

Full Length Research Paper

Diatomaceous earths: Alternative insecticides to Malathion in the Soudano-Guinean agro-ecological zone of Cameroon against stored maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae)

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Experiments were carried out in ambient laboratory conditions to evaluate the effect of Fossil-Shield and SilicoSec, and Malathion against *Sitophilus zeamais* on stored Cameroonian maize variety CMS8501. The DE products were applied at 0, 0.5, 1, 1.5 and 2 g/kg of maize grains and Malathion at 0.5 g/kg of maize. Adult mortality, F₁ progeny emergence, population increase, grain damage, grain weight loss, and seed germination were evaluated. Mortality was recorded at four exposure periods, followed by F₁ progeny production. Weevil population increase, grain damage, grain weight loss, and seed germination were recorded after four months of storage. Like Malathion, Fossil-Shield caused complete mortality of *S. zeamais* in 14 days of exposure at 1.5 g/kg, and at 2 g/kg SilicoSec achieved 96.67% mortality. Like Malathion, Fossil-Shield (99.80%) and SilicoSec (99.30%) completely inhibited the F₁ progeny emergence at 2 g/kg. Fossil-Shield was the best maize grain protectant with no live insects emerging, no seed damage and no grain weight loss, SilicoSec showed similar performance at 1.5 g/kg. The DE products do not affect the seed viability. Considering these results, Fossil-Shield and SilicoSec could replace Malathion used in stored maize grains according to their same efficacy and long term protection against the weevils.

Key words: Storage, diatomaceous earth formulations, effectiveness, *Sitophilus zeamais*, Malathion 5%, CMS8501.

INTRODUCTION

Subsistence farmers comprise some of the poorest and marginalized people across Africa and the most vulnerable to malnutrition. Their principal needs are

simple: food security in terms of production and storage. Unfortunately, stored product insects infest different agricultural products ranging from farm to industrial

products (Babarinde et al., 2013). In Cameroon and many other African countries, maize (*Zea mays* L.) is an important food crop (Nukenine et al., 2007) which is widely cultivated and consumed. It is a major source of dietary carbohydrates for both humans (Mebarkia et al., 2010) and animals. Unfortunately, one of the major problems encountered in agriculture in developing countries is postharvest losses which usually occur during storage (Adedire et al., 2011). Insect pests of stored products are a problem worldwide because they reduce the quantity and quality of stored grains (Rojht et al., 2012). The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), causes considerable damage to stored products, particularly in developing countries (Wanyika et al., 2009). Its attack starts from field, and becomes more important during storage. This insect is one of the major pests of stored maize grains in Cameroon. The control of *S. zeamais* populations around the world relies heavily on the continued applications of conventional insecticides (Nukenine et al., 2011). The organophosphate, Malathion is registered for use in grain storage in several Africa countries including Cameroon. However, its current application for the control of storage insect pests is limited because of resistance development by the pest, chemical residues in food, widespread environmental hazards, side effects on non-target organisms, and the associated high costs of application (Cherry et al., 2005). This situation requires a serious effort to develop safe, viable, environmentally friendly and effective substitutes to these chemicals for stored product protection.

The use of inert dusts such as diatomaceous earth (DE) formulations that are dry, chemically inert powders have been used as potential alternatives (Phillips and Throne, 2010; Shah and Khan, 2014; Kavallieratos et al., 2015). DE is one of the safest and most effective naturally occurring insecticides (Ebeling, 1971). The DE is an abrasive powder (Korunić, 2013). In fact, DE's mode of action is mainly by dehydration or desiccation. The DE's adsorptive capacity for lipids and its insecticidal efficacy are affected by size, shape and surface topography of diatom species, uniformity of particles, the purity of the formulation, ambient temperature and relative humidity, and type of grains stored (Rohitha et al., 2003; Korunić, 2013). During the past two decades, abundant literature on the efficacy of DE products to control stored product insects have been published. However, only a few studies were dedicated to the use of DE against *S. zeamais* or other insect pests in Cameroon despite the important destructive nature of *S. zeamais* on various maize varieties. Many maize varieties are cultivated in Cameroon, and the assessment of

insecticidal efficacy of diatomaceous earths on another important Cameroonian maize variety CMS8501 against *S. zeamais* is necessary. The objective of this study was to compare the insecticidal efficacy of Fossil-Shield or SilicoSec with Malathion 5% against *S. zeamais* on maize variety CMS8501 in the Soudano-Guinean agro-ecological zone of Cameroon.

