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## RESEARCH ARTICLE

### EFFECT OF WOOD ASH AND LEAF POWDER ON THE FECUNDITY AND DEVELOPMENT OF IMMATURE STAGES OF *SITOPHILUS ZEAMAI* (COLEOPTERA: CURCULIONIDAE)

\*<sup>1,2</sup>Jean Wini Goudougou, <sup>2</sup>Elias Nchiwan Nukenine, <sup>3</sup>Christopher Suh and <sup>2</sup>Dieudonné Ndjonka

<sup>1</sup>Department of Biological Sciences, University of Bamenda, Bamenda, Cameroon  
<sup>2</sup>Department of Biological Sciences, University of Ngaoundéré, Ngaoundéré, Cameroon  
<sup>3</sup>Crop Protection Laboratory, IRAD, Bambui, Cameroon

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#### ABSTRACT

The maize weevil, *Sitophilus zeamais* is largely widespread maize pest across the world. It causes important losses of stored maize grain since infestation starts in field and continues in storage. Therefore, an environmentally safe and economically feasible pest control practice needs to be available. The effect of *Acacia polyacantha* and *Hymenocardia acida* wood ashes, *Plectranthus glandulosus* leaf powder and diatomaceous earth (Fossil Shield) was assessed on the development of the immature stages (egg, larva and pupa) and the fecundity of *S. zeamais*. Ten couples of insects were introduced in maize treated with sublethal contents to allow egg-laying. The number of eggs was counted after staining of infested grain. The batches of infested grain with different immature stages were treated with three contents of each powder. The number of adult emerged was recorded. The number of eggs laid by treated insects decreased slightly by increasing of product content. The application of wood ashes, leaf powder and diatomaceous earth did not suppress completely the development of immature stages. However the number of insects emerging from different stages was considerably reduced compared to the control (0 g/kg). Not significant difference ( $P > 0.05$ ) was observed concerning the number of insects emerging from different stages at 0 g/kg. The four insecticidal materials substantially reduced the fecundity and the development of immature stages.

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#### INTRODUCTION

The continuous growth of world population makes essential the mobilization of substantial resources for production of foodstuffs (Gwinner *et al.*, 1996; Ngamo *et al.*, 2007). The protection of stored food products occupies in fact a significant place. During storage, foods are currently destroyed by insects and other pests (Ngamo *et al.*, 2007). The insufficiencies of different storage methods in developing countries did not stop to cause grain losses and that in unacceptable proportions (Gwinner *et al.*, 1996). It is profitable and essential for the conservation of the environment to protect harvests from damage. An increase in production aiming at compensating for the losses of postharvest mobilizes additional capital, labour and natural resources. Consequently harvests protection is at same time the active protection of environment. Their role is to ensure on the one hand availability of food reserves in the zones touched by penury, and in addition to make possible

make possible to the peasants to delay the sale of their surpluses, that allows them to obtain an advantageous price. Maize weevil, *S. zeamais* is largely widespread in tropical and subtropical zones and as meets in moderate areas but it is not as resistant as *S. granarius* to cold temperature. *S. zeamais* can attack maize seed since field and infestation continues in storage. The adult insect is small size; it measures from 2.5 to 4 mm length and it is brown dark. It has a characteristic length rostrum and antennae bent out of bludgeons (Bijlmarkers and Verhoek, 1995). The development passes by following stages; egg, larva, pupa, nymph and then adult. The larva (not only the lava) develops inside the grain until emergence of adult and digs the latter as it grows (Danho and Haubruge, 2003). The larvae develop to the detriment of grain, where they consume the endosperm. The quantitative losses of stored products are due to the modifications of grains' water content during storage period, or to a deterioration of grains by harmful organisms (Gwinner *et al.*, 1996). *S. zeamais* causes several damages, which are qualitative and quantitative. The qualitative losses can take various forms. There are amongst other the change of colour, the modification of odour and taste,

\*Corresponding author: Jean Wini Goudougou,  
Department of Biological Sciences, University of Bamenda, Bamenda,  
Cameroon.

the loss in nutritional value (degradation of proteins and the vitamins), contamination of food products by mycotoxins or pathogenic agents and the loss of germinative ability of seeds (reference). Between harvest and consumption, more than 30% of the production is lost. This proportion is higher in sahelian zones according to the long period of storage (Ngamo and Hance, 2007).

After a survey to the farmers, Nukenine *et al.* (2002) recorded losses of 30% of stored cereals caused by *S. zeamais* in Adamaoua region in Cameroun. The damage of grains can reach up to 90% after five months of storage, when the food products are not protected (Nukenine *et al.*, 2002). Many methods are used to reduce the damage cause by maize weevil on stored cereals. Under many circumstances, the easiest, most rapid and economical method of controlling insects is the use of insecticides (Buxton *et al.*, 2014). However effective, the use of chemicals affects non-target organisms, leads to the development of insects' resistance and may even have effect on the users (Obeng-Ofori, 2008). Furthermore, the majority of farmers in Africa is resource-poor and has neither the means nor the skills to obtain and handle pesticides appropriately.

