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Research Article
Influence of Maize Grain Moisture Content on the Insecticidal Efficacy of Wood Ash, Leaf Powder and Diatomaceous Earth Against Maize Weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae)

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Abstract
Background: *Sitophilus zeamais* Motschulsky is one of the most important maize pests in developing countries. The application of natural insecticidal powders like inert materials and leaf powders are amongst the many approaches that are used during storage to limit grain losses and are promoted in developing countries. Many factors affect the efficacy of insecticidal powder including the grain moisture content. **Materials and Methods:** The effect of maize moisture content on the efficacy of *Hymenocardia acida* Tulasne and *Acacia polyacantha* Willdenow wood ash, fossilshield (diatomaceous earth) and *Plectranthus glandulosus* Hook f. leaf powder on adult mortality, progeny inhibition, population suppression and damage reduction of *S. zeamais* after storage was assessed. **Results:** The results showed that, at a given dosage within the same period, the mortality of *S. zeamais* adult decreased when the grain moisture content increased. *H. acida*, *A. polyacantha* and *P. glandulosus* leaf powder at their highest content (40 g kg^{-1}) and fossilshield (2 g kg^{-1}) caused respectively 90, 77.50, 61.25 and 100% mortality at 11% moisture content after 14 days exposure while at 17% moisture content, 56.25, 46.25, 35 and 87.50% mortality was recorded for the respective products. The progeny production was weakly inhibited by the different powders when the grain moisture content increased. Maximum suppression of population as well as the reduction of damage was observed at the lowest moisture content (11%). **Conclusion:** The studied products are efficient to control infestation by *S. zeamais*, but their efficacy decreased by the rise of grain moisture content. Therefore for a better grain storage, good drying of maize grains combined with the application of botanical powders is recommended.

Key words: *Sitophilus zeamais*, moisture content, wood ash, leaf powder, fossilshield

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Maize is amongst the most cultivated crops and consumed cereals around the world especially in Africa where it constitutes the essential food cereal for many sub-Saharan people[1]. The inhabitants of rural African regions cultivate this crop for their nutrition and also store it in their traditional facilities for the future use and market supplying[2]. It constitutes the basic food of many populations in Africa and Latin America. Maize is cultivated for its grain value and is rich in starch (approximately 63%). The crop is grown in all the 10 regions of Cameroon country in Central Africa. This cereal is consumed in all the regions of the country. The insufficiency of different storage methods in developing countries has resulted to grain losses at an unacceptable proportion[3]. An increase in production aiming the compensation of postharvest losses, mobilizes additional resources.

Storage is successful, if at long term stored products do present depreciation neither in quality nor in quantity[4]. Unfortunately, damages are always observed during storage in tropical zones. These damages are in general the result of several factors quite exogenous as well as endogenous. The exogenous factors refer to the pests (insects, fungus and rodents) which affect the stored products directly[5]. Insects are the most significant pests; not only because they cause qualitative and quantitative damage but also because they create favourable conditions to the attack and proliferation of micro-organisms.

During storage, maize is seriously damaged by insect pests, especially the primary pest: Maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), which is one important cosmopolitan pest of stored cereals[6] and the most detrimental insect[7]. From harvest to consumption, the farmers loose more than 30% of their production without any protection[4]. This proportion is higher in sahelian zones according to the long period of storage[4]. In Cameroon, especially in Adamaoua region, Nukenine *et al.*[8] recorded 30% of stored cereals losses caused by *S. zeamais*. This pest can provoke up to 90% damage of foodstuffs after five months of storage if any protection measure has been taken[8].

The management of grain moisture content is important measure to ensure good grain storage. Generally, high moisture content compared to the normal necessary for grain conservation leads to pest damage in grain storage. The moisture content of seed may influence the effectiveness of insecticidal powders. It tends to reduce the effectiveness by diluting the active ingredients of powdery insecticides. The determination of appropriate moisture content for a successful conservation of a given produce (cereals or pulses) is needed. The determination of the effective concentration of a given insecticidal powder on grain with various moisture contents is also very significant for a successful storage.

Certain plants, because of their aromatic properties have been used by peasants for a long time in protecting stored food products[8,9]. They act as repellent, anti-appetite agents, cause death of insect pests, inhibit adult insect's fertility thus reducing the pest population[9]. *Plectranthus glandulosus* Hook, Lamiaceae is one of the 18 known species of the genus *Plectranthus* known[10]. This plant is found in the flora of West Africa[11] and also in the Cameroonian flora[11-13]. The ethno botanic studies reveal the use of their leaves in the conservation of stored maize and beans against insect damage[13].

The use of chemically inert materials, such as diatomaceous earths and botanicals powders in large quantities to fill up the interstitial space in grain bulks to provide a physical barrier to insect movement is quite widespread[14].

Diatomaceous Earth (DE) is a deposit constituted by the fossilised skeletons of siliceous unicellular organisms in particular diatoms and other algae from sea and fresh water. This fossilised deposit is transformed in fine powder after quarrying, crushing and milling. The obtained powder is made up by the porous particles, which are abrasive and able to absorb lipids to about three or more times the particle mass. In dry condition, inert dusts do not have any interaction with the grain and remain effective for long period[15-17]. Many researchers have reported the effectiveness of wood ash as a grain protectant[18,19]. The insecticidal properties of ashes vary according to the plant species[20]. That justified the use of *A. polyacantha* and *H. acida* wood ash in the present study.