MATERIALS AND METHODS

Insect rearing

Adults of *S. zeamais* were collected from a colony kept since 2010 in the laboratory of Applied Zoology, Faculty of Science, University of Ngaoundere. The weevils were reared on disinfested maize grains in 900 ml glass jars kept under ambient laboratory conditions (temperature: 23.73 - 26.26°C and relative humidity: 46.26 - 77.22%).

Maize variety

The maize used was a Cameroonian variety CMS8501 free of insecticides, which is the most cultivated variety in the Centre, South, East, Littoral, Southwest and North Regions of Cameroon. This maize grain has white color and rough texture. Grains were purchased from the Institute of Agricultural Research for Development, Nkolbisson-Yaounde, Cameroon. The grains were cleaned and disinfested by keeping them in a freezer at -14°C for 21 days prior to the bioassays. The maize was then kept under the experimental conditions for 2 weeks before use to allow its acclimatisation. The moisture content of maize seeds was 11.50%.

Insecticidal materials

The two DE formulations used were Fossil-Shield and SilicoSec obtained from Bein GmbH (Eiterfeld, Germany) and Biofa GmbH (Münsingen, Germany), respectively. Fossil-Shield is brown in color with a particle size of 5 to 30 µm and is composed of 73% amorphous SiO₂, 3% aerosol and other mineral compounds (Rohitha et al., 2003). SilicoSec is a formulation of diatomaceous earth containing 92% SiO₂, 3% Al₂O₃, 1% Fe₂O₃, 1% Na₂O, with 8 to 12 µm particle size (Ziaee et al., 2007) and is white in color. Malagrain composed of 5% Malathion was purchased from phytochemical inputs shop at the local market of Ngaoundere. It was applied at its recommended dosage (0.5 g/kg) and served as a reference insecticide. The two DE formulations and Malathion 5% were stored in the ambient laboratory conditions, until the beginning of the bioassays (approximately for a month).

Mortality test and F₁ progeny production

The mortality was obtained by adding separately 0.025, 0.5, 0.075, and 0.1 g of each DE formulation to 50 g of maize, giving the application rates of 0.5, 1, 1.5, and 2 g/kg in 1 L glass jars (Gabriel et al., 2016). Malathion served as positive control at the

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recommended dosage of 0.5 g/kg by adding 0.025 to 50 g of maize. The jars were shaken manually for 2 min to achieve uniform distribution of the dust in the entire grain mass. Untreated maize (50 g) served as negative control. Twenty unsexed weevils 7 to 14 days old were introduced into each jar. The jars were capped with perforated metal lids. The experiments were replicated four times. All treatments were maintained under ambient laboratory conditions and the temperatures ranging from 21.7 to 25.6°C and 76.1 to 79% of relative humidity. Mortality was recorded 1, 3, 7 and 14 days after infestation. The percentage mortality was corrected for control mortality using Abbott's formula (Abbott, 1925). At 14 days, weevil mortality was counted, and all weevils were removed in treated and untreated maize grains. The jars containing maize grains after discarding of weevils were kept in the same laboratory conditions for another 35 days to determine *S. zeamais* F1 progeny emergence. The counting of F₁ progeny was carried out once a week for 5 weeks. After each counting session, the weevils were removed from the jars. The inhibition rate of F₁ progeny (%IR) was calculated as:

$$\%IR = \frac{Cn - Tn}{Cn} \times 100$$

where Cn is the number of newly emerged insects in the untreated jar and Tn is the number of insects in the treated jar (Rajashekar et al., 2010).

Population increase and seed damage

About 0.5, 1, 1.5 and 2 g/kg of Fossil-Shield and SilicoSec were admixed separately with 200 g of maize seeds as described earlier. Thirty unsexed insects 7 to 14 days old were introduced into each glass jar containing treated and untreated maize grains. Each treatment was replicated four times. Four months after infestation, the number of live and dead insects, numbers of damaged and undamaged seeds were recorded. The percentage of grain damage was calculated using the following formula:

$$\text{Seed damage (\%)} = \frac{\text{Number of seeds damaged}}{\text{Total number of seeds}} \times 100$$

The weight of damaged seeds and that of undamaged seeds was recorded and the percentage weight loss was calculated according to Nukenine et al. (2010a):

$$\text{Percentage weight loss} = \frac{(\text{Initial weight} - \text{final weight})}{\text{initial weight}} \times 100$$

