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Efficacy of mixed powders of *Piper guineense* and *Zingiber officinale* as maize grain protectants against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae)

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ABSTRACT

This study was carried out to evaluate the effects of mixed powders of *Piper guineense* (Piperaceae) and *Zingiber officinale* (Zingiberaceae) on *Sitophilus zeamais* population and seed weight loss percentage of stored maize. Experiment was conducted under laboratory conditions (26.94 ± 0.81 °C, 74.76 ± 4.51% r.h. and 12 h photoperiod). In a completely randomized design with three replications, powders of *P. guineense* and *Z. officinale* were applied separately and in mixtures at the ratios of Zg100%:Pg0% (T1), Zg0%:Pg100% (T2), Zg30%:Pg70% (T3), Zg70%:Pg30% (T4) and Zg50%:Pg50% (T5), respectively. The mortality rate of *S. zeamais* adults was recorded and compared with the control at 24, 48, 72, 96, 120 and 168 hours after treatment. Maize grain damage and seed weight loss percentage were determined. Results showed highly significant differences with *S. zeamais* mortality rate between treatments, treatment exposure duration and their interactions (p<0.001). *P. guineense*, alone, showed the best efficacy (mortality rate = 96.66 ± 5.77% at 96 hours after treatment) and mixed with *Z. officinale* at the ratio 70%Pg: 30Zo (mortality rate = 93.33 ± 5.77%). Taking into account this high performance of *P. guineense* seed powder, it can be recommended as stored maize grain protectants.

Keywords: Botanical insecticides, maize storage, *Sitophilus zeamais*, weight loss, mortality rate.

INTRODUCTION

Cereals are the major sources of nutrition for one-third of the world's population especially in developing and underdeveloped countries of Sub-Saharan Africa and South-east Asia (Gemechu et al., 2013). Among the cereal crops, rice, wheat and maize constitute about 85% of total global production (Sofia et al., 2009). Specially, maize (*Zea mays* L.) is an important cereal crop in Africa serving as a source of food, animal feed and industrial raw materials. It is also an important food crop grown commercially on a large scale and a

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smallholder base throughout the world (Meseret, 2011). However, its market and nutritional value is hampered due to many biotic and abiotic factors both in the field and during storage (Ouedraogo et al., 2016). Postharvest insect pests are the primary causes of loss for maize grains during storage (Waongo et al., 2013). In southern Benin for instance, the damage due to insect pests on stored maize reached 40% of losses (Adegbola et al., 2011). Crop losses due to insect pests constitute a great constraint for food security worldwide (FAO, 2011). In Africa, the constant availability of maize in adequate quantities is fundamental to food security (Nukenine et al., 2013). Therefore, in order to meet the food demand for the ever increasing world population, we need to address the issue of maize grain losses due to insect pests' attacks during the storage.

Many pests of stored maize are coleopterans and the most destructive tropical species for maize belong to the genus *Sitophilus* and *Tribolium* (Bello et al., 2000). The losses arise from larvae's penetration inside the grains, and their subsequent feeding actions causing weight loss, lower nutritional value, and germination failure (Barbosa et al., 2002). The maize weevil, *Sitophilus zeamais* Motsch**.** (Coleoptera : Curculionidae) is a serious and the most important insect pest of stored maize (Nukenine et al., 2013). It poses a serious threat to food security, particularly in developing countries (Tefera et al., 2011). The weevil together with other insects causes an estimated 24.5% loss of maize (Napaleao et al., 2013). Declining food production, coupled with huge losses resulting from *S. zeamais* attack during maize storage, exposes farmers to different levels of food shortage (Nwosu and Nwosu, 2012). To overcome the threat of insect pests, control methods are mainly ensured with the use of synthetic chemical pesticides. Under optimal conditions, their effectiveness in controlling pests is assured. However, they have many drawbacks, including addiction insects and the selection of resistant strains (Benhalima et al., 2004), poisoning, environmental pollution and ecological disorders (Regnault-Roger et al., 2002). All these reasons militate in favor of the search for alternative control methods. The use of plant extracts with insecticidal or repellency properties that are inexpensive, effective and easy to adopt for smallholder farmers can be a good alternative (Toumnou et al., 2012).