The objective of this study was to evaluate the effect of moisture content on the efficacy of plant powder and inert dusts for maize preservation against weevils. The study was conducted therefore to assess the effect of grain moisture content on the efficacy of *H. acida* and *A. polyacantha* wood ash, fossilshield (diatomaceous earth) and *P. glandulosus* leaf powder on *S. zeamais* regarding adult mortality, progeny production and suppression of damage and population increase.

MATERIALS AND METHODS

Insects rearing: Adults of *S. zeamais* were obtained from a colony maintained in rearing since 2005 in the Applied Chemistry Laboratory of the University of Ngaoundere, Cameroon. The insect culture was then transferred and kept in the Crop Protection Laboratory of IRAD (Institut des

Recherches Agronomiques pour le Développement) Bambui in Cameroon. The weevils were reared on disinfested maize in 900 mL glass jars and kept under ambient laboratory conditions (temperature (t) = 18.5-26°C, relative humidity (r.h.) = 78.5-89%). Fifty adult weevils of mixed sex were added in glass jars of 900 mL containing each 500 g of cleaned maize grain. Then the jars were closed with perforated lids allowing ventilation and displayed on shelves. Twenty jars were used for this issue. After 14 days of infestation, the grains were sieved and the insects were discarded. Then the jars containing the infested grains were kept under the same laboratory conditions. Each week, it was carried out observations. Only from the 7th week post-infestation, the insect emergence was observed. From this period, the second sieving was carried out and the insects were discarded. Then from this step the sieving was carry out once a week and the insects were collected for the bioassays. For all bioassays, insects aged between 7-14 days old were used.

Source of maize grains: The maize variety "Shaba" was used during the experiment which was provided by IRAD Wakwa in Adamawa region, Cameroon. Before experimentation, the broken grains and other impurities were removed from the stock. The maize was then kept in the freezer at -4°C for 14 days to allow for complete disinfestation. After disinfestation, the grains were kept in ambient laboratory conditions (t = 18.5-26°C, r.h. = 78.5-89%) for 14 days to allow for acclimatization. After all these steps, the maize grains were ready for bioassays.

Conditioning maize grain to different moisture content (m.c.): Initial moisture content of the grain was 12.65% as determined by the digital moisture computer (Insto, serial number 52795, Insto Auburn, IL., USA). Several moisture content levels have been identified in storage facilities. Among these, three prominent ones (11, 14 and 17%) were chosen. The grains were then reconditioned to 11, 14 and 17% m.c., levels. To obtain grains with 11% m.c., maize was dried in a ventilated oven at 35°C and the m.c., determined at intervals of 30 min till the required m.c. levels were achieved. For a m.c., above 12%, pre-determined amounts of distilled water were added to the grains which were thoroughly mixed until 14 and 17% m.c. were achieved. The amount of water added was calculated according to the following formula[21]:

$$\text{Quantity of water required (g)} = \frac{\text{weight of grain} \times (mc_f - mc_i)}{100 - mc_f}$$

where, mc_f is final grain moisture content, mc_i is initial grain moisture content.

Maize grains (500 g) were weighed into 1 L glass jar allowing sufficient headspace for mixing and then the calculated amount of distilled water was added. The water was measured out as volume since 1 g of water occupies 1 mL. The jars were tumbled daily for three weeks before being kept in the refrigerator maintained at 4°C to avoid mould growth and grain germination[2]. At the end of 3 weeks, the final m.c. of the grains was determined as earlier described.

Preparation of *Acacia polyacantha* and *Hymenocardia acida* wood ash: Woods of *A. polyacantha* and *H. acida* were collected respectively in Kousseri, Far-North region and Dang subdivision of Ngaoundéré, Adamaoua, Cameroon. The identification of the plants was confirmed at the Cameroon National Herbarium in Yaoundé, where voucher samples were deposited. The *A. polyacantha* and *H. acida* are registered respectively on No. 36699/HNC and 50114/HNC. Woods were air-dried until completely moisture lost and burnt separately in a traditional kitchen normally used locally. For each plant material, 1 kg of ash was obtained. The ashes were packaged in glass jars, labelled and kept in a refrigerator (at -4°C) until subsequent use in the bioassays.

Preparation of *Plectranthus glandulosus* leaf powder: The leaves of *P. glandulosus* were collected in July, 2012 in Ngaoundéré located in the Vina Division of the Adamaoua, Cameroon. The identification of the plant was confirmed at the Cameroon National Herbarium in Yaoundé on No. 7656/SRFCam. 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 and then stored in a freezer at -4°C until needed for bioassays. This operation was carried out from December, 2011 to February, 2013.

Diatomaceous earth: The diatomaceous earth product fossilshield (FS 90.0s, Bein GmbH, Germany) has a particle size of 5-30 µm and is composed of 73% amorphous SiO_2, 3% aerosol, water content of approximately 2% and other mineral compounds. The fossilshield used during all experiment was brown in colour.