Seed germination

A total of 30 undamaged seeds (seeds that showed no visual damage) were picked randomly from each jar after separation of damaged and undamaged grains. The seeds were put on moistened sand in perforated plastic plates. These seeds were collected from those stored for 4 months as described earlier (population increase and damage). Each treatment was replicated four times. The number of germinated seeds was recorded after 10 days (Gabriel et al., 2016). The percentage of germination was determined using the following formula:

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds selected}} \times 100$$

Statistical analysis

Abbott (1925) formula was used to correct for control mortality before Probit analysis and ANOVA. Data on percentage cumulative corrected mortality, percentage reduction in F₁ progeny, percentage seed damage, and percentage germination were transformed using arcsine [(square root (x/100))] and the number of progeny produced was log-transformed (x+1) before conducting ANOVA statistics using the Statistical Analysis System (SAS Institute, 2003). Tukey's test ($P = 0.05$) was applied for mean separation. The toxicities of the two DE formulations were compared using Student's *t*-test.

RESULTS

Toxicity of Fossil-Shield and SilicoSec against *S. zeamais*

The mortality of *S. zeamais* increased with level of dosage and exposure periods for the two DE formulations (Figure 1). At 0.5 g/kg, no mortality was observed at 1 day of exposure in the maize grains treated with Fossil-Shield or SilicoSec. At the highest dosage (2 g/kg), mortality of *S. zeamais* adults was 10.70% in maize treated with Fossil-Shield after 1 day of exposure, whereas in grain treated with SilicoSec, the mortality was 3.30%. At 14 days of exposure, like Malagrain (Malathion 5%), Fossil-Shield caused complete mortality at 1.5 g/kg, while SilicoSec caused 96.70% mortality of the weevils at the highest dosage (2 g/kg). No significant differences in terms of mortality rates caused by Fossil-Shield and SilicoSec against *S. zeamais* adults were recorded at all the exposure periods and dosages (*t*-value; $P > 0.05$) (Figure 1).

F₁ progeny inhibition

Compared to untreated controls, the two DE formulations significantly inhibited F₁ production at all tested dosages (Table 1), which was dose dependent ($F_{(5, 35)} = 126.06$; $P = 0.0001$). The results revealed a significant difference between the two DE formulations at 1.5 g/kg in terms of the percent of inhibition in F₁ emergence ($t_{(3, 24)} = 3.29$; $P = 0.03$).

Similar to Malagrain (Malathion 5%), there was almost complete inhibition of F₁ progeny production (99.80%) observed in the maize grain treated with Fossil-Shield at the dosage of 1.5 g/kg and with SilicoSec (99.30%) at its highest dosage of 2 g/kg.

Population increase and grain damage

Fossil-Shield and SilicoSec significantly reduced the population growth of *S. zeamais* compared to untreated control ($P = 0.0001$) (Table 2). This suppression was dose-dependent for the two DE formulations. Like to Malathion 5% at 0.5 g/kg, Fossil-Shield at all tested

