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Evaluation of some Plant Powders as Natural Biopesticides Against Maize Weevil *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae)

Mamudou Jallow[1,3] and Olufemi O. R. Pitan[1,2]

[1]Centre of Excellence in Agricultural Development and Sustainable Environment, Federal University of Agriculture, Abeokuta, Nigeria.
[2]Department of Crop Protection, Federal University of Agriculture, Abeokuta, Nigeria.
[3]School of Arts and Sciences, Department of Physical and Natural Sciences, University of The Gambia.

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ABSTRACT
The study investigated the bioactivity of *Curcuma longa, Azadirachta indica, Eucalyptus globulus, Capsicum annum, Icacina senegalensis, Moringa oleifera and Piper guineense* plant powders obtained in the Gambia against the maize weevil *Sitophilus zeamais* at concentrations of 2.5, 5.0 and 10.0% (w/w) in a Randomized Complete Block Design with four replicates. Data were collected on adult mortality, F_1 progeny emergence, number of eggs laid, grain weight loss and damage to determine the potency of the botanicals. *Piper guineense*, *Azadirachta indica* and *Eucalyptus globulus* were the most bioactive against *S. zeamais* at 10%. The application of *P. guineense* caused higher mortality and inhibition rates and lower weight loss and oviposition, with respective values of 100, 98.50, 0.03, and 0.00%. *Piper guineense* was the most potent plant powder against the maize weevil, hence promising for its control.

Keywords: Bioactivity, The Gambia, Potency, Sitophilus zeamais, Control

1. Introduction

The maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) is one of the most devastating insect pests of stored cereals in tropical and sub-tropical regions (Throne, 1994). Although it is frequently found on maize, it can also feed on other cereal grains, including wheat, barley, sorghum, rye, and rice. Its host range is comparable to that of the rice and granary weevil and has been shown to feed on processed grain products including pet food and pastas.

In a survey conducted in The Gambia, 47% of farmers were reported to use conventional insecticides for storage of products against pests (personal communication), but these pose problems of availability, dilution by traders, environmental and health risks (Stevenson *et al.,* 2012). Since then, interest has grown in conducting research into botanical insecticides for crop protection, especially as small-scale farmers in sub-Saharan Africa often rely on various plant-based pest management methods (Poswal and Akpa, 1991; Bekele *et al.,* 1996). Therefore, the present study is aimed at assessing the bioactivity of seven plant powders against the maize weevil, *Sitophilus zeamais* in The Gambia.

2. Materials and Methods

2.1. Study location

The study was carried out at the National Agricultural Research Institute (NARI) in Brikama (Latitude 13^{0}16' N, Longitude 16^{0}38W), situated 24 metres above sea level in the Kombo Central District of the Western Coast Region of The Gambia, which is part of the Sudan-Guinean Zone and 35 kilometres west of the capital, Banjul.The study location is characterized by a single rainfall pattern between the months of June and October and a long dry season from November to June, with an average annual rainfall of 1004 mm and an annual average temperature of 25.9^{0}C [Access Gambia, 2019].

Corresponding Author: Mamudou Jallow, Centre of Excellence in Agricultural Development and Sustainable Environment, Federal University of Agriculture, Abeokuta, Nigeria.
E-mail: mjallow@utg.edu.gm

2.2. Insect cultures

Sitophilus zeamais were obtained from infested maize samples collected from Brikama, Barra and Basse in The Gambia and cultured on Swan-2 maize seeds previously cleaned and disinfested in a deep freezer at -20 ± 2 °C for two weeks. Fifty unsexed adult *S. zeamais* of mixed ages were cultured in 500 ml capacity plastic containing 250 g of the seeds. The jars were covered with muslin cloth, held in place with rubber band for ventilation and to prevent the weevils from escaping. The weevils were allowed to oviposit for 7 days, thereafter; all adults were removed and placed in another set of jars and the newly emerged adult *S. zeamais* used for the different experiments (Adedire and Lajide, 1999).

2.3. Collection and Preparation of Plant Materials

The seven botanicals evaluated in the study are shown in Table 1. *Curcuma longa* rhizomes were procured from the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria, while the other six were obtained from The Gambia. The plant materials were air-dried for two weeks and pulverised with a Panasonic MX-T1GN electric grinder. The plant powders were then stored in a refrigerator in air-tight plastic bags until when needed for the experiment.

2.4. Sex determination

The sexes of *S. zeamais* were determined using the method of Kranz *et al.,* (1978). Female snouts are usually longer and thinner compared to those of the males which are shorter and fatter. In addition, the females have smooth textured snout while that of the males are rough.