Therefore, an environmentally safe and economically feasible pest control practice needs to be promoted. Diatomaceous earths and botanicals are relatively environmentally safe. Diatomaceous earths are inert dust, which are extremely stable and do not react with other substances of the environment (Korunic, 1998). The use of natural products has been found to be very effective against stored product insect pests (Buxton *et al.*, 2014). Several plant products such as leaf powders, wood ashes, essential oils are used to protect stored maize seed against infestation by *S. zeamais*. Many strategies control are focused on the control of adult stage of this insect pest which is exogenous. Whereas the immature stages (egg, larvae, pupa) have an endogenous development. These stages develop and feed inside the grain; they are more harmful to stored grain. Thus the understanding and the management of immature stages and life cycle are the important parameters to insure a good preservation of stored gain. The objective of this study was, therefore, to assess the ability of wood ashes from *A. polyacantha* and *H. acida*, *P. glandulosus* leaf powder and diatomaceous earth (FossilShield) to reduce the development of the immature stages (egg, larva, pupa) and the fecundity of *S. zeamais* infesting maize grain.

## MATERIALS AND METHODS

### Insects rearing

Adults *S. zeamais* were obtained from a colony maintained in rearing since 2005 in the Applied Chemistry Laboratory of the University of Ngaoundere.

Then the insects' culture was transferred and kept in the Crop Protection laboratory of IRAD Bambui. The weevils were reared on disinfested maize in 900 ml glass jars and kept under ambient laboratory conditions ( $t = 23.08 \pm 2.05^\circ \text{C}$ ,  $rh = 74.67 \pm 14.36\%$ ). This culture was maintained and used as source of *S. zeamais*.

### Maize grains

The variety of maize used during all experimentation was "Shaba". That variety was provided by IRAD Wakwa, Adamaoua region in Cameroon. Before experimentation, the broken grain, the dirt such as piece of stone, sand was removed from the stock. Then the maize was kept in the freezer at  $-4^\circ \text{C}$  for 14 days to allow its disinfestation from all types of living organisms. The grains were kept in ambient conditions of laboratory for 14 days to allow its acclimatisation before using for bioassays.

### Preparation of ashes of *Acacia polyacantha* and *Hymenoptera acida*

Woods of *A. polyacantha* and *H. acida* were collected respectively in Kousseri, Logone and Chari Division, Far North Region and in Ngaoundéré, Adamaoua Region, Cameroon. The identification of both plants was confirmed by the Cameroon National Herbarium in Yaounde, where voucher samples were deposited. Woods were air-dried until completely moisture lost and burnt separately in a traditional kitchen normally used in the two zones. The obtained ashes were packaged in glass jars, labelled and kept in a refrigerator (at  $-4^\circ \text{C}$ ) until subsequent use for bioassays.

### Preparation of *Plectranthus glandulosus* leaf powder

The leaves of *P. glandulosus* were collected in July 2012 from Ngaoundere located in the Vina Division of the Adamawa region of Cameroon. The identification of the plant was confirmed at the Cameroon National Herbarium in Yaounde. The leaves were dried at room temperature for seven days, and then crushed. The crushed leaves were ground until the powder passed through a 0.20 mm sieve. Part of powder was stored in a deep-freezer at  $-20^\circ \text{C}$  until bioassays and the other part was used for essential oil extraction.

### Diatomaceous earth

The diatomaceous earth (DE) commercially known as Fossil Shield (FS 90.0s, Bein GmbH, Germany) was used. It has a particle size of 5–30  $\mu\text{m}$  and is composed of 73% amorphous  $\text{SiO}_2$ , 3% aerosol, water content of approximately 2% and other mineral compounds. The fossil Shield used during all experiment was brown.

### Analysis of volatile compounds for *Plectranthus glandulosus* leaf powder

The essential oil was extracted by hydrodistillation during 4 h using a Clevenger type apparatus. The extracted oil was kept in brown bottle at  $4^\circ \text{C}$  until its use for GC-MS analysis. The GC-MS analysis were carried out thanks to a chromatograph model Agilent 7890A GC equipped with an automatic injector and a column HP-1MS (15 m  $\times$  0.25 mm d.i; 0.25  $\mu\text{m}$  film thickness) coupled to a mass detector Agilent 7890A MSD. The molecules were bombarded by an electronic beam of 70 eV. The gaze vector was helium (1 mL/min) with a pressure of 25 psi at the beginning of column.

