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Potential of seed dusts of *Jatropha curcas* L., *Thevetia peruviana* (PERS.), and *Piper guineense* SCHUMACH. against the maize weevil, *Sitophilus zeamais* (MOTSCHULSKY, 1855) (Coleoptera: Curculionidae) in storage of corn grain

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ABSTRACT. A laboratory evaluation was carried out to determine the efficacy and phytochemical composition of powdered seeds of Physic nut *Jatropha curcas*, Yellow oleander *Thevetia peruviana* and West African black pepper *Piper guineense* at different dosages (2.5, 5.0, 7.5 and 10.0 g) for the management of *Sitophilus zeamais*. Treatments were mixed with 50 g of maize and infested with 10 adult *S. zeamais* in 200 ml air-tight glass vials and kept under ambient conditions (25-30°C and 70-90 RH) for a period of 28 days in August 2015. The trial was laid out in a completely randomized design in four replicates. Results from data analyses showed that treated maize grains in storage recorded significantly ($P < 0.05$) higher mean mortality levels of adult *S. zeamais* than the untreated controls. However, there were no significant differences in mean mortality of the weevil at 7, 14, 21 and 28 DAT, except on those stored only for 1 DAT. Batches treated with higher doses (10.0, 7.5, 5.0 g) suffered a higher total mortality of adult *S. zeamais*; this was significantly ($P < 0.05$) higher compared with treatments using smaller dosages (2.5 and 0.0 g). Phytochemical analysis of treatments revealed high levels of alkaloids, tannins and phenols in all the plant extracts. The flavonoid contents were also high in *Piper guineense* and Permethrin, but lower in *Jatropha curcas* and *Thevetia peruviana*. These compounds are known to possess insecticidal properties that may have been responsible for the mortality of *Sitophilus zeamais.*

KEY WORDS: Botanicals, efficacy, phytochemical, S*itophilus zeamais*.

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INTRODUCTION

Maize (*Zea mays* L.) is an important staple cereal crop for most people in sub-Saharan Africa (NUKENINE et al. 2002). Besides being a major source of food for both human and animals worldwide, it is also processed into various industrial products such as fuel (ethanol) and starches (OGUNSINA et al. 2011).

In the past, the efficacy of a particular pest control measure was the most important consideration to all stakeholders in the grain value-chain, but at present, the safety of workers, effects on the environment and product quality are issues of high priority. The bulk of African grains come from small-scale farmers' holdings. These traditional farmers use different kinds of plant products to control pests. The precise strategy used by different communities varies from place to place and appears to depend partly on the type and efficacy of suitable materials available in different locations (GREENBERG et al. 2005). Many African plants are potential sources of pesticides and have been shown to contain either anti-feedant, repellent or insecticidal compounds that enable the crude plant material or an extracted active compound to protect stored products (KUBO & NAKANISHI 1977). If sufficiently exploited, botanical pesticides can play a big role in reducing pollution, health risks and crop losses to pests (KAMATENESI-MUGISHA et al. 2008).

LALE (2010) observed that insect pests of grains can seldom be separated from their grains. The presence of *Sitophilus zeamais* (MOTSCHULSKY, 1855) has been established in the maize seed (LALE 2002, OBENG-OFORI & AMITEYE 2005, DANLOYE et al. 2008). In most of Africa, the greater proportion of maize is produced by resource-poor farmers in remote villages with poor road networks and poor post-harvest storage facilities, which often results in their incurring substantial losses (NTONIFOR & MONAH 2001). ISLAM (2006) estimated grain loss in Africa due to insect pest damage in storage at 20 to 30%. According to NUKENINE et al. (2002), *S. zeamais* causes more than 20% grain loss for untreated maize worldwide. Storage ensures food availability and security, profit maximization and seed availability for the next planting season.

In Nigeria, synthetic insecticides such as Lindane, methyl-bromide iodofenphos, malathion, permethrin, actellic sumithion, whose modes of action are broad-spectrum, have been marketed under different trade names to control stored-product pests. Farmers, produce-merchants and housewives store food grains with these insecticides in various facilities. The use of synthetic insecticides has been reported to leave residues (TOTEJA et al. 2006, UYGUN et al. 2007). A variety of botanicals such as *Jatropha curcas* L., *Thevetia peruviana* (L.) LIPPOLD, *Piper guineense* SCHUMACH. and *Carica papaya* L. are being used by resource-poor farmers in different parts of Nigeria (GADZIRAYI et al. 2006), but information on their efficacy against *Sitophilus zeamais* on stored maize in view of the crop's economic importance is scarce. The objectives of this study were therefore to determine the effectiveness and phytochemical composition of these botanicals for the control of *S. zeamais*, which is a major pest of stored maize.

MATERIALS AND METHODS

The experiment was conducted in August 2015 to determine the efficacy of selected botanicals (*Jatropha curcas*, *Thevetia peruviana* and *Piper guineense*) to control *Sitophilus zeamais* at the Post-Graduate Laboratory of the Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike (latitude 05°26’ to 5°25’ N and longitude 07°34’ to 7°36’ E), Abia State, Nigeria.

Insect Culture

The adult *S. zeamais* used for the experiment were cultured in plastic buckets covered with muslin cloth in the laboratory at 27±2 °C, 60 to 65% relative humidity and 12 h: 12 h light: dark regime. *S. zeamais* were obtained from infested stocks of maize from small scale farmers at Umuokrika Ekwerazu, Ahiazu Mbaise, Imo State. The *S. zeamais* used were reared for six weeks; only adults were used for this study.