2.5. Preparation of maize seeds

Freshly harvested maize seeds (Jeka variety) were procured, cleaned and disinfested in a deep freezer at -20 ± 2 °C for two weeks after which the seeds were air dried and kept in 500 ml air-tight plastic jars in the laboratory.

2.6. Test for toxicity of the plant powders against *Sitophilus zeamais*

A sample of 100 g disinfested Jeka seed maize variety was placed in 500 ml plastic jar and treated with each of the seven plant powders, at 2.5, 5.0, and 10.0% (wt/wt) concentrations while Fenitrothion dust applied at 1% concentration served as the check. The control was maize seeds without treatment application. The set up was a 7 X 3 factorial experiment arranged in a Randomised Complete Block Design with four replicates in a shelf (Plate 4). All the plastic jars containing treated maize seeds were thoroughly shaken for even spread of the treatments. Thereafter, 10 unsexed *S. zeamais* adults (7-10 days old) were introduced in each jar and covered with a muslin cloth held in place by a rubber band. Seven days after introduction of weevils, data on adult mortality was collected by removing and counting the number of dead and life weevils. Weevils were considered dead if no movement was observed when prod with a pin.

The experiment was allowed to continue in order to determine the offspring percent inhibition rate of the plant powders against *S. zeamais*. The number of insects that emerged from each treatment were counted every two days, starting from appearance of the first F_1 progeny, for 56 days after infestation (Nwana and Akibi-betts,1982).

Percentage reduction in emergence of F_1 adults was calculated using the formula (Tapondjou *et al.,* 2002):

$$\% \text{ Inhibition rate} = \frac{(\text{Cn} - \text{Tn})}{\text{Cn}} \times 100$$

Where Cn= number of newly emerged insects in the control maize seed samples
Tn= number of insects emerged in the treated seed samples

2.7. Test for oviposition deterrence on *Sitophilus zeamais*

A sample of 50 g disinfested Jeka maize variety was placed in 500 ml plastic jars and treated with plant powders at 2.5, 5.0, and 10.0% (wt/wt) concentration, while the control was untreated maize seeds. Five pairs of *S. zeamais* adults (7-10 days old) sexed as stated in 2.4, were introduced into the seed samples arranged in Randomised Complete Block Design with four replicates.

Weevils were removed seven days after oviposition, after which the experimental set-up were observed for oviposition deterrence by staining 50% maize seeds of each treatment with acid fuchsin for egg counting (Frankenfeld, 1948).

Distilled water of 750 ml and 250 ml glacial acetic acid were mixed to form a solution in a 1.5 L beaker and 3.5 g acid fuchsin was added and stirred. The sample of seeds to be tested was treated with warm water for 2-5 minutes and placed in a suitable shallow dish container large enough to adequately hold (immerse) the sample and the acid fuchsin dye solution. The seeds were left in this solution for about 2-5 minutes, then the dye poured, and the seeds washed in running tap water until all of the excess dye has been removed. After staining, the grains were examined for egg plugs (Plate 5) using a digital microscope connected to computer (Frankenfeld, 1948).

2.8. Test for feeding deterrence on *Sitophilus zeamais*

A sample of 100 g disinfested Jeka maize seeds was placed in 500 ml plastic jars and treated with the plant powders at 2.5, 5.0, and 10.0% (wt/wt) concentrations, while the control was untreated maize seeds.

Ten unsexed *Sitophilus zeamais* adults (7-10 days) were introduced into the seed samples arranged in Randomised Completely Block Design in four replicates. Weevils were allowed to feed, mate and oviposit for 30 days, after which the content of the jars (treatments) was separated into seeds, insects and dust using 1.0 mm and 4.7 mm sieves. The number and weight of damaged and undamaged seeds were recorded and used to determine percentage grain damage and percentage weight loss as follows (Dobie, 1991);

$$\%\ \text{Grain damage} = \frac{\text{Total number of bored grains}}{\text{Total number of grains}} \times 100$$

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

Where Wu = weight of undamaged; Nd = number of damaged;
Wd = weight of damaged; Nu = number of undamaged

2.9. Statistical Analyses

Data collected on insect count were subjected to square root transformation ($\sqrt{x+1}$) and analysis of variance (ANOVA) using SAS (JMP 14) method. Means were separated with Student's Newman-Keuls tests at 5% probability, whilst percent grain damage, weight loss and percent inhibition rate were angular transformed (arcsine √proportion). The transformed data were analysed using analysis of variance (ANOVA) with SAS (JMP 14) method.

