student. Additionally, regardless of the initial number of adults exposed to treated maize, percentage of progeny reduction in treatment, compared to control, was higher for the two maize varieties treated with each DE in all exposure periods (1, 3, 7 and 14). FossilShield® and SilicoSec® were more effective on CLH103 (total reduction from 1.5 g/kg). This could be due to the total mortality of parents in the mortality test at these concentrations. Effective control of protectants is qualified as mortality of adult and/or immature, confirmed by lack of progeny generation (Hertlein *et al.*, 2011). On the other hand, on SHABA treated with each DE formulation, the percentage reduction in adult emergence was not total even at 2 g/kg. this could be due to the higher level of grain moisture content (13.20%) which is one of the most important factors influencing efficacy of DE in pest control products (Khakame *et al.*, 2012). Our results agree with those of Mewis and Ulrichs (2001) who did not recorded total suppression progeny emergence of *S. granaries*, insect of the same family as *S. zeamais* exposed to FossilShield® despite the total mortality recorded.

Seed damaged was significantly reduced for the two maize varieties treated with each diatomaceous earth after four months of storage. Matti and Awaknavar (2009) observed no percentage of seed damage in the sorghum treated with Protect-It at the dosage of 0.1 g. The studies of the previous authors agree with ours in the case of CLH103 variety treated with both DEs which recorded no percentage of seed damage at the concentration of 2 g/kg. In the SHABA variety, damage observed on grains may be attributed to the increase of population. Furthermore, the grains moisture content is one of the most important factors affecting efficacy of DE in pest control products (Khakame *et al.*, 2012). The moisture content of the two maize varieties used here were respectively 11.30% and 13.20% for CLH103 and SHABA under the ambient laboratory conditions (t ≈ 22.6-25.6°C; r.h. ≈ 70.7-80%). For that reason, the higher moisture content of SHABA variety compared to CLH103 may decrease the efficacy of DEs and permitted the development of *S. zeamais* (Snelson, 1987; Afridi *et al.*, 2001). In additional to reduce seed damages, since the mode of action of DE dusts is the desiccation, lower grain moisture content also increased their efficacy (Fields and Korunic, 2000). Khakame *et al.* (2012) observed in their study that when moisture content increased from 10 to 16%, the progeny emerged in grain treated with Actellic Super® dust increased from 0 to 0.5 compared to the untreated grain.

Generally seed viability was not affected by each DE. Matti and Awaknavar (2009) reported that Protect-it did not have negative effects on seed germination even exposed at different temperature and relative humidity. Also Nukenine *et al.* (2010) recorded percentage of germination of maize treated with SilicoSec® ranged between 66.7-70.0%. Our results suggest that the viability of the two maize varieties was not affected by each DE. However, the percentage of seed germination varies according the maize variety. Akob and Ewete (2007) observed that *E. grandis* ash at 2 g/2kg of maize protected grains for 6 months without any adverse affect on seeds germination. According to Couturon (1980), when environmental conditions are not well controlled, germination rate decreases quickly. That could partly explain the loss of viability.

Conclusion

The study showed that like the Malathion (positive control), FossilShield® and SilicoSec® is very effective against *Sitophilus zeamais* on the grains of two maize varieties (CLH103 and SHABA). Student *t*-test showed significant difference between the two maize varieties treated with FossilShield® and SilicoSec® in the reduction of adult emergence. Additionally, they can protect stored grains of the two maize varieties for 4 months without affecting the seed germination power**.** However, the two diatomaceous earths seem to have protected CLH103 variety more than SHABA. Therefore, FossilShield® and SilicoSec® could be favorably considered as alternative solution to Malathion against *Sitophilus zeamais*.

Abbreviations

CLH: Cameroon Lowland Hybrid; LC: Lethal Concentration.

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