Evidently, plants have long been used by farmers for their flavor in foods and to protect harvested crops (Djossou, 2006). The aim of this present study was to evaluate the susceptibility of *S. zeamais* with two local spices commonly used in Benin, *Piper guineense* (Piperaceae) and *Zingiber offinicale* (Zingiberaceae).

MATERIALS AND METHODS

Experiment was conducted in the Laboratory of Applied Biology Research at University of Abomey-Calavi, Benin. *S. zeamais* was cultured in the laboratory at 26.94 ± 0.81 °C, 74.76 ± 4.51%r. h. and 12 h photoperiod.

Collection and preparation of botanical products

Z. officinale rhizomes and *P. guineense* seeds were locally purchased from the international market, Dantokpa of Cotonou. All plant materials were brought to the laboratory and were air-dried for two weeks in a well-ventilated place. Their powders were obtained by using Thomas (model ED-5) milling machine, after which they were sieved through an 80 µm laboratory sieve. The powders were labeled and kept separately in glass containers and stored at an ambient temperature prior to use.

Rearing of *S. zeamais*

Adults of *S. zeamais* were obtained from agriculture office of Porto Novo (Benin). They were cultured in a laboratory rearing room, at 26.94 ± 0.81 °C, 74.76 ± 4.51%r.h. and photoperiod 12 h light and 12 h dark. Whole maize grains bought from local farmers were disinfested in an oven at 60 °C for 1 hour (Asmanizar and Idris, 2008) before being used as substrates for insect rearing. Fifty pairs of *S. zeamais* were introduced into 560 ml glass jars containing 250 g of maize grains. Bottles were covered with muslin cloth to allow air circulation and secured with rubber bands. Parent weevils were sieved out after oviposition. Afterwards, the seeds were kept in an incubator and were monitored for adult emergence. F1 progeny (14 days old emerged adults) was used for maize infestation during the experiment.

Experimental set up

Plant powders were thoroughly mixed with 20 g of maize grains in 250-ml plastic containers. 5g of both powders of *Z. officinale* and *P. guineense* was used in the proportions of Zg100%:Pg0% T1), Zg0%:Pg100% (T2), Zg30%:Pg70% (T3), Zg70%:Pg30% (T4) and Zg50%:Pg50% (T5) and then maize grains were thoroughly mixed with the powder formulations. Untreated maize grains (Zg0%:Pg0%) were used as control.

Containers with their contents were gently shaken to ensure thorough mixture of the maize grains and treatment powders. Ten unsexed *S. zeamais* adults were randomly selected and introduced to each of the containers. Three replicates of the treatments and untreated controls were laid out in a completely randomized design.

Data collection

Mortality

Mortality of the adult *S. zeamais* was assessed at 24h, 48h 72h, 96h, 120h and 168h after the treatments application. All insects, both dead and alive, were removed from each container. Percentage of mortality was determined using the following formula:

$$\text{Mortality percentage} = \frac{\text{Number of dead insects}}{\text{Total number of insects}} \times 100$$

F1 Progeny emergence

Forty five days after infestation with adult *S. zeamais,* the containers were checked for adult emergence and recorded. Percentage reduction in adult emergence or inhibition rate (% IR) was calculated as:

$$(\%)\ \text{IR} = \frac{C_n - T_n}{C_n} \times 100$$

Where C_n is the number of newly emerged insects in the untreated (control) jar and T_n is the number of insects in the treated jar.

Percentage of grain damage

Percentage of grain damage was calculated on the 45th day after *S. zeamais* adults' introduction to each experimental unit. Numbers of perforated grains were recorded and the beneath general formula was used to determine the percentage of grain damage:

$$(\%)\ \text{grain damage} = \frac{\text{Number of perforated grains}}{\text{Total number of grains counted}} \times 100$$

Percentage of seed weight loss

The weight loss due to insects was calculated for each storage period using the formula of "Count and Weigh Method" (Adams and Schulten, 1978):

$$(\%)\ \text{Weight loss} = \frac{(W_u \times N_d) - (W_d \times N_u)}{W_u \times (N_d + N_u)} \times 100$$

Where W_u = Weight of undamaged fraction in the sample, N_d = Number of damaged grains in the sample, W_d = Weight of damaged fraction in the sample and N_u = Number of undamaged grains.