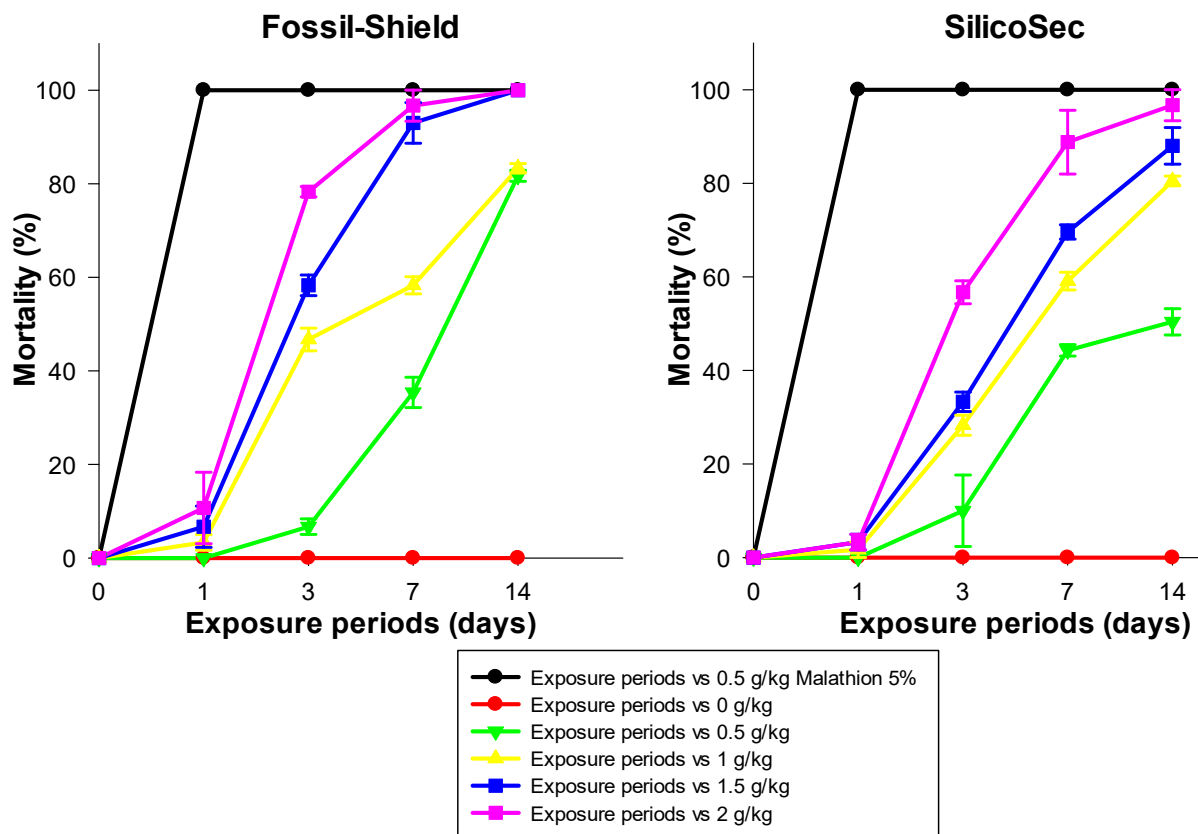


Figure 1. Corrected cumulative mortality (mean ± SE) of *S. zeamais* exposed to Fossil-Shield and SilicoSec.

Table 1. Progeny production of *S. zeamais* in maize grains treated with DE Fossil-Shield and SilicoSec.

Contents (g/kg)	Mean number of F ₁ progeny		t-value
	Fossil-Shield	SilicoSec	
0	124.30 ± 11.68 ^a	124.30 ± 11.68 ^a	
0.5	16.30 ± 5.46 ^b	19.30 ± 3.93 ^b	-0.45 ^{ns}
1	10.00 ± 5.20 ^{bc}	9.70 ± 1.20 ^b	0.06 ^{ns}
1.5	1.30 ± 0.67 ^c	7.70 ± 2.19 ^{bc}	-2.77 [*]
2	0.30 ± 0.33 ^c	1.00 ± 1.00 ^{cd}	-0.63 ^{ns}
Malathion 5%	0.00 ± 0.00 ^c	0.00 ± 0.00 ^d	
M ± MSE	25.40 ± 11 ^A	27.00 ± 10.81 ^A	
F value	55.89 ^{***}	109.8 ^{***}	
	%inhibition in adult emergence relative to control		
0	0.00 ± 0.00 ^c	0.00 ± 0.00 ^d	
0.5	87.20 ± 3.60 ^b	84.30 ± 2.89 ^c	0.62 ^{ns}
1	92.60 ± 3.78 ^{ab}	89.50 ± 3.27 ^b	0.10 ^{ns}
1.5	99.00 ± 0.49 ^a	93.90 ± 1.48 ^{ab}	3.29 [*]
2	99.80 ± 0.24 ^a	99.30 ± 0.73 ^a	0.65 ^{ns}
Malathion 5%	100.00 ± 0.00 ^a	100.00 ± 0.00 ^a	
M ± MSE	79.80 ± 8.75 ^A	78.30 ± 10.60 ^A	
F value	337.38 ^{***}	750.40 ^{***}	

Mean ± SE in the same column for same category of insecticide, followed by the same letter do not differ at *P* = 0.05 (Turkey's test). ^{ns}*P* > 0.05; ^{*}*P* < 0.05; ^{***}*P* < 0.001.

Table 2. Damage parameters and seed germination of maize admixed with four dosages of Fossil-Shield and SilicoSec and stored for four months.