Toxicity bioassay: The toxicity bioassays were carried out in ambient laboratory conditions (t = 18.5-26°C, r.h. = 78.5-89%). During experimentation the temperatures and the relative humidity were recorded using a data logger (Data logger Model EL-USB-2, LASCAR, China). Four concentrations of each product were considered. The masses of 0.25, 0.5, 1 and 2 for *P. glandulosus* leaf powder, *A. polyacantha* and *H. acida* wood ashes and 0.025, 0.05, 0.075 and 0.1 g for fossilshield were separately added to 50 g of maize in glass jars to constitute respectively the contents of 5, 10, 20 and 40 g kg^{-1} and 0.5, 1, 1.5 and 2 g kg^{-1}. Twenty 7-14 days old adult weevils of mixed sex were introduced into each jar. The control consisted of treatments without insecticidal products. All treatments were replicated four times and the experiment was arranged on the shelf. Adult mortality was recorded 14 days after exposure.

F_1 progeny bioassay: After the 14 days mortality recordings, all insects and products were separated from grains and discarded. The grains were left inside the jars and all the F_1 progeny was counted subsequently[22]. The counting of F_1 adults was carried out once a week for 5 weeks starting 6 weeks post-infestation. The emergence started only after the 5th week after infestation. After each counting session, the insects were removed from the jars.

Damage and population increase bioassay: The two lowest contents of each product as described above for adult mortality bioassay were considered. Thirty unsexed weevils (7-14 days old) were introduced into each jar. Each treatment had four replications. After three months storage, the numbers of live and dead insects were counted. Damage assessment was performed by measuring and counting the number of damaged and undamaged grains using the method of Adams and Schulten[23] formula:

$$\text{Weight loss (\%)} = \frac{(\text{Wu}\times\text{Nd})-(\text{Wd}\times\text{Nu})}{\text{Wu}\,(\text{Nd}+\text{Nu})}\times 100$$

where, Wu is the weight of undamaged grains, Nd the number of damaged grains, Wd the weight of damaged grain and Nu the number of undamaged grain.

Data analysis: Data on percentage corrected mortality, reduction in F_1 progeny, grain damage and weight loss were arcsine-transformed [(square root(×/100)] and the number of F_1 progeny produced was log-transformed (x+1) to normalize and homogenize variances. The transformed data were subjected to the analysis of variance (ANOVA) procedure using the statistical analysis system[24,25]. Tukey's test ($p = 0.05$) was applied for mean separation. Probit analysis[25,26] was conducted to determine lethal dosages causing 50% (LC_{50}) and 95% (LC_{95}) mortality of *S. zeamais* at 14 days after treatment application. Abbott's formula[27] was used to correct for control mortality before ANOVA.

RESULTS

Toxicity: The four products induced significant mortality of *S. zeamais* adult (Table 1). Mortality increased with ascending dosage but decreased by the augmentation of grain moisture content ($F_{(2, 95)} = 105.11$, $p<0.0001$). The efficacy was different according to the powder ($F_{(3, 95)} = 288.57$, $p<0.0001$).The total mortality was achieved when fossilshield was applied at its highest concentration level (2 g kg^{-1}) on maize with the lowest moisture content (11%). The lowest mortality rate (25%) was recorded in *P. glandulosus* leaf powder at its lowest concentration (10 g kg^{-1}) on the maize with the highest moisture content (17%). The four powders induced highest mortality at the highest concentration on the maize with lowest moisture content (11%), The highest concentration level (40 g kg^{-1}) of *P. glandulosus* leaf powder, *A. polyacantha* and *H. acida* wood ashes induced, respectively 61.25, 77.50 and 90% at 11% moisture content within 14 days after exposure. But with the same insecticidal materials at the same concentration within the same exposure period the mortality rate decreased with increasing moisture content to induce in the same order at 17% moisture content respectively 35.00, 46.25 and 56.25%. The similar tendency was observed with diatomaceous earth (Fossilshield), at 0.5 g kg^{-1}. The mortality rate varied to 95.00, 63.75 and 61.25% on maize grain with 11, 14 and 17% moisture content respectively. The lethal content for the same powder increased when the grain moisture content increased (Table 2). The lowest values of LC_{50} were recorded on maize with lowest moisture content, whereas the highest LC_{50} were obtained in grains with the highest moisture content (17%) for all the four tested products.

Inhibition of progeny production: All the four insecticidal materials significantly inhibited the production of progeny (Table 3), but this efficacy was significantly reduced by the augmentation of grain moisture content. The significant inhibition of F_1 progeny was recorded at 11% moisture content while the lowest reduction of offspring rate was observed at the highest moisture content (17%). Fossilshield remained effective at all the moisture contents. There was less influence of moisture content as compared to other powders (wood ashes and *P. glandulosus* leaf powder). Complete inhibition (100%) was observed by Fossilshield at its highest concentration level (2 g kg^{-1}) on maize grain with 11% moisture content. *Plectranthus glandulosus* leaf powder did not reach 50% reduction of F_1 progeny relative to the control at all its concentration levels when the grain moisture content

Table 1: Mortality of *Sitophilus zeamais* induced by the different powders on maize grains with different moisture content after 14 days of exposure (t = 22.14±1.88°C, r.h = 77.09±9.40%)