Statistical analysis

Data were analyzed using statistical program R 3.3.1 (R Core Team, 2016). Data generated on mortality of *S. zeamais* were subjected to analysis of variance (ANOVA) at 0.05 level of significance with aov function of agricolae package (de Mendiburu, 2015) to compare the various treatments. Means separation was performed using Student Newmann and Keuls test by function SNK.test of agricolae package (de Mendiburu, 2015).

RESULTS

Mortality of *S. zeamais*

Significant differences ($p<0.001$) for *S. zeamais* mortality were observed between treatments. Significant time interaction was also observed (Table 1). Results for mortality of *S. zeamais* at 96 hours after treatment, indicated that mean mortality count of *S. zeamais* adults is significantly higher ($p<0.05$) in maize seeds treated with *P. guineense* powder (Pg100%) (96.66 ± 5.77) compared to those of *Z. officinale* powder (Zg100%) (16.66 ± 15.27). The lowest mean mortality (0.00 ± 0.00) was recorded in the control (Table 2).

Mortality rate increased with the duration of exposure time (Table 2). Mean mortality count of *S. zeamais* adults is highest in maize seeds treated with the powder mixture of Zg30%:Pg70% (93.33 ± 5.77) followed by Zg70%:Pg30% (86.66±15.27) and Zg50%:Pg50% (86.66 ± 15.27) mixtures. The lowest mortality count (0.00 ± 0.00) was recorded in the control treatment at 120 hours after treatment.

Adult emergence, seed weight loss and grain damage percentages

After 45 days, emergence inhibition rate, low percentage weight losses and damaged grain percentage were observed for the different treatments (Figure 1). Powder mixture of Zg0%:Pg100%, Zg30%:Pg70%, Zg70%:Pg30% and Zg50%:Pg50% ensured absolute protection of maize seeds against *S. zeamais* (IR=100 ± 0.00%, Figure 1a, and grain damage percentage = weight loss percentage = 0 ± 0.00% Figure 1b).

Table 1: Analysis of variance (ANOVA) for *S. zeamais* mortality.

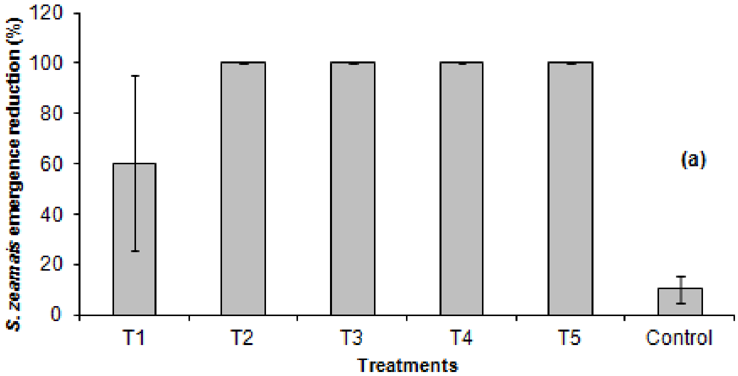
Source of variation	Degrees of freedom	Sum of squares	Mean square	F Ratio	p-value
Treatment	5	77438	15488	273.310	2.10^{-16}
HAT	5	63538	12708	224.252	2.10^{-16}
Rep	2	1757	879	15.507	$3.73.10^{-6}$
Rep × Treatment	10	2976	298	5.252	$1.65.10^{-5}$
HAT × Treatment	25	28012	1120	19.773	2.10^{-16}
Residuals	60	3400	57		

HAT: hours after treatment, Rep: Replication.

Table 2: Mortality of *S. zeamais* with treatments exposure duration.