Insecticidal products (g/kg)	Number of live insects	Seed damage (%)	Weight loss (%)	Seed germination (%)
Fossil-Shield				
0	733.70 ± 34.74 ^a	99.90 ± 0.00 ^a	86.5 ± 0.22 ^a	0.00 ± 0.00 ^b
0.5	0.00 ± 0.00 ^b	0.20 ± 0.16 ^b	0.00 ± 0.02 ^b	76.70 ± 6.96 ^a
1	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	87.80 ± 4.00 ^a
1.5	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	90.00 ± 1.91 ^a
2	0.00 ± 0.00 ^b	0.40 ± 0.41 ^b	0.00 ± 0.03 ^b	85.60 ± 5.57 ^a
Malathion 5%	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	88.90 ± 2.94 ^a
F value	1679.93 ^{***}	1204.93 ^{***}	99999.99 ^{***}	65.57 ^{***}
SilicoSec				
0	733.70 ± 34.74 ^a	99.90 ± 0.00 ^a	86.5 ± 0.22 ^a	0.00 ± 0.00 ^b
0.5	48.30 ± 19.84 ^b	6.80 ± 2.11 ^b	1.00 ± 0.40 ^b	66.70 ± 5.11 ^a
1	0.00 ± 0.00 ^c	0.29 ± 0.29 ^c	0.00 ± 0.02 ^c	75.60 ± 4.83 ^a
1.5	2.30 ± 1.86 ^c	0.41 ± 0.21 ^c	0.10 ± 0.04 ^c	77.80 ± 6.19 ^a
2	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	0.00 ± 0.03 ^c	74.40 ± 6.78 ^a
Malathion 5%	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	88.90 ± 2.94 ^a
F value	262.06 ^{***}	677.92 ^{***}	35475.95 ^{***}	56.53 ^{***}

Means ± SE in the same column within the same group of treatments, followed by the same letter do not differ significantly at $P = 0.05$ (Tukey's test). ^{ns} $P > 0.05$; ^{**} $P < 0.01$; ^{***} $P < 0.001$.

dosages achieved complete suppression of the population and no damaged grains were recorded. The same result was observed with SilicoSec at 1 g/kg. SilicoSec at the lowest content of 0.5 g/kg had 48.30 weevils and less than 6.8% of the grains were damaged. Even at lowest content of SilicoSec, treated grains appeared more protected than untreated ones.

Germination rate

The maize treated with either DE had significantly higher germination percentage whereas no grain germinated in the untreated control (Table 2). The percentage of seed germination of maize seeds treated with the two DE formulations was not significantly different from the percent recorded with positive control (Malathion 5%). The lowest germination percentage (66.70 and 76.70%, respectively for SilicoSec and Fossil-Shield) in treated maize grains was recorded at the lowest content (0.5 g/kg). The highest percentage seed germination (90%) was noticed at 1.5 g/kg in maize grains treated with Fossil-Shield, and seeds treated with SilicoSec at 1.5 g/kg recorded 77.80%. While the Malathion 5% recorded 88.90% seed germination.

DISCUSSION

Fossil-Shield and SilicoSec caused significant mortality of

S. zeamais in the maize grain. The insecticidal efficacy of the DE formulations was highly influenced by dosages, time of exposure and type of DE tested in this study. Previous investigations showed an increase mortality of stored-product beetles exposed to inert dusts with increasing dosages and time of exposure (Demissie et al., 2008; Wakil et al., 2011; Jean et al., 2015; Tofel et al., 2021). Therefore, at their higher dosage, the adsorption of wax and abrasiveness caused by the insecticidal products occurs faster, causing death in a shorter time compared with those at lower dosages. The death of insects caused by DE could be attributed to the dehydration provoked by the abrasiveness of the small particles of this inert dust and by adsorption of oils from the body of the insect (Korunić, 1997; Fields and Korunic, 2000; Athanassiou et al., 2007) which breaks the layer of wax on the epicuticle, exacerbating the fatal loss of water as reported by Subramanyam and Roesli (2000). Fossil-Shield is constituted by smaller particle size than that of SilicoSec, facilitating the adhesion on the surface of the gains and weevil (Subramanyam and Roesli, 2000). Besides, the different additives present in each DE formulation contribute to differentiate the efficacy of one diatomaceous earth to another (Athanassiou et al., 2007). This could explain the difference of efficacy of the DE formulation used in the present study. Nukenine et al. (2010b) reported that SilicoSec caused 100% mortality to *S. zeamais* within 14 days exposure in the Shaba maize variety at the dose rate of 2 g/kg. The results of these authors are similar to those recorded in the present study