The injector temperature was 250°C. The programming of temperature consisted to the increasing of 60 to 230° C with the stage of 2° C/min then 35°C/min to reach 230° C. The injection was done by split mode with the coefficient of 1/180. The injected quantity of essential oil of *P. glandulosus* was 0.2 µl. The detection was done by a quadripolar analyser constituted by an assembling of four parallel cylindrical electrodes. The temperature of source was 150°C. The bombardment of essential oil with the electronic beam of 70 eV induced its ionisation and its fragmentation. Then the positive ionic fragments formed the characteristic mass spectrum of compound. The obtained spectra were compared with computerized data base using NIST/EPA/NIH Mass Spectral Library (NIST, 1999), Wiley Register of Mass Spectral Data (Mc Lafferty and Stauffer, 1994; König *et al.*, 2001).

#### Determination of chemical composition of wood ashes

Ashes were subjected to a mineralisation in order to determine the mineral contents (Pauwels *et al.*, 1992). The sample of each ash was burnt at 450°C for a complete mineralisation. Burnt ash was dissolved in nitric acid (HNO<sub>3</sub>) 1M for digestion then carried to boiling. The solution was filtered after cooling. The filtrate obtained was used to proportion the following cations: P, K, Ca, Mg, Na, Fe, Mn, Zn and Pb. Ca, K, Na were proportioned by flame photometry using the extract obtained by mineralisation. Mg, Fe, Mn, Zn and Pb were proportioned by atomic absorption spectrometry. Concerning phosphate, its content was measured by molecular absorption spectrophotometry.

#### Assessment of the effect of insecticidal powders on Fecundity and fertility

To examine the possible reproductive impact of the insecticidal products on the treated insects, ten 1 to 2 days old couples of the insect were introduced in 250 ml glass bottles containing 50 g of maize. The sublethal contents of 0.05; 0.1 and 0.15 g for each product were considered (Stamopoulos *et al.*, 2007) were introduced each separately in each glass jars containing 50 g/kg to constitute the contents of 1; 2 and 3g/kg. Fossil Shield was used at the doses of 0.005; 0.01 and 0.015 g corresponding to 0.1; 0.2 and 0.3 g/kg respectively. Controls consisted of substrate without insecticidal product. Each treatment was replicated four times.

After seven days of exposure to the products, the surviving insects were removed and placed on 50 untreated maize grains and left for seven days for oviposition to occur. The number of eggs laid was then counted, for both batches of maize (treated and untreated). The method described by Holloway (1985) and used by Danho and Haubruge (2000) was applied to count eggs laid by female. The grains were introduced first in water for one minute to humidify them and then placed for two minutes in a solution of acid fuchsine 0.5% which colored mucilaginous plugs in red cherry. The exceeding of color was reduced by introducing the grain in clean water for one minute. The grains were then placed on paper to dry them and the count of the eggs was done with a microscope.

#### Determination of immature stages development

The procedure of Obeng-Ofori and Amiteye (2005) with little modification was employed for the bioassay. Three jars containing each 2 kg of maize were infested with 200 adults *S. zeamais* of mixed sex. After 6 days of oviposition, the parent adults were removed. One day after adult removal, the infested maize grain from the first jar was divided and 50 g of grain were introduced in small glass jars. Then, they were treated with 0.5, 2 and 4g of *P. glandulosus* leaf powder, *A. polyacantha* and *H. acida* wood ashes and 0.05, 0.075 and 0.1 g of Fossil Shield.

The same treatment was carried out for the second and the third jars after 14 and 21 days respectively of parents' removal. These different post infestation periods used for this bioassay permitted to determine the toxicity of the treatments on the egg, larval and pupal stages. Observations were done every week and the counting of F<sub>1</sub> progeny was carried out once a week for 5 weeks starting from the sixth weeks after infestation. Each treatment was replicated four times.

#### Data analysis

The number of eggs and F<sub>1</sub> progeny emerging from different stages were log-transformed (x + 1). The transformed data were subjected to the ANOVA procedure using the Statistical Analysis System (Zar, 1999; SAS Institute, 2003). Tukey's test (P = 0.05) was applied for mean separation.

## RESULTS

#### Chemical composition of *P. glandulosus* essential oil

The volatile constituents of the essential oils from the leaf powder of *P. glandulosus* were identified by their retention indices and mass spectra in comparison with those of standard synthetic compounds. The results of the chemical analysis are presented in Table 1.

The dominated chemical constituents were terpenic compounds. The major compounds were Thymol (10.1%),  $\alpha$ -Terpineol (10.8%),  $\alpha$ -Pinene (11.5%), 3-Carene (8.7%),  $\beta$ -Myrcene (9.7%), Pinene (11.5%),  $\alpha$ -Pinene (11.2%) and L- $\beta$ -Pinene (11.5%) which are monoterpenes. The only major constituents, which were not monoterpenes, were  $\beta$ -Caryophyllene oxide (9.4%) which is oxygenated sesquiterpene and Acetophenone (5.5%) which is aromatic compound.

#### Mineral content of wood ashes

The mineral content of wood ash was different according to the species of plant (Table 2) except Sodium, Zinc, lead and Manganese where the two wood ashes were found in the same proportion.