	Mortality count of *S. zeamais* at:					
Treatment	**24 HAT**	**48 HAT**	**72 HAT**	**96 HAT**	**120 HAT**	**168 HAT**
T1	3.33±1.77b*	6.66±1.77b	10.00±10.00b	16.66±15.27c	20.00±17.32b	23.33±20.81b
T2	16.67±5.77a	60.00±1.32a	83.33±15.27a	96.66±5.77a	100±0.00a	100±0.00a
T3	0±0.00b	0±0.00b	66.66±23.09a	80.00±10.00ab	93.33±5.77a	100±0.00a
T4	6.66±1.54ab	16.66±5.81b	56.66±15.27a	70.00±10b	86.66±15.27a	100±0.00a
T5	0±0.00b	16.66±5.77b	53.33±11.54a	70.00±10b	86.66±15.27a	96.66±5.77a
Control	0±0.00b	0±0.00b	0±0.00b	0±0.00c	0±0.00b	0±0.00c

*Means in each column bearing the same letter (s) are not significantly different at the 5% level of probability by Student Newmann and Keuls test. T1 : Zg100%:Pg0%, T2 : Zg0%:Pg100%, T3 : Zg30%:Pg70%, T4 : Zg70%:Pg30%T5 : Zg50%:Pg50%. HAT: Hour after treatment.



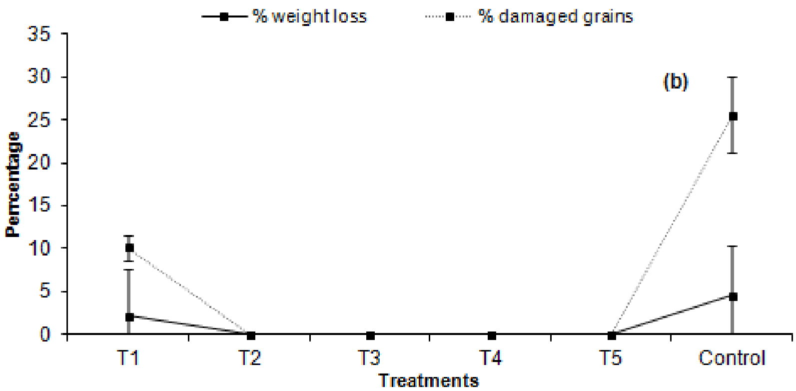


Figure 1: (a) Adults of *Sitophilus zeamais* emergence reduction with treatments; (b) Seed weight loss and damaged grain percentages of *Zea mays* with different treatments. T1 : Zg100%:Pg0%, T2 : Zg0%:Pg100%, T3 : Zg30%:Pg70%, T4 : Zg70%:Pg30%T5 : Zg50%:Pg50%.

DISCUSSION

Results showed effect of *P. guineense* seed powder on *S. zeamais* mortality. The levels of mortality obtained in this study are consistent with the results of Lale and Alga (2000), Udo (2000), Udo et al. (2011) who indicated that plant powders of *P. guineense* be used in suppressing *S. zeamais*. Lale and Alga (2000) and Udo (2000) reported that oviposition deterrence and depression of adult emergence of *S. zeamais* are due to active components in *P. guineense* seeds such as piperine, chavicine and piperidine.

Udo (2005), has suggested that the toxicity of *P. guineense* has also been suggested may cause death of insect pests by anoxia or interference in normal respiration resulting in suffocation. Our results revealed that application of *P. guineense* seed powder only and then mixture of *Z. officinale* rhizome and *P. guineense* seed powders increased adult mortality and emergence inhibition of *S. zeamais*.

In this study, results showed high mortality and 100% inhibition rate of *S. zeamais* adult emergence with mixed powders according to the following ratio Zg30%:Pg70%, Zg70%:Pg30% and Zg50%:Pg50%. The highest mortality and inhibition rate in treatment with mixed powders of *Z. officinale* and *P. guineense* can mean that there were interactive positive effects from mixing two plant powders. Similar findings were obtained from Dawudo and Ofuya (2000) and Akunne et al. (2014) who reported that the mixture of seed powder of *P. guineense* and rhizome powder of *Z. Officinale* in equal proportion (50%:50%) significantly caused mortality, reduced oviposition and adult emergence of *Callosobruchus maculatus* (Coleoptera: Chrysomelidae, Bruchinae).