within the same exposure period and dosage. Mewis and Ulrichs (2001) recorded complete mortality of *Tribolium confusum* and *Tenebrio molitor* in 13- and 14-days exposure on plywood plates with food, respectively, at 4 g/m² of Fossil-Shield. The exposure time is crucial for the effectiveness of DE, because the movement of insects increase the contact of the cuticle with dust particles (Athanassiou et al., 2005). In the current study, complete mortality was observed with Fossil-Shield at 1.5 g/kg in 14 days of exposure in maize variety CMS8501. The same tendency was recorded by Jean et al. (2015) using the same DE against *S. zeamais* on maize variety Shaba. These authors reported that SilicoSec was very efficient by causing 100% mortality of *S. zeamais* within 14 days exposure period.

In addition to causing adult mortality, the two DE formulations significantly reduced progeny emergence. Previous studies with SilicoSec demonstrated that this DE formulation is effective against several beetle species in several grain commodities, such as *Sitophilus oryzae* (L.) and *T. confusum* on oat, rye and triticale (Athanassiou et al., 2004), and *T. confusum* (Shams et al., 2011), and *S. zeamais* on maize (Nukenine et al., 2010b). Kavallieratos et al. (2005) reported that SilicoSec was effective against *Rhyzopertha dominica* in maize, wheat, barley, oats, and rice and *Callosobruchus maculatus* and *Sitophilus granarius* on wheat (Shams et al., 2011). The present work demonstrated that SilicoSec was also effective against *S. zeamais* in the CMS8501 maize variety. But the level of protection changes with the commodities and insect species. Gabriel et al. (2016) reported that, Fossil-Shield and SilicoSec better protected maize variety CLH103 than Shaba variety with total reduction in adult emergence. Athanassiou and Korunic (2007) emphasized that progeny production in a treated substrate is perhaps more important than parent mortality, because a grain protectant should protect the grain for a long storage period. In this study during four months of storage, the complete population suppression was obtained on maize treated with Fossil-Shield for all the dosages tested while in the maize treated with SilicoSec, the complete suppression of population was achieved at 1 g/kg. Rigaux et al. (2001) reported the intraspecific differential susceptibility of DE on the red flour beetle *T. castaneum*, and their studies showed wide variation in susceptibility to DE within one species. These results may explain why different researchers obtain different results while using the same source of DE formulation and the same insect species (Arnaud et al., 2005). The different insect species strains may have different susceptibilities to a given insecticide formulation. The reduction of damaged grain was observed in all treatments and no seed weight loss was recorded. Khakame et al. (2012) found a satisfactory level of protection against *S. zeamais* for nine months storage period using Dryacide dust. According to the results of the current work, maize may be effectively protected for at least four months in Ngaoundere-Cameroon by using

the two DE formulations tested in this study. In Cameroon, SilicoSec and Fossil-Shield have been tested on the maize variety Shaba against *S. zeamais* and given good results during four months of storage (Gabriel et al., 2016).

The percent seed germination was generally not affected by Fossil-Shield and SilicoSec. The difference was noticed between the percentage germination in treated maize seeds compared to the untreated seeds. This shows that, like Malathion 5%, Fossil-Shield and SilicoSec have no adverse effect on seed germination and protect stored seeds against damage by maize weevils. Stathers et al. (2002) also reported that DE products did not have any negative effects on seed germination. In other words, DEs do not inhibit the viability of treated maize seeds.

Conclusion

The results obtained from this work indicated that Fossil-Shield and SilicoSec have the same effectiveness like Malathion 5% when they are used against the major pest of maize *S. zeamais* for the long-term periods and they can prevent infestation of stored Cameroonian maize variety CMS8501 by this pest. Considering environmental issues, low mammalian toxicity and low effects on food, these DE formulations could serve as a good alternative to synthetic residual insecticides and could favourably replace Malathion which is commonly used to protect stored commodities in Cameroon for a period due to the negative effect of this conventional insecticide on human and environment. Therefore, there is the need to promote the use of these DE formulations for the biorational and sustainable protection of stored grains in the Soudano-Guinean agro-ecological zone of Cameroon against stored maize weevils. However, future studies should focus on the collection and testing of DEs from Cameroon for stored product protection.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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