The amount of calcium, iron and phosphor were higher in *H. acida* ash than in *A. polyacantha* ash. But the content of Magnesium and Potassium were higher in *A. polyacantha* wood ash than those in *H. acida* wood ash.

Table 1. Chemical constituents of essential oil from *Plectranthus glandulosus* leaf powder

Retention time (min)	Compounds	% composition
Hydrocarbonated monoterpenes		
13.890	(+)-4-Carene	0.3
24.209	1R- $\alpha$ -Pinene	11.2
25.809	3-Carene	8.7
37.267	$\beta$ -Phellandrene	tr.
38.102	$\gamma$ -Terpinene	0.1
42.938	$\beta$ -Thujene	0.2
54.825	2-Methyladamantane	0.8
62.678	$\beta$ -Myrcene	9.7
63.183	$\beta$ -Pinene	11.5
63.526	$\alpha$ -Terpinene	1.3
63.569	Paracymene	7.5
63.967	Limonene	2.5
64.085	(E)- $\beta$ -Ocimene	0.3
64.396	(E)-4, 8-Dimethyl-1, 3, 7-nonatriene	tr.
Hydrocarbonated sesquiterpenes		
16.661	7- $\beta$ -[H]-silphiperfol-5-ene	1.2
18.715	Silphin-1-ene	tr.
20.041	Silphiperfol-5, 7(14)-diene	0.1
22.366	$\alpha$ -Copaene	2.7
43.445	Pethybrene	0.3
44.499	Modhephene	0.3
45.649	$\alpha$ -Isocomene	0.2
51.982	$\alpha$ -Curcumene	0.2
52.914	$\gamma$ -Gurjumene	tr.
53.418	(Z, E)- $\alpha$ -Farnesene	2.5
54.670	$\beta$ -Selinene	0.1
Oxygenated monoterpenes		
11.860	Thymol	10.1
11.939	O-Acetylthymol	0.8
15.998	Neryle acetate	0.3
21.231	(E)- $\beta$ -Damascenone	0.4
62.872	p-Methoxycumene	tr.
62.978	4'-Methoxyvalerophenone	tr.
64.643	$\alpha$ -Terpineol	10.8
65.783	8, 9-Dihydrothymol	tr.
65.868	$\beta$ -Cyclocitral	0.1
66.451	Bornyle acetate	tr.
77.876	(E, E)-2, 4-Decadienal	0.1
80.351	$\alpha$ -Terpenyle	0.1
Oxygenated sesquiterpenes		
54.849	Cubebol	tr.
56.464	$\beta$ - Caryophyllene oxide	9.4
59.493	$\beta$ -Oplophenone	tr.
Aromatic compounds		
26.652	3,4-Xylenol	tr.
59.505	2-(2-Butynyl)- Cyclohexanone	0.1
62.721	Acetophenone	5.5
Aldehydes		
49.354	Lauraldehyde	0.1
64.149	Nonenal	0.3
64.460	(E)-2-nonanal	0.1
64.675	Decanal	0.1
65.910	(E)-2-Decenal	0.1
72.190	Undecanal	0.2
Ketones		
53.168	Tridecan-2-one	tr.
66.526	Decan-2-one	tr.
Ester		
63.419	cis-hex-3-enyl-acetate	0.5

tr.: &lt; 0.1

### Eggs laid by untreated parents on treated grain with low contents of products and their development in adult

The number of eggs laid on treated grain decreased slightly by increasing of products content levels (Table 3). The highest eggs laid were observed on untreated maize grain. The same tendency was observed concerned the eggs hatched, which was assessed by F<sub>1</sub> adult coming from those eggs (Table 3).

The lowest number of eggs laid was recorded at the highest content for each powder. The ash of *H. acida* and Fossil Shield achieved almost complete inhibition of egg development. There were recorded the lowest number of eggs and adult emerging on the grain treated with highest content of *H. acida* ash (3 g/kg) and Fossil Shield (0.3 g/kg). Even at the lowest content, the four insecticidal materials considerably reduced the number of eggs laid and their development in adult compared to the control.

**Table 2. Chemical composition of *Hymenocardia acida* and *Acacia polyacantha* wood ashes**

Cations	<i>H. acida</i>	<i>A. polyacantha</i>
	Content (mg/kg)	
Calcium	5800	2640
Magnesium	851	3742
Potassium	997	1118
Sodium	289	289
Iron	778	608
Zinc	786	786
Lead	0.0019	0.0019
Manganese	0.011	0.011
Phosphor	2782	1828

**Table 3. Number of eggs laid by untreated parents on maize grain treated with low contents of *Acacia polyacantha* and *Hymenocardia acida* wood ashes, *Plectranthus glandulosus* leaf powder and FossilShield and their development in adult ( $t = 24.13 \pm 1.96^\circ \text{C}$ ;  $hr = 67.32 \pm 10.16\%$ )**