Maize Used for the Experiment

The *Zea mays* used for the experiment was purchased from Ndoro market at Ikwuano L.G.A of Abia State. A pre-experiment was conducted to ascertain whether the maize was free from chemicals to avoid altering the results in the laboratory for 28 days. No mortality indicated that the maize grains were free from chemicals before use. Furthermore, the grains were heated in an oven at 60 ^{o}C for 4 hrs to destroy any hidden infestation and then maintained at ambient temperature and relative humidity.

Preparation of the Botanic Dusts

Two hundred grams of mature fresh seeds of the botanicals were collected from Umuokrika Ekwerazu and Amawom at Ikwuano L.G.A, Abia State. The seeds were sun-dried, ground separately into powder with a hand grinder and then sieved to obtain fine powders. The powders were put into dark air-tight containers to ensure that their active ingredients were not lost, and stored in a cool dry place until needed.

Synthetic Insecticide Used

The synthetic insecticide (Permethrin) used for the experiment was bought from a chemical dealer in Umuahia.

Methodology

The treatments, i.e. *Jatropha curcas*, *Thevetia peruviana* and *Piper guineense* (Table 1), the check (Permethrin) of different dosages (2.5, 5.0, 7.5 and 10.0 g), and an untreated control were tested against *Sitophilus zeamais* in stored maize. Each treatment dosage was mixed with 50 g of pristine maize grain and shaken thoroughly by agitating the vials manually. 1 g of talcum powder was also added to increase adhesion (binding) and treatment efficacy. The Completely Randomized Design (CRD) was adopted for the experiment and each treatment was replicated four times.

Table 1. Tested plant products, part used and their bioactive compounds.

Tested plant products	Part used	Bioactive compounds	References
Piper guineense West African black Pepper	Seed	Piperine	LALE & ALAGA (2000)
Jatropha curcas Physic nut	Seed	Phorbol esters (PEs)	MARTINEZ-HERRERA et al. (2004), GOEL et al, (2007)
Thevetia peruviana Yellow oleander	Seed	Unknown	–

Ten unsexed adults of *Sitophilus zeamais* were introduced into each vial and covered with muslin cloth held tightly in place by a rubber band to prevent the insects from escaping and/or predators entering.

Using the method reported by OMOTOSO (2014), the mortality counts of the weevils were recorded at 1, 7, 14, 21 and 28 days after treatment (DAT). The numbers of dead insects (insects which did not respond to pin probes) were recorded and removed from all the experimental vials. The counting was done by pouring the contents of each vial onto a small white tray and sorting the dead insects using a camel-hair brush and pin probe.

Phytochemical Analysis of the Plant Extract and the Synthetic Insecticides

The botanicals and Permethrin dust were extracted using ethanol, then subjected to preliminary screenings for the presence of secondary metabolites (alkaloids, tannins, flavonoids, phenols and saponins) using standard procedures (TREASE & EVANS 1989) with some modifications.

Test for Alkaloids

To test for alkaloids, 5 ml of 2% HCl was added to 2 ml of each of the samples in a test tube placed on a steam bath and warmed. This was filtered and a few drops of Wagner's Reagent (potassium iodide + iodine solution) were added to the filtrate in a test tube. The observed reddish brown precipitate indicated the presence of alkaloids (NWOKOCHA et al. 2011).

Test for Tannins

To test for tannins, 5 ml of each of the samples were treated with 2 ml of HCl and boiled for 5 minutes. A red precipitate confirmed the presence of tannins (NWOKOCHA et al. 2011).

Test for Flavonoids

To test for flavonoids, 1 ml of each of the samples was treated with 1 ml of dilute NaOH. A cloudy precipitate confirmed the presence of flavonoids (NWOKOCHA et al. 2011).

Test for Saponins

To test for saponins, 1 ml of each of the samples was added to 4 ml of distilled water and shaken. A stable frothing of foam indicated the presence of saponins (NWOKOCHA et al. 2011).

Test for Phenols

To test for phenols, 1 ml of each of the samples was added to 1 ml of 10% FeCl and mixed. A blue precipitate confirmed the presence of phenols (NWOKOCHA et al. 2011).

Data Analysis

Count data such as numbers of dead adult *Sitophilus zeamais* were transformed to square root values to improve the normality of the variable (variance stability), after which two-way analysis of variance was applied to compare means. Significant means were separated using the SNK test at a 5% error limit using SAS software version 8(2) (SAS 2001).

RESULTS

Effect of treatments on mean total mortality of *Sitophilus zeamais* in stored maize

Treated maize grains in storage caused significantly higher mean mortality than the untreated control (Fig. 1). Mean mortality at all treatment doses was higher with Permethrin and *Piper guineense* than with *Jatropha curcas* and *Thevetia peruviana*, although the differences were not significant.

There were no significant differences in mean adult mortality of treated maize grains stored at 7, 14, 21 and 28 DAT, except for those stored only for 1 DAT. Similarly, the treatments tested were not significantly different from each other, except for the batches treated with *T. peruviana*, where mean mortality increased significantly from 7 DAT (2.35) to 14 DAT (2.58) but decreased significantly at 21 DAT (2.14) (Fig. 2).