3. Results

3.1 Test for toxicity of the plant powders against *Sitophilus zeamais*

Significant ($p<0.05$) differences in toxicity were observed among the plant powders tested. There was 100% mortality with *Piper guineense* at 10.0% concentration and 92.5% and 97.5% mortality at 2.5 and 5.0% concentrations, respectively and are comparable to the check. Powders of *Eucalyptus globulus* and *Azadirachta indica* applied at 10% level caused 72.5 and 67.5% mortality of the weevils, whereas they recorded lower ($p<0.05$) mortality rates at 2.5 and 5% levels. Very low or no mortality was recorded for *Capsicum annum, Curcuma longa*, *Moringa oleifera* and *Icacina senegalensis* at all concentrations (Table 1).

Treatments showed significant differences in inhibition rate ($p<0.05$), *Piper guineense* recorded similar percentage inhibition rate to Fenitrothion (check) with 91, 98.5 and 97% inhibition at 2.5, 5 and 10%, respectively. Fenitrothion recorded 100% inhibition at 1.0%. *A. indica*, *E. globulus* and *C. longa* recorded significantly similar percentage inhibition rate of 83.25, 76.25 and 65.50% at 10%, respectively, but at 2.5 and 5% level, *A. indica* and *E. globulus* inhibited almost 62 to 65% F_1 progeny, whilst *C. longa* was most effective at 5% level with 71.75% inhibition rate. *M. oleifera*, *C. annum,* and

I. senegalensis affected F_1 progeny development minimally at all concentration levels with inhibition rates of about 14.25 to 37.5% (Table 1).

Table 1: Bioactivity and toxicity of plant powders tested against the *Sitophilus zeamais*

Treatment	Concentration (%)	% Mortality	% Inhibition rate
	2.5	30.00e	65.00bcde
Azadirachta indica	5	40.00d	62.25bcde
	10	67.50b	83.25abc
	2.5	47.50cd	49.50defg
Eucalyptus globulus	5	52.50c	54.50cdef
	10	72.50b	76.25abcd
	2.5	0.00f	26.75fghi
Capsicum annum	5	0.00f	37.50efgh
	10	0.00f	30.50fghi
	2.5	0.00f	50.50defg
Curcuma longa	5	0.00f	71.75abcd
	10	0.00f	65.50bcde
	2.5	0.00f	14.25hi
Icacina senegalensis	5	0.00f	17.50hi
	10	0.00f	26.25fghi
	2.5	92.50a	91.00ab
Piper guineense	5	97.50a	98.50a
	10	100.00a	97.00a
	2.5	0.00f	19.25hi
Moringa oleifera	5	0.00f	23.75ghi
	10	0.00f	19.50hi
Control	0	0.00f	0.00i
Fenitrothion (Check)	1	100.00a	100.00a

Means followed by same letter in a column for a parameter are not significantly different ($p>0.05$) using Student Neuman Keuls test.

3.2. Determination of the LD_{50} of selected plant powders against *Sitophilus zeamais*

The lethal dose (LD_{50}) is the quantity of the tested powders that would provide 50% mortality to the maize weevil. The positive slope regression indicates that mortality increases as the concentration is raised (i.e. dose-dependent) within 7 days (Table 2). *Piper guineense* was the most toxic powder among the tested plants with an LD_{50} (0.74 g/100g) as compared to *E. globulus* (3.89 g/100g) and *A. indica* (5.75 g/100g). *C. longa, C. annum, M. oleifera,* and *I. senegalensis* recorded either one or no mortality against the maize weevil. The LD_{50} values of the plant powders indicated that *P. guineense* was the most toxic followed by *E. globulus* and *A. indica* (Table 2).

Table 2: Lethal dose (LD_{50}) of the three most bioactive plant powders against *S. zeamais* after 7 days exposure

Plant powder	Slope (±S. E)	Intercept (±S. E)	LD_{50} (g/100 g seed)
P. guineense	2.67±0.45	5.34±0.33	0.74g
A. indica	1.64±0.43	3.75±0.31	5.75g
E. globulus	1.43±0.19	4.15±0.14	3.89g

3.3. Test for oviposition deterrence of selected plant powders on *Sitophilus zeamais*

Significant differences ($p<0.05$) were observed among the plant powders tested for oviposition. *Piper guineense* recorded zero mean number (0.00±1.11) of eggs at 5 and 10% and recorded only 1.50±1.11 mean number of eggs at 2.5% and this was statistically similar to *C. longa* with a mean number (2.75±1.11) of eggs at 10% level (Figure 1). Maize seeds treated with *M. oleifera, I. senegalensis, C. annum* and the control were not significantly different ($p>0.05$) in the mean number of eggs recorded at all concentrations whilst *C. longa, A. indica,* and *E. globulus* had similar number of eggs at 2.5 and 5.0% (Figure 1).