Conclusion

This study showed that *P. guineense* seed powder allowed the control of *S. zeamais*. It can be used as bio-insecticide for maize grains storage. Further studies should be conducted to reveal the repellent effects of these plant materials on developmental stages of the pest. In addition, further researches are needed to determine efficacy of the active components of *P. guineense* seed and *Z. officinale* rhizome on a wide range of other common insect pests of stored products in Benin.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

DCC contributed to manuscript writing and statistical analysis. YABZ wrote proposal and manuscript draft. SEDS set up experience and collected the data. CHA and AA conducted statistical analysis and the correction of manuscript.

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REFERENCES

Adams JM, Schulten GGM. 1978. Losses caused by insects, mites and microorganisms. In *Post-harvest Grain Loss Assessment Methods*, Harris KL, Lindblad CJ (ed). Ameri Association of Cereal Chemists: St Paul, Minnesota, USA; 83-95.

Adegbola PY, Arouna A, Ahoyo NRA. 2011. Acceptabilité des structures améliorées de stockage du maïs au Sud-Bénin. In : Bulletin de la Recherche Agronomique du Bénin, Numéro spécial 2, Aspects économiques du stockage et de la conservation du maïs au Sud-Bénin. Institut National des Recherches Agricoles du Bénin ; 9-20.

Akunne CE, Afonta CN, Mogbo TC, Ononye BU, Ngenegbo UC. 2014. Evaluation of the efficacy of mixed powders of *Piper guineense* and *Zingiber Officinale* against *Callosobruchus Maculatus* (F.) (Coleoptera: Bruchidae). *Ameri Journal of Biology and Life Sciences*, **2**(2):63-67.

Asmanizar DA, Idris AB. 2008. Effect of selected plant extract on mortality of adult *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), pest of stored rice grains. *Malaysian Applied Biology*, **37**(2):41 – 46.

Barbosa FR, Yokoyama M, Pereira PAA, Zimmermann FJP. 2002. Controle do caruncho-do-feijoeiro *Zabrotes subfasciatus* com óleos vegetais, munha, materiais inertes e malathion. *Pesquisa Agropecuária Brasileira*, **37**: 1213-1217.

Bello GD, Padina S, Lastrab CL, Fabrizio M. 2000. Laboratory evaluation of chemical biological control of rice weevil, *Sitophilus oryzae* L. in stored grain. *Journal of Stored Products Research*, **37**: 77-84.

Benhalima H, Chaudry MQ, Mills KA, Price NR. 2004. Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. *Journal of Stored Product Research*, **40**: 241-249. DOI: 10.1016/s0022-474x(03)00012-2

Dawudo EO, Ofuya TI. 2000. Effects of mixing powders of *Piper guineense* Schumand Thonnand *Dennettia tripetala* Baker on oviposition and adult emergence of *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae) infesting cowpea seeds in storage. *Appl. Trop. Agric.*, **5**: 156-160.

de Mendiburu F. 2015. Agricolae tutorial version 1.2-2. Statistics and informatics, Department of Economic, Faculty of National, University of Agriculture, Molina. Peru. 78p.

Djossou J. 2006. Etude des possibilités d'utilisation des formulations à base de fruits secs de *Xylopia aethiopica* Dunal (Annonaceae) pour la protection des stocks de niébé contre *Callosobruchus maculatus* Fabricius (Coleoptera : Bruchidae). Mémoire d'ingénieur agronome à la Faculté des Sciences Agronomiques de Gembloux Belgique, 27p.

FAO. 2011. FAOSTAT for 2011. Food and Agricultural organization of the United Nations: Rome.

Gemechu F, Sori W, Santiago DR. 2013. Efficacy of botanical powders and cooking oils against Angoumois grain moth, *Sitotroga cereallela* O. (Lepidoptera: Gelechiidae) in stored maize. *Afri Journal of Biotechnology*, **12**(16):1978-1986.

Lale NES, Alga KA. 2000. Exploring the insecticidal, larvicidal and repellent properties of *Piper guinnense* schum and thonn seed oil for the control of rust-red flour beetle *Tribolium castaneum* (Herbst) in stored pearl millet *Pennisetum glaucum* (L.) R.Br. *Journal of Plant Diseases and Protection*, **108**(3): 305-313.