	Percentage of mortality (Mean±SE)			
	Moisture (%)			
Products and contents (g kg^{-1})	11	14	17	$F_{(2, 9)}$
A. polyacantha				
0	0.00±0.00^{Ca}	0.00±0.00^{Ba}	0.00±0.00^{Ca}	-
10	56.25±7.18^{Ba}	48.75±2.39^{Aab}	27.50±5.95^{Bb}	5.51*
20	72.50±2.50^{ABa}	50.00±6.12^{Ab}	42.50±2.50^{Ab}	41.23***
40	77.50±4.33^{Aa}	63.75±3.15^{Ab}	46.25±3.15^{Ac}	19.09**
$F_{(3, 12)}$	115.81***	128.87***	82.34***	
H. acida				
0	0.00±0.00^{Ca}	0.00±0.00^{Ca}	0.00±0.00^{Ca}	-
10	66.25±2.39^{Ba}	46.25±3.15^{Bb}	40.00±2.89^{Bb}	23.37**
20	72.50±2.50^{Ba}	63.75±3.75^{Aa}	51.25±1.25^{ABb}	14.73*
40	90.00±3.54^{Aa}	67.50±1.44^{Ab}	56.25±5.54^{Ab}	20.82**
$F_{(3, 12)}$	270.73***	283.10***	149.55***	
P. glandulosus				
0	0.00±0.00^{Ca}	0.00±0.00^{Ba}	0.00±0.00^{Ba}	-
10	38.75±3.75^{Ba}	32.50±2.50^{Aab}	25.00±3.54^{Ab}	4.40*
20	50.00±3.54^{Aba}	35.00±2.89^{Ab}	32.50±2.50^{Ab}	9.62*
40	61.25±2.39^{Aa}	46.25±7.18^{Aab}	35.00±2.89^{Ab}	7.59*
$F_{(3, 12)}$	193.35***	62.23***	108.07***	
Fossilshield				
0	0.00±0.00^{Ba}	0.00±0.00^{Da}	0.00±0.00^{Ba}	-
0.5	95.00±2.89^{Aa}	63.75±3.75^{Cab}	61.25±17.12^{Ab}	5.30*
1	98.75±1.25^{Aa}	82.50±2.50^{Ba}	75.00±11.70^{Aa}	1.66^{ns}
2	100.00±0.00^{Aa}	95.00±2.04^{Aa}	87.50±12.50^{Aa}	0.88^{ns}
$F_{(3, 12)}$	191.68***	193.00***	9.43*	

Means±SE followed by the same capital letter in columns and the same lower letter in rows 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, ***p<0.0001, t: Temperature, r.h: Relative humidity, SE: Standard error

Table 2: Fourteen days lethal contents inducing 50 and 95% of *Sitophilus zeamais* adult mortality in maize lots of different moisture contents and treated with wood ash of *Acacia polyacantha* and *Hymanocardia acida*, leaf powder of *Plectranthus glandulosus* and fossilshield in ambient laboratory conditions (t = 22.14±1.88°C, r.h = 77.09±9.40%)

Products and moisture content (%)	Slope±SE	R^2	LC_{50} (95% FL) (g kg^{-1})	LC_{95} (95% FL) (g kg^{-1})	χ^2
A. polyacantha					
11	1.01±0.31	0.91	6.34 (1.06, 10.45)	269.90 (95.53, 1702)$^{\beta}$	0.74^{ns}
14	0.58±0.30	0.69	13.13$^{\#}$	-	0.18^{ns}
17	0.83±0.30	0.89	45.16 (28.33, 391.48)$^{\beta}$	4434 (457.81, 44E^{5})$^{\beta}$	1.02^{ns}
H. acida					
11	1.35±0.34	0.91	5.53 (1.63, 8.86)	91.13 (50.84, 472.64)$^{\beta}$	2.20^{ns}
14	0.92±0.30	0.95	11.02 (3.34, 16.17)	683.61 (165.43, 4235.08)$^{\beta}$	1.20^{ns}
17	0.68±0.30	0.95	21.49 (10.03, 66.92)	5567 (407.44, 9204)$^{\beta}$	0.27^{ns}
P. glandulosus					
11	0.95±0.30	0.99	20.00 (12.86, 31.09)	1080 (230.06, 6791.18)$^{\beta}$	-
14	0.60±0.30	0.89	65.70 (32.20, 88.39)	-	0.50^{ns}
17	0.48±0.31	0.92	229.59$^{\#\beta}$	-	0.22^{ns}
Fossilshield					
11	2.36±1.11	0.86	0.10 (0.07, 0.25)	0.51 (0.04, 0.75)	0.20^{ns}
14	1.42±0.34	0.98	0.32 (0.12, 0.48)	2.08 (1.55, 3.71)	0.07^{ns}
17	2.11±0.39	0.96	0.35 (0.20, 0.46)	4.59 (2.63, 19.38)	0.11^{ns}

^{ns}p>0.05, -: LC values are too large or estimation impossible due to inadequate mortality, $^{\beta}$LC values are obtained by extrapolation, $^{\#}$Fudicial limit for these LC could not be computed due to very low variations among the different contents of insecticidal material, LC: Lethal content, FL: Fudicial limit, SE: Standard error

was 17%. *Acacia polyacantha* and *H. acida* wood ashes inhibited significantly F_1 progeny compared to the control at 11 and 14% moisture contents. At 17% m.c., the efficacy of the two wood ashes slightly decreased.