Products and contents (g/kg)	Eggs (mean $\pm$ SE)	Adult $F_1$ (mean $\pm$ SE)
<i>Acacia polyacantha</i>		
0	22.75 $\pm$ 0.85 <sup>a</sup>	16.75 $\pm$ 1.03 <sup>a</sup>
1	13.50 $\pm$ 2.63 <sup>b</sup>	7.00 $\pm$ 0.41 <sup>b</sup>
2	6.25 $\pm$ 1.11 <sup>c</sup>	3.50 $\pm$ 0.65 <sup>c</sup>
3	4.00 $\pm$ 0.82 <sup>c</sup>	2.25 $\pm$ 0.48 <sup>c</sup>
$F_{(3; 12)}$	29.95***	91.96***
<i>Hymenocardia acida</i>		
0	22.75 $\pm$ 0.85 <sup>a</sup>	16.75 $\pm$ 1.03 <sup>a</sup>
1	6.75 $\pm$ 2.02 <sup>b</sup>	3.75 $\pm$ 1.11 <sup>b</sup>
2	4.50 $\pm$ 1.85 <sup>b</sup>	2.50 $\pm$ 1.04 <sup>b</sup>
3	2.25 $\pm$ 1.44 <sup>b</sup>	0.50 $\pm$ 0.25 <sup>b</sup>
$F_{(3; 12)}$	33.74***	59.98***
<i>Plectranthus glandulosus</i>		
0	22.75 $\pm$ 0.85 <sup>a</sup>	16.75 $\pm$ 1.03 <sup>a</sup>
1	8.25 $\pm$ 2.59 <sup>b</sup>	5.00 $\pm$ 1.41 <sup>b</sup>
2	5.75 $\pm$ 1.11 <sup>b</sup>	3.50 $\pm$ 0.65 <sup>b</sup>
3	2.75 $\pm$ 0.95 <sup>b</sup>	1.25 $\pm$ 0.48 <sup>b</sup>
$F_{(3; 12)}$	32.86***	51.71***
FossilShield		
0	22.75 $\pm$ 0.85 <sup>a</sup>	16.75 $\pm$ 1.03 <sup>a</sup>
0.1	6.75 $\pm$ 2.10 <sup>b</sup>	4.00 $\pm$ 1.08 <sup>b</sup>
0.2	4.75 $\pm$ 0.75 <sup>bc</sup>	2.75 $\pm$ 0.48 <sup>b</sup>
0.3	1.25 $\pm$ 0.75 <sup>c</sup>	0.75 $\pm$ 0.48 <sup>b</sup>
$F_{(3; 12)}$	58.07***	78.23***

Means  $\pm$  S.E. followed by the same letter in column do not differ significantly at  $P < 0.05$  (Tukey's test). S.E.: standard error.  
\*\*\*  $P < 0.0001$ .

### Eggs laid by treated parents with low content of products on untreated maize grain and their development in adult

The treated parents conserved their ability to lay eggs and that of development of eggs in adult (Table 4). However, the number of eggs decreased according to the increasing of contents. Generally, not all the eggs hatched and development in adult. The important eggs number was observed for all the products even at the highest content level. The untreated insect laid 48 eggs, where 36.75 developed in adult  $F_1$ . The highest number of eggs laid (37.25) and insects emerging (28.50) from treated insects were recorded with *P. glandulosus* leaf powder at content of 1g/kg. Whereas the lowest number of eggs laid and insects emerging from treated parents were observed with Fossil Shield at 0.3 g/kg. The same performance was practically observed for insects parents treated with *H. acida*

ash at 3 g/kg. The insects treated with *A. polyacantha* and *H. acida* wood ashes had almost the same performance.

**Table 4. Eggs laid by parents first treated with low contents of *Acacia polyacantha* and *Hymenocardia acida* wood ashes, *Plectranthus glandulosus* leaf powder and FossilShield on untreated maize grain and their development in adult ( $t = 24.13 \pm 1.96^\circ \text{C}$ ;  $hr = 67.32 \pm 10.16\%$ )**