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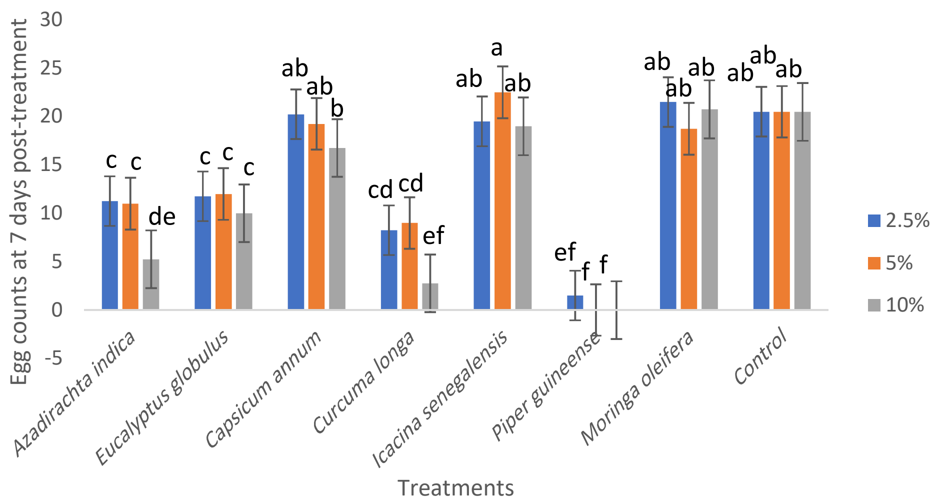

Fig.1: Oviposition deterrence effects of seven botanicals against the *Sitophilus zeamais*

3.4. Test for feeding deterrence of selected plant powders on *Sitophilus zeamais*

Treatments were significantly different ($p<0.05$) in percent weight loss and grain damage. Percent weight loss was significantly higher (0.89%) in the control, while seeds treated with *M. oleifera* (0.83%) and *I. senegalensis* (0.75%) were statistically similar at 2.5% concentration level. At all levels of concentration, maize seeds treated with *P. guineense* recorded the lowest percent weight loss from *S. zeamais* damage ($p<0.05$) and this was similar to Fenitrothion (check) at 1% level (Table 3). *A. indica*, *E. globulus* and *P. guineense* were statistically similar at 10% level, recording 0.14, 0.12, and 0.05% weight loss, respectively.

Table 3: Effect of selected botanicals on damage indices of *Sitophilus zeamais* at different concentration levels on maize grains

Treatment	Concentration (%)	% Weight loss	% Grain damage
Azadirachta indica	2.5	0.31d	3.12de
	5	0.30d	2.95def
	10	0.14e	2.04f
Eucalyptus globulus	2.5	0.28d	2.98def
	5	0.28d	2.98def
	10	0.12e	2.04f
Capsicum annum	2.5	0.42cd	3.73d
	5	0.45c	3.42d
	10	0.51c	3.67d
Curcuma longa	2.5	0.45c	3.90d
	5	0.38cd	3.83d
	10	0.39cd	3.67d
Icacina senegalensis	2.5	0.75ab	6.94abc
	5	0.73b	6.17c
	10	0.75ab	6.35bc
Piper guineense	2.5	0.06e	0.88g
	5	0.03e	0.32g
	10	0.05e	0.44g
Moringa oleifera	2.5	0.83ab	7.24ab
	5	0.86ab	6.88abc
	10	0.84ab	6.52bc
Control	0	0.89a	7.58a
Fenitrothion (Check)	1	0.00e	0.00g

Means followed by same letter in a column for a parameter are not significantly different ($P<0.05$) using Student Neuman Keuls test.