Suppression of population growth and reduction of grain damage: The two concentrations of each insecticidal powder significantly reduced the number of living insects at the three used moisture contents compared to the control (Table 4).

Table 3: Reduction of progeny production of *Sitophilus zeamais* relative to the control induced by the four insecticidal materials (t = 22.14±1.88°C, r.h = 77.09±9.40%)

	Percentage in reduction of progeny production (Mean±SE)			
	Moisture content (%)			
Products and content (g kg⁻¹)	11	14	17	$F_{(2, 9)}$
A. polyacantha				
0	0.00±0.00^{Ca}	0.00±0.00^{Ba}	0.00±0.00^{Ca}	-
10	55.99±7.16^{Bab}	60.27±8.20^{Aa}	27.51±2.66^{BCb}	5.76*
20	59.83±9.68^{Ba}	66.97±4.57^{Aa}	50.80±11.41^{ABa}	0.80^{ns}
40	88.16±2.05^{Aa}	79.22±1.74^{Aa}	66.45±9.44^{Aa}	3.70^{ns}
$F_{(3, 12)}$	64.45***	100.62***	20.90***	
H. acida				
0	0.00±0.00^{Da}	0.00±0.00^{Da}	0.00±0.00^{Ba}	-
10	55.25±2.89^{Ca}	44.13±4.66^{Ca}	60.93±18.03^{Aa}	0.62^{ns}
20	73.41±2.14^{Ba}	66.55±2.73^{Ba}	63.37±18.44^{Aa}	0.22^{ns}
40	85.72±1.41^{Aa}	79.16±2.53^{Aa}	71.19±20.64^{Aa}	0.37^{ns}
$F_{(3, 12)}$	611.73***	234.16***	6.28*	
P. glandulosus				
0	0.00±0.00^{Ca}	0.00±0.00^{Da}	0.00±0.00^{Ba}	-
10	18.12±4.81^{Ba}	16.92±2.78^{Ca}	14.04±3.30^{Aa}	0.32^{ns}
20	44.66±6.53^{Aa}	34.00±5.31^{Ba}	28.33±8.28^{Aa}	1.95^{ns}
40	63.71±9.72^{Aa}	55.55±4.42^{Aab}	30.91±3.05^{Ab}	5.33*
$F_{(3, 12)}$	33.88***	68.62***	18.36***	
Fossilshield				
0	0.00±0.00^{Ca}	0.00±0.00^{Da}	0.00±0.00^{Ca}	-
0.5	96.13±0.33^{Ba}	86.52±1.06^{Ca}	82.42±1.62^{Bb}	38.79***
1	98.16±0.82^{Aa}	95.85±0.58^{Ba}	91.48±1.37^{Ab}	11.89*
2	100.00±0.00^{Aa}	99.27±0.42^{Aa}	95.31±1.00^{Ab}	31.71***
$F_{(3, 12)}$	1187.14***	1143.95***	947.03***	

Means±SE followed by the same capital letter in columns and the same lower letter in rows do not differ significantly at p<0.05 (Tukey's test). Each datum represents the mean of four replicates, ^{ns}p>0.05, *p<0.05, ***p<0.0001, t: Temperature, r.h: Relative humidity, SE: Standard error

Table 4: Number of live insects recorded after three months storage on maize grains with different moisture content treated with *Acacia polyacantha* and *Hymenocardia acida* wood ashes, *Plectranthus glandulosus* leaf powder and fossilshield in ambient conditions of laboratory (t = 22.14±1.88°C, r.h = 77.09±9.40%)

	No. of live insects (Mean±SE)			
	Moisture content (%)			
Products and content (g kg⁻¹)	11	14	17	$F_{(2, 9)}$
A. polyacantha				
0	72.75±11.34^{Aa}	94.00±10.34^{Aa}	121.25±18.22^{Aa}	3.12^{ns}
10	42.00±8.75^{ABb}	76.25±11.20^{ABab}	97.00±8.88^{ABa}	8.24*
20	23.50±6.90^{Ba}	51.75±2.17^{Ba}	52.50±18.02^{Ba}	2.17^{ns}
$F_{(2, 9)}$	7.35*	5.69*	4.96*	
H. acida				
0	72.75±11.34^{Aa}	94.00±10.34^{Aa}	121.25±18.22^{Aa}	3.12^{ns}
10	19.75±6.29^{Bb}	25.25±2.29^{Bb}	84.25±15.76^{Aa}	13.09*
20	8.00±1.41^{Bb}	29.25±4.48^{Bb}	75.75±12.78^{Aa}	19.44**
$F_{(2, 9)}$	20.98**	33.77***	2.36^{ns}	
P. glandulosus				
0	72.75±11.34^{Aa}	94.00±10.34^{Aa}	121.25±18.22^{Aa}	3.12^{ns}
10	67.00±10.59^{Aa}	77.50±11.81^{Aa}	107.25±17.66^{Aa}	2.32^{ns}
20	58.25±13.92^{Aa}	73.75±14.05^{Aa}	86.25±14.63	0.98^{ns}
$F_{(2, 9)}$	0.37^{ns}	0.78^{ns}	1.09^{ns}	
Fossilshield				
0	72.75±11.34^{Aa}	94.00±10.34^{Aa}	121.25±18.22^{Aa}	3.12^{ns}
0.5	5.50±3.57^{Bc}	41.50±5.61^{Ba}	66.75±5.71^{Ba}	36.65***
1	0.25±0.25^{Bb}	8.25±1.18^{Cb}	39.75±5.92^{Ba}	35.82***
$F_{(2, 9)}$	34.68***	39.87***	12.94*	