Products and contents (g/kg)	Eggs (mean $\pm$ SE)	Adult $F_1$ (mean $\pm$ SE)
<i>Acacia polyacantha</i>		
0	48.00 $\pm$ 4.08 <sup>a</sup>	36.75 $\pm$ 3.12 <sup>a</sup>
1	34.00 $\pm$ 2.35 <sup>b</sup>	23.25 $\pm$ 1.60 <sup>b</sup>
2	26.75 $\pm$ 1.03 <sup>bc</sup>	18.25 $\pm$ 0.75 <sup>bc</sup>
3	22.00 $\pm$ 0.58 <sup>c</sup>	15.00 $\pm$ 0.58 <sup>c</sup>
$F_{(3; 12)}$	21.82***	27.83***
<i>Hymenocardia acida</i>		
0	48.00 $\pm$ 4.08 <sup>a</sup>	36.75 $\pm$ 3.12 <sup>a</sup>
1	34.25 $\pm$ 5.34 <sup>ab</sup>	25.00 $\pm$ 4.90 <sup>ab</sup>
2	22.50 $\pm$ 4.97 <sup>b</sup>	15.25 $\pm$ 3.54 <sup>b</sup>
3	18.50 $\pm$ 6.54 <sup>b</sup>	13.50 $\pm$ 4.50 <sup>b</sup>
$F_{(3; 12)}$	6.24*	7.25*
<i>Plectranthus glandulosus</i>		
0	48.00 $\pm$ 4.08 <sup>a</sup>	36.75 $\pm$ 3.12 <sup>a</sup>
1	37.25 $\pm$ 6.74 <sup>ab</sup>	28.50 $\pm$ 1.50 <sup>ab</sup>
2	34.25 $\pm$ 2.66 <sup>ab</sup>	23.25 $\pm$ 1.84 <sup>b</sup>
3	29.50 $\pm$ 2.22 <sup>b</sup>	20.25 $\pm$ 1.60 <sup>b</sup>
$F_{(3; 12)}$	3.33 <sup>ns</sup>	11.66**
FossilShield		
0	48.00 $\pm$ 4.08 <sup>a</sup>	36.75 $\pm$ 3.12 <sup>a</sup>
0.1	34.00 $\pm$ 1.68 <sup>ab</sup>	23.50 $\pm$ 1.19 <sup>b</sup>
0.2	22.50 $\pm$ 4.92 <sup>b</sup>	14.25 $\pm$ 3.33 <sup>b</sup>
0.3	18.50 $\pm$ 3.75 <sup>b</sup>	12.50 $\pm$ 2.60 <sup>b</sup>
$F_{(3; 12)}$	12.13**	16.37***

Means  $\pm$  S.E. followed by the same letter in column do not differ significantly at  $P < 0.05$  (Tukey's test). SE: standard error

<sup>ns</sup>  $P > 0.05$ , \*  $P < 0.05$ , \*\*  $P < 0.001$ , \*\*\*  $P < 0.0001$ .

### Effect of different products on the development of immature stages

The effect of the four powders to inhibit the development of immature stages (eggs, larvae, pupae) is shown in Table 5. The reduction of number of adult emergence, where the insecticidal treatments were applied varied according to the products and insect stages. For a given treatment on different stages, the number of emerged insects decreased when the content increased. The emerged adult from different treated stages was lower for all content levels compared to the control. The number of emerged adult at 0 g/kg from eggs (18.25), larvae (18.75) and pupae (17.25) did not show any difference ( $P > 0.05$ ).

However, there was not complete inhibition of development of immature stages in adult by *A. polyacantha* ash, *H. acida* ash, *P. glandulosus* leaf powder and Fossil Shield. The lowest emergence of adults (2.25 insects) was obtained when grains were treated with *A. polyacantha* (20 g/kg) at egg stage. *H. acida* and *P. glandulosus* recorded 4.25 and 5.00 insects respectively at their highest content (20 g/kg) as the egg stage was concerned. At the same egg stage, the number of emerging *S. zeamais* in maize treated with highest content (1.5 g/kg) of Fossil Shield was 5.02 insects. The emerging adult from treated larvae was significantly reduced. However the number of adults emerging from this stage remained important compared to the others stages.

**Table 5.** Effect of *Acacia polyacantha* and *Hymenocardia acida* wood ashes, *Plectranthus glandulosus* leaf powder and Fossil Shield on immature stages of *Sitophilus zeamais* ( $t = 22.98 \pm 1.98^\circ \text{C}$ ;  $hr = 70.40 \pm 7.36\%$ )