4. Discussion

According to Jallow and Pitan (2021), *S. zeamais* is a major pest of maize in The Gambia and any number of infestations greater than 5 weevils could result to severe damage to stored seeds if prompt and effective control measures are not instituted. The toxicity activity of the seven botanical powders tested to control *S. zeamais* varied significantly with the concentrations used. Powders of *P. guineense, A. indica* and *E. globulus* at 10% concentration exhibited significantly higher potent toxicity activity at 10% concentration than the other botanical powders. *Piper guineense* powder was highly effective with LD_{50} of 0.74 g/100 g, recording 100% mortality at 7 days post-treatment, mortality was similarly high at 2.5 and 5% levels with 92.5 and 97.5%, respectively. Spices and their extracts are known to have similar effects on stored product insects due to their characteristic volatile oils which are potent sources of botanical pesticides (Singh and Upadhyay, 1993; Kim and Ahn, 2001 and Owolabi *et al.,* 2009). Shazia *et al.* (2006) established that *P. guineense* powder gave significantly better results than the control in suppressing bruchid survival, higher numbers of undamaged seeds and fewer holes per seed. *Eucalyptus globulus* with an LD_{50} of 3.89g/100g recorded 72.5% mortality in maize weevils 7 days post-treatment at 10% concentration, but recorded low mortality at lower concentrations of 2.5 and 5%, suggesting that mortality of the maize weevil increased with rates applied. Mbaiguinam *et al.,* (2006) reported that ground *E. saligna* leaves releases their insecticidal effect on weevils more effectively, whereas *E. tereticornis* leaf powder significantly reduced the number of maize weevils by 65% (Muzemu *et al.,* 2013). Neem powder with an LD_{50} of 5.75g/100g, was effective against maize weevil at 10% concentration causing 67.5% mortality but the lower concentrations recorded mortality below 50%. This was contrary to the findings of Erenso and Berhe (2016), who observed that neem leaf powder at 1, 2 and 3% levels killed 61.13, 68.76 and 77.75% adult maize weevils, respectively. Achiri *et al.,* (2020) found that mortality of adult maize weevils increased with increasing concentrations from 2 to 10g w/w. This proves that toxicity of botanical insecticides is concentration-dependent for unitary formulations. An increased *S. zeamais* mortality was observed at higher concentrations of unitary formulations.

Three of the botanicals used in this study exhibited oviposition deterrent activity against *S. zeamais. Piper guineense* treated maize grains recorded 0.00 mean number of egg plugs at 5 and 10 % concentration (ww), whereas the control provided the least deterrent activity with a mean number of 20.5 egg plugs. *C. longa* at 10% concentration was very effective, exhibiting oviposition deterrent with 2.75 mean number of egg plugs. The effects of the plant powders on oviposition by *S. zeamais* could be due to metabolic alteration and consequently other systems of the body of the insects (Ileke, 2014). Bamaiyal *et. al.,* (2007) reported that the semiochemicals nature, pungent and peppery odour of the plant products might alter the behaviour and physiology of beetles adversely and thus prevent them from oviposition, *C. longa, Z. officinale, V. trifolia, M. cajuputi, H. caronarium, P. guajava,* and *H. cordata* were reported to possess high potential of oviposition deterrent property against *Aedes aegypti* at a concentration of 0.01% (v/v) (Tawatsin *et al.,* 2006).

After 30 days of maize weevil exposure, *I. senegalensis* and *M. oleifera* showed the largest percent weight loss among the treatments, recording mortalities that were quite similar to the control. Maize grains treated with *P. guineense* powder were similar to the synthetic Fenitrothion dust, incurring the least percent weight loss at all concentrations. Percent weight loss of maize seeds treated with neem and eucalyptus powder were low but significantly different from the untreated control. Ground plant extracts were reported to dehydrate and suffocate maize weevils and hence, reduce grain damage and weight loss (Hall, 1990; Parwada *et al.,* 2012). The maize grains treated with *P. guineense* recorded the least number of damaged seeds due to its high toxicity against the maize weevil and this was similar to the synthetic Fenitrothion dust that recorded zero grain damage. Neem and eucalyptus also exhibited effective grain protection against the weevil and were different from turmeric, chili pepper, *Icacina senegalensis* and the control that incurred higher grain damage percentages.

According to Jallow and Pitan (2022), seven of the eleven maize varieties cultivated by farmers in the Gambia are susceptible to the maize weevil infestations. Consequently, it is crucial for farmers to safeguard their grain stocks by applying effective natural biopesticides like *Piper guineense* and *Eucalyptus globulus*, which have demonstrated efficacy in countering this prevalent threat.

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4. Conclusion

The study found out that black pepper, neem, eucalyptus and turmeric could serve as maize grain protectants for farmers in rural Gambia, hence, minimizing the use of the synthetic Fenitrothion dust as grain protectant. These botanicals are readily available at a little cost to the farmer and they are environmentally friendly and easy to prepare.

Competing interest: Authors have declared that no competing interests exist.

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