Means±SE 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), Each datum represents the mean of four replicates, ^{ns}p>0.05, *p<0.05, **p<0.001, ***p<0.0001, t: Temperature, r.h: Relative humidity, SE: Standard error

However, this reduction was different with respect to the powders. For the same powder, the reduction was considerably influenced by grain moisture content. The number of living insects decreased for a same concentration of a given product when the grain moisture content increased. Without insecticidal treatment, the number of living insects was more than 70, 90 and 120 insects respectively at 11, 14 and 17% moisture content. At 20 g kg^{-1}, *P. glandulosus* leaf powder, *A. polyacantha* and *H. acida* wood ashes recorded more than 20, 8 and 55 living insects, respectively at 11% moisture content. When the moisture content was 17%, the same powders at the same order recorded more than 50, 75 and 85 living insects. Fossilshield achieved complete reduction of insects (<1) at 1 g kg^{-1} at the lowest moisture content (11%), but at the moisture content of 17% it recorded more than 35 living insects.

The four insecticidal materials greatly reduced grain damage and then grain weight losses (Table 5) compared to the control. The number of perforated grains and the weight loss were related. Higher was the number of perforated grains,

Table 5: Damage parameters recorded after three months storage for maize grains with different moisture contents treated with *Acacia polyacantha* and *Hymenocardia acida* wood ashes, *Plectranthus glandulosus* leaf powder and fossilshield in ambient conditions of laboratory (t = 22.14±1.88°C, r.h = 77.09±9.40%)

		Moisture content (%)			
Products	Contents (g kg^{-1})	11	14	17	$F_{(2, 9)}$
Percentage of damaged gains (Mean±SE)					
A. polyacantha	0	26.38 ± 0.37^{Ab}	32.37 ± 123^{Ab}	53.15 ± 2.77^{Aa}	60.37***
	10	23.50 ± 2.59^{Ab}	30.33 ± 2.93^{Aab}	38.66 ± 1.94^{Ba}	9.04*
	20	15.07 ± 2.45^{Bb}	26.81 ± 1.83^{Aa}	30.66 ± 3.09^{Ba}	10.44*
	$F_{(2, 9)}$	7.76*	1.76^{ns}	18.53**	
Percentage of weight loss (Mean±SE)					
	0	4.14 ± 1.24^{Ac}	8.23 ± 0.57^{Ab}	12.61 ± 0.82^{Aa}	54.42***
	10	2.34 ± 0.37^{Ba}	3.25 ± 0.83^{Ba}	3.940 ± 0.23^{Aa}	1.02^{ns}
	20	0.51 ± 0.13^{Ba}	2.12 ± 0.93^{Ba}	2.500 ± 0.23^{Ba}	3.48^{ns}
	$F_{(2, 9)}$	7.77*	21.25**	117.15***	
Percentage of damaged gains (Mean±SE)					
H. acida	0	26.38 ± 0.78^{Ab}	32.37 ± 1.23^{Ab}	53.15 ± 2.77^{Aa}	60.37***
	10	12.07 ± 2.16^{Bb}	18.00 ± 5.75^{Bab}	30.43 ± 3.95^{Ba}	4.93*
	20	8.680 ± 0.49^{Bb}	10.28 ± 1.22^{Bb}	29.97 ± 3.50^{Ba}	30.18***
	$F_{(2, 9)}$	47.87***	10.45*	14.81*	
Percentage of weight loss (Mean±SE)					
	0	4.14 ± 1.24^{Ac}	8.23 ± 0.57^{Ab}	12.61 ± 0.82^{Aa}	54.42***
	10	1.82 ± 0.29^{Ba}	2.55 ± 2.00^{Ba}	3.270 ± 1.04^{Ba}	0.28^{ns}
	20	0.78 ± 0.19^{Bb}	1.60 ± 0.28^{Bab}	2.600 ± 0.59^{Ba}	5.34*
	$F_{(2, 9)}$	7.02*	9.96*	99.26***	
Percentage of damaged gains (Mean±SE)					
P. glandulosus	0	26.38 ± 0.78^{Ab}	32.37 ± 1.23^{Ab}	53.15 ± 2.77^{Aa}	60.37***
	10	22.98 ± 1.55^{Ab}	25.59 ± 1.22^{Ab}	40.08 ± 2.09^{Ba}	21.82**
	20	22.85 ± 0.58^{Aa}	26.09 ± 2.91^{Aa}	30.46 ± 1.98^{Ca}	3.46^{ns}
	$F_{(2, 9)}$	3.65^{ns}	2.89^{ns}	24.36**	
Percentage of weight loss (Mean±SE)					
	0	4.14 ± 1.24^{Ac}	8.23 ± 0.57^{Ab}	12.61 ± 0.82^{Aa}	54.42***
	10	2.01 ± 0.66^{Ba}	2.74 ± 0.35^{Ba}	5.41 ± 1.64^{Ba}	2.95^{ns}
	20	1.25 ± 0.35^{Ba}	1.50 ± 0.19^{Ba}	1.77 ± 0.18^{Ba}	1.07^{ns}
	$F_{(2, 9)}$	16.89**	64.67***	28.42***	
Percentage of damaged gains (Mean±SE)					
Fossilshield	0	26.38 ± 0.78^{Ab}	32.37 ± 1.23^{Ab}	53.15 ± 2.77^{Aa}	60.37***
	0.5	5.08 ± 1.26^{Bc}	14.24 ± 1.29^{Bb}	22.47 ± 0.87^{Ba}	56.30***
	1	3.92 ± 0.91^{Bb}	3.83 ± 0.19^{Cb}	15.17 ± 0.95^{Ca}	72.63***
	$F_{(2, 9)}$	198.64***	194.84***	130.16***	
Percentage of weight loss (Mean±SE)					
	0	4.14 ± 1.24^{Ac}	8.23 ± 0.57^{Ab}	12.61 ± 0.82^{Aa}	54.42***
	0.5	1.21 ± 0.75^{Ba}	1.53 ± 0.37^{Ba}	2.44 ± 0.51^{Ba}	4.24^{ns}
	1	0.55 ± 0.06^{Ba}	0.71 ± 0.18^{Ba}	1.67 ± 0.13^{Ba}	3.40^{ns}
	$F_{(2, 9)}$	14.08*	135.07***	119.13***	