Products and Content (g/kg)	Treated insect stage / Number of progeny emerged (mean $\pm$ SE)			
	Eggs	Larvae	Pupae	F <sub>(2, 9)</sub>
<i>Acacia polyacantha</i>				
0	18.25 $\pm$ 2.75 <sup>Aa</sup>	18.75 $\pm$ 0.48 <sup>Aa</sup>	17.25 $\pm$ 0.85 <sup>Aa</sup>	0.90 <sup>ns</sup>
5	15.25 $\pm$ 2.71 <sup>ABa</sup>	15.03 $\pm$ 0.21 <sup>ABa</sup>	6.25 $\pm$ 1.03 <sup>Bb</sup>	9.32*
10	8.00 $\pm$ 1.08 <sup>BCab</sup>	11.75 $\pm$ 1.70 <sup>Ba</sup>	5.25 $\pm$ 1.11 <sup>Bb</sup>	6.23*
20	2.25 $\pm$ 0.25 <sup>Ca</sup>	6.23 $\pm$ 1.63 <sup>Ca</sup>	3.01 $\pm$ 0.41 <sup>Ba</sup>	4.08 <sup>ns</sup>
F <sub>(3, 12)</sub>	13.00***	20.16***	57.68***	
<i>Hymenocardia acida</i>				
0	18.25 $\pm$ 2.72 <sup>Aa</sup>	18.75 $\pm$ 0.48 <sup>Aa</sup>	17.75 $\pm$ 0.85 <sup>Aa</sup>	0.90 <sup>ns</sup>
5	7.75 $\pm$ 0.48 <sup>Ba</sup>	12.00 $\pm$ 1.08 <sup>Ba</sup>	11.03 $\pm$ 1.87 <sup>Ba</sup>	3.03 <sup>ns</sup>
10	6.00 $\pm$ 0.82 <sup>Bb</sup>	10.25 $\pm$ 0.85 <sup>Ba</sup>	4.25 $\pm$ 0.25 <sup>Ca</sup>	19.59**
20	4.25 $\pm$ 1.25 <sup>Bab</sup>	8.75 $\pm$ 1.55 <sup>Ba</sup>	4.02 $\pm$ 0.41 <sup>Cb</sup>	5.20*
F <sub>(3, 12)</sub>	16.06***	17.23***	38.24***	
<i>Plectranthus glandulosus</i>				
0	18.25 $\pm$ 2.72 <sup>Aa</sup>	18.75 $\pm$ 0.48 <sup>Aa</sup>	17.75 $\pm$ 0.85 <sup>Aa</sup>	0.90 <sup>ns</sup>
5	9.75 $\pm$ 3.01 <sup>ABa</sup>	12.07 $\pm$ 1.15 <sup>Ba</sup>	7.75 $\pm$ 0.25 <sup>Ba</sup>	1.32 <sup>ns</sup>
10	6.75 $\pm$ 1.44 <sup>Bab</sup>	11.03 $\pm$ 1.07 <sup>Ba</sup>	4.75 $\pm$ 0.63 <sup>Cb</sup>	8.43*
20	5.00 $\pm$ 1.08 <sup>Bb</sup>	9.02 $\pm$ 1.10 <sup>Ba</sup>	3.05 $\pm$ 0.41 <sup>Cb</sup>	11.20*
F <sub>(3, 12)</sub>	7.02*	19.19***	128.29***	
FossilShield				
0	18.25 $\pm$ 2.72 <sup>Aa</sup>	18.75 $\pm$ 0.48 <sup>Aa</sup>	17.75 $\pm$ 0.85 <sup>Aa</sup>	0.90 <sup>ns</sup>
0.5	7.25 $\pm$ 0.63 <sup>Bb</sup>	16.00 $\pm$ 0.71 <sup>ABa</sup>	9.25 $\pm$ 1.25 <sup>Bb</sup>	25.65***
1	5.75 $\pm$ 0.48 <sup>Bb</sup>	12.05 $\pm$ 1.22 <sup>BCa</sup>	7.50 $\pm$ 0.65 <sup>BCb</sup>	14.53*
1.5	5.02 $\pm$ 0.41 <sup>Bb</sup>	11.12 $\pm$ 1.47 <sup>Ca</sup>	4.02 $\pm$ 0.71 <sup>Cb</sup>	15.18**
F <sub>(3, 12)</sub>	18.76***	11.77**	42.52***	

Means  $\pm$  S.E. followed by the same capital letter in column and the same lower letter in the line do not differ significantly at  $P < 0.05$  (Tukey's test). SE: standard error

<sup>ns</sup>  $P > 0.05$ . \*  $P < 0.05$ . \*\*  $P < 0.001$ . \*\*\*  $P < 0.0001$ .

At 20 g/kg, the number of adult emerging from larvae stage was 6.23, 8.75 and 9.02 respectively for *A. polyacantha*, *H. acida* and *P. glandulosus*. Concerning the same parameter, Fossil Shield at its highest content (1.5 g/kg) recorded 11.12 adults. The number of progeny emerged from pupae in maize treated with insecticidal materials was in general lower than those emerging from eggs and larvae (Table 5). At 10 g/kg, 5.25 insects were registered when maize was treated with *A. polyacantha* at pupal stage, whereas at same content at the eggs and larvae stages, the number of insects was respectively 8.00 and 11.75. *H. acida* and *P. glandulosus* at same content recorded respectively 4.25 and 4.75 insects when applied at pupae stage.

The same powders in the same order recorded 6.00 and 6.75 insects when the treatment was applied at the eggs stage and 10.25 and 11.03 insects at larvae. The same tendency was observed for Fossil Shield. At content level of 1.5 g/kg, there were recorded 5.02, 11.12 and 4.02 respectively from eggs when Fossil Shield was applied from larvae and pupae stages. The development of larvae in maize treated with Fossil Shield into adult was important compared to the others stages. At 0.5 g/kg of Fossil Shield, the number of emerging insects from larvae was 16.00 insects whereas those emerging from eggs and pupae were 7.25 and 9.25 respectively. Generally the larval stage was more tolerant than egg and pupal stages to the different treatments. Egg and pupal stages had practically the similar susceptibility to the four insecticidal materials.

## DISCUSSION

The parameters of development and growth of insect population are very important to take in consideration for a best insect pest management.