Meseret B. 2011. Effect of fermentation on Quality Protein Maize-soybean blends for the production of weaning food. MSc thesis, Addis Ababa University, Addis Ababa, Ethiopia, p.116.

Napaleao TH, Belmonte BDR, Pontual EV, Albuquerque de LP, Sa RA, Paiva LM, Coelho LCBB, Paiva PMG. 2013. Deleterious effects of *Myracrodruon urundeuva* leaf extract and lectin on maize weevil, *Sitophilus zeamais* (Coleopera: Curculionidae). *J. Stored Prod. Res.*, **54**: 26-33.

Nukenine EN, Chouka FP, Vabi MB, Reichmuth C, Adler C. 2013. Comparative toxicity of four local botanical powders to *Sitophilus zeamais* and influence of drying regime and particle size on insecticidal efficacy. *Int. J. Biol. Chem. Sci.,* **7**(3): 1313-1325. DOI : http://dx.doi.org/10.4314/ijbcs.v7i3.36

Nwosu LC, Nwosu UI. 2012. Assessment of maize cob powder for the control of weevils in stored maize grain in Nigeria. *Journal of Entomological Research*, **36**: 21-24.

Ouedraogo I, Sawadogo A, Neibie RCH, Dakouo D. 2016. Evaluation de la toxicité des huiles essentielles de *Cymbopogon nardu*s (L) et *Ocimum gratissimum* (L) contre *Sitophilus zeamais* Motsch et *Rhyzopertha dominica* F, les principaux insectes nuisibles au maïs en stockage au Burkina Faso. *Int. J. Biol. Chem. Sci.*, **10**(2): 695-705. DOI: http://dx.doi.org/10.4314/ijbcs.v10i2.20

R Core Team 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org/.

Regnault-Roger C, Philogène BJR, Vincent C. 2002. *Biopesticides d'Origines Végétales*. Tec & Doc Eds : Paris.

Sofia PS, Wanil SA, Rather AG, Wani SH. 2009. Quality Protein Maize (QPM): Genetic manipulation for the nutritional fortification of maize. *Journal of Plant Breeding and Crop Science*, **1**(6): 244-253.

Tefera T, Kanampiu F, De Groote H, Hellin J, Mugo S, Kimenju S, Beyene Y, Boddupalli PM, Shiferaw B, Banziger M. 2011. The metal silo: an effective grain storage technology for reducing postharvest insect and pathogen losses in maize while improving smallholder farmers' food security in developing countries. *Crop Prot*., **30**: 240-245.

Toumnou AL, Seck D, Namkosserena S, Cisse N, Kandioura N, Sembene M. 2012. Utilisation des plantes indigènes à effet insecticide pour la protection des denrées stockées contre des insectes ravageurs à Boukoko (Centrafrique). *Int. J. Biol. Chem. Sci.,* **6**(3): 1040-1050. DOI: http://dx.doi.org/10.4314/ijbcs.v6i3.11

Udo IO, Ekanem MS, Inyang EU. 2011. Laboratory evaluation of west afri black pepper (*Piper guineense*) seed powder against maize weevil *Sitophilus zeamais* (Mots.). *Munis Entomology and Zoology*, **6**(2): 1003-1007.

Udo IO. 2000. Efficacy of dle wood, *Zanthoxylum xanhoxyloides* Lam for the control of three stored product pests. M. Phil thesis, University of Ghana, Legon, Accra, p.81.

Udo IO. 2005. Evaluation of the potentiel of some local spicies as stored grain protectants against the maize weevil *Stophilus zeamais* Motschulsky (Coleoptera : Curculionidae). *Journal of Applied Science and Environmental Management*, **9**(1): 165-168.

Waongo A, Yamkoulga M, Dabire-Binso CL, BA MN, Sanon A. 2013. Conservation post-récolte des céréales en zone sud-soudanienne du Burkina Faso : Perception paysanne et évaluation des stocks. *Int. J. Biol. Chem. Sci*., **7**(3): 1157-1167. DOI: http://dx.doi.org/10.4314/ijbcs.v7i3.22