Means±SE followed by the same capital letter in column and the same lower letter in the line for a given product concerning a damage parameter do not differ significantly at p<0.05 (Tukey's test). Each datum represents the mean of four replicates, $^{ns}p>0.05$, *p<0.05, **p<0.001, ***p<0.0001, t: Temperature, r.h: Relative humidity, SE: Standard error higher was the weight loss. The damage was very important when the grain moisture content was highest. The maize grain was more protected by the different powders when their moisture content was lowest. *Plectranthus glandulosus* leaf powder recorded the highest damage compared to other products at three chosen moisture contents. However, the reduction of damage remained significant compared to the control even at the highest moisture content. At 17% m.c., *P. glandulosus* leaf powder recorded about 30% number of perforated grains and 1.77% weight loss at concentration of 20 g kg^{-1}, whereas the control recorded more than 50% number of perforated grains and 12.61% weight loss. *Acacia polyacantha* and *H. acida* wood ashes had practically the same performance except for 14% m.c. The grain treated with fossilshield for all the moisture contents recorded the lowest grain damage and weight loss compared to the other powders. At 1 g kg^{-1} concentration, grain treated with Fossilshield recorded less than 5% of perforated grains and almost no weight loss at 11 and 14% m.c. But at 17% m.c., more than 15% of grain perforated and about 2% weight loss were recorded.

DISCUSSION

The four experimental powders caused significant mortality to adult *S. zeamais*, this mortality reduced when the moisture content increased. *Acacia polyacantha* and *H. acida* wood ashes, *P. glandulosus* leaf powder and Fossilshield have insecticidal properties but reduced by high grain moisture content. The increase in insecticidal efficacy under dried conditions may be due to the desiccative properties of DE[28] and wood ash[29]. The efficacy of the two wood ashes was different; *H. acida* was more effective than *A. polyacantha* at different moisture content levels. The efficacy of wood ash varied according to the plant species. Akob and Ewete[20] found that the cation composition varied among *Cupressus arizonica*, *Eucalyptus grandis*, *Ocimum gratissimum* and *Vetiveria zizanioides* ashe's. This variation could be the consequence of the discrepancy in their insecticidal properties as observed for *A. polyacantha* and *H. acida* wood ashes. Many studies[22,30] reported the lethal effect of plant on insect pests. *Plectranthus glandulosus* leaf powder was less effective than the three other powders, however, this powder considerably caused mortality to adult *S. zeamais*. Adesina *et al.*[31] reported that the leaf powder of *Secamone afzelii* induced significant mortality to *S. zeamais* at 2 g leaf powder for 20 g of maize grain within 15 days of infestation. Fresh, dried or processed plant materials can be applied as insecticides or to repel the pest insects[32]. Concerning the mode of action, powders have properties to disturb the respiratory system by blocking the spiracles of insects then causing death[33]. *Plectranthus glandulosus* powder contains several monoterpenes[11,22], 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[34]. Nukenine *et al.*[35] found that essential oil of *P. glandulosus* leaf contains certain chemicals such as thymol, fenchone which were toxic against *Prostephanus truncatus* and *S. zeamais*. Rozman *et al.*[36] observed that some Lamiaceae plants *Lavandula angustifolia*, *Rosmarinus officinalis*, *Thymus vulgaris* and *Laurus nobilis* were effective to suppress adult of *Rhyzopertha dominica*, *S. oryzae* and *Tribolium castaneum*. The chemical analysis of essential oils of these plants revealed the presence of chemical compounds such as eugenol, camphor, thymol, linalool, cineol and borneol. These compounds used individually provoked significant mortality of *S. oryzae* and *R. dominica* adult. That revealed the insecticidal effectiveness of Lamiaceae family which *P. glandulosus* belongs to.

In addition to causing mortality in adult, wood ashes for *A. polyacantha* and *H. acida*, leaf powder for *P. glandulosus* and diatomaceous earth considerably inhibited production of F_1 progeny indicating that the different powders could contain the inhibiting property. *Plectranthus glandulosus* leaf could contain some chemical compounds that reduced progeny production. Olaifa and Erhun[37] found that higher concentration of the powder of *Piper guineense* significantly reduced oviposition. That oviposition disturbance can be due to the physiological and behavioural changes occurring in the adults of *Callosobruchus maculatus*. This mechanism can also be considered for reduction of *S. zeamais* production by *P. glandulosus* leaf powder. The diatomaceous earth and the wood ashes act almost on progeny inhibition by the same mechanism. The abrasive properties of the inert dusts may play some part in preventing the development of pest; the dust inhibit insect behaviour, affecting movement and reproduction by blocking air space between grains. Gemu *et al.*[29] found lowest number of F_1 progeny emerged from maize kernels treated with 15% wood ash (w/w). The same observation was noticed in the present study but in maize grains with lowest moisture content. In maize grains with highest moisture content, the reduction of F_1 progeny decreased considerably. Khakame *et al.*[2] reported that a diatomaceous earth, dryacide recorded 6.1 and 27.4 *S. zeamais* progeny respectively at 10 and 16% maize grain moisture content at 0.9% w/w. A similar finding was reported in the present study with little difference that could be due to the formulation of DE, maize variety or *S. zeamais* strain. The inert dusts efficacy decreases with increase in grain moisture content level[28]. Parsaeyan *et al*.[38] found that the diatomaceous earth reduced the fecundity, adult longevity and egg hatching rate of *C. maculatus*. Shayesteh and Ziaee[39] also reported that the reproduction potential of adult *Tribolium castaneum* was significantly suppressed in treatments containing silicosec.

The four powders suppressed *S. zeamais* population increase and reduced grain damage. Effective control of protectants is qualified as mortality of adult and/or immature, confirmed by lack of progeny generation[40]. Mutambuki[41] showed that the activity of silica dust decreased when the grain moisture content rose above 15%. The population growth and damage (perforated grains and weight losses) were important at the highest moisture content for the four powders. But when the concentration level of powders was increased especially for inert dusts (DE and ashes), the damage and proliferation of *S. zeamais* were considerably reduced. Added in sufficient quantity, wood ash can effectively protect grain stored in small lots[18]. Higher was the grain moisture content, lower was the efficacy of insecticidal powder. The ash and DE absorb the water contained in grains that makes them inactive. The loss or drop in potency of inert dusts could probably be attributed to slower capacity to absorb the oily or waxy epicuticular lipid layers by direct contact under wet conditions produced by the high grain moisture content[42]. In addition, the absorption of water contained in grain by the particles, diluted the active compounds and consequently the concentration of insecticidal powder. Sometimes, the seeds are traditionally mixed with ash, sand or other dry fine dusts that act as a physical barrier to limit insect movement[43]. At harvest, the grain generally has higher moisture content levels than those required for storage, therefore, it is dried to acquire moisture content level suitable for storage. The absorption of water contained in grains by the powders especially botanicals, results in the loss of insecticidal ability, creates favourable conditions for the proliferation of fungi, moulds and other micro-organisms thus destroying the stored grains.

CONCLUSION

Plectranthus glandulous leaf powder, diatomaceous earth (Fossilshield) and *A. polyacantha* and *H. acida* wood ash were effective to control infestation of the maize grain by *S. zeamais* in the fluctuating environment conditions. But this effectiveness was significantly reduced by the increase of the grain moisture content. In the different regions of Cameroon, the people use to storage their grain in the traditional storage facilities characterized by the uncontrolled conditions. Then the humidity of storage facilities uses to fluctuate according to the region and for the same region according to the season. This situation induces the variation of grain moisture content and then the adjustment of the doses of insecticidal powders is imperative to insure good storage especially for absorbent and abrasive powders such as ashes and inert dusts like diatomaceous earth. The management of grain moisture content especially in the humid conditions of the western highlands of Cameroon is an important factor to take into account for successful grain storage.

SIGNIFICANCE STATEMENTS

It is already established that wood ash, diatomaceous earth and some plant powders have properties to control insect pest infestation. But this study highlights the effect of grain moisture on these powders efficacy. Sometime, farmers mix grains with high quantity of powder which in turn leave residues on grains whereas a small quantity of the plant product is sufficient. Laboratory experiment is necessary to measure the doses which protect them against insect attack. Grain moisture is among the factors that affect the efficiency of insecticidal powders in general. The present study shows how different grain moisture contents influence the effectiveness of the studied dusts. The results of this study will help the small farmers to have their free-cost and less hazardous insecticides which can be more effective if applied on grains at its recommended moisture content.

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