In addition to cause mortality of adult insects, it is important for insecticidal to reduce fecundity and fertility and to inhibit the development of immature stages. The number of eggs laid by non-treated parents introduced on treated maize was less than that laid by the same survival parents after their introduction in untreated maize grain. Without treatment the number of eggs laid by the female *S. zeamais* was different. The female aged to 0-2 days old laid less eggs. But the same female after 7 days then 7-9 days old laid more eggs. The female of *S. zeamais* lays fewer eggs just after emergence, the number of eggs increase by increasing of age up to the optimum where the egg-laying is cancelled.

According to Delobel and Tran (1993) under optimum conditions of life, the female *S. zeamais* lays two eggs per day and when it is 22 days old, it lays approximately six eggs per day to the maximum, about its third month this number is cancelled (Delobel and Tran, 1993). The fewer number of eggs laid on treated grain could be explained by the dissuasive effect that inert dusts and leaf powder induced when they are mixed with the grain. The number of eggs was reduced for the treated insects compared to the control (non-treated insect). The F<sub>1</sub> adult emerged from these two batches followed the same tendency as the egg-laying. Not all the eggs hatched even those laid by untreated insects on untreated maize grain. Danho et al. (2002) reported that not all the eggs laid by *S. zeamais* developed into adult weevils. The four insecticidal material exhibited different efficacy levels on the development and the fecundity of *S. zeamais*. The two wood ashes were highly toxic and this toxicity was different according to the plant species. *H. acida* wood ash was more effective than those of *A. polyacantha*. Gwinner et al. (1996) reported that the effect of ash varies according to the type of ash. The desiccant powders such as wood ash and diatomaceous earth could make the grain

harder and non-suitable for development of offspring. Khanam *et al.* (2014) observed that the fecundity of *T. castaneum* and *T. confusum* was seriously reduced by application of plants extracts such as *Trichosanthes palmata* seeds and *Zingiber cassumunar* rhizome. The efficacy of plant was significant because the eggs of genus *Tribolium* are not laid out of the grain that allows the extracts to have a good bioactivity.

But in *S. zeamais*, after laying eggs, the female deposits the eggs into the grain and covers it with gelatinous material that close the ovoposited hole for protection and waits for hatching (Hall, 1991; Jood *et al.*, 1992; Umoetok, 2004). *A polyacantha* and *H. acida* wood ashes, *P. glandulosus* leaf powder and diatomaceous earth (FossilShield) influenced negatively the development of immature stages. The inhibition action varied according to the stages. The larval stage revealed more tolerant to the four products than the egg and pupal stages.

All the *S. zeamais* development stages except adult occur inside the grain. Then to disturb the development of *S. zeamais*, it is necessary for insecticidal materials to make the grain non-suitable for development or to diffuse inside the grain. Many works (Nukenine *et al.*, 2007; Nukenine *et al.*, 2010a; Tofel *et al.*, 2014) reported that *P. glandulosus* leaf contains some terpenes such as thymol, mentol, caryophyllene, pinene as observed in the present chemical analysis of the essential oil of this plant. However for the same plant species, there was obtained different chemical composition, Nukenine *et al.* (2010a) found that the major compounds of *P. glandulosus* were fenchone, cis-piperitone, piperitone oxide, piperitone epoxide, terpineolene and thymol. Whereas in the present study the majors compounds were  $\alpha$ -pinene,  $\beta$ -pinene,  $\beta$ -myrcene,  $\alpha$ -terpineol, thymol,  $\beta$ -caryophyllene.

The composition of essential oil of given plant species can vary dramatically according to the part of the plant from which the oil is extracted (leaf, fruits, flowers, stem, etc), the phenological state of the plant, the season, the climate, the soil type (Zhao *et al.*, 2014; Onyambu *et al.*, 2015). Chaubey (2012) found that caryophyllene; pinene reduced significantly oviposition potential of adults inhibited pupation and adult emergence in larvae of *Tribolium*. That earlier reported findings could support the result obtained for *P. glandulosus* leaf powder. Malarvannan (2001) observed leaf powder of *Argemone mexicana* (Papaveraceae) at moderate doses significantly reduced pupation in *Helicoverpa armigera* (Hubner) (Noctuidae: Lepidoptera). It was reported that the ground seeds of *Jatropha curcas* at a dose of 5% (w/w) significantly reduced *S. zeamais* progeny emergence in treated maize and cowpea (Adabie-Gomez *et al.*, 2007). The inert materials such as ash and diatomaceous earths cannot diffuse inside the grain; the possible mechanism involved may be the previous cited one. Inert dusts are most effective against exposed stages, especially first instars hatching from the eggs. However, against *Sitophilus* species, effectiveness is determined to a large extent by the dust's ability to kill adults before they can mate, and before females can lay the eggs inside the kernels (Subramanyam and Roesli, 2000). Therefore, applying inert dusts to grains infested with *Sitophilus* species could not provide a complete protection as the immature development occurs inside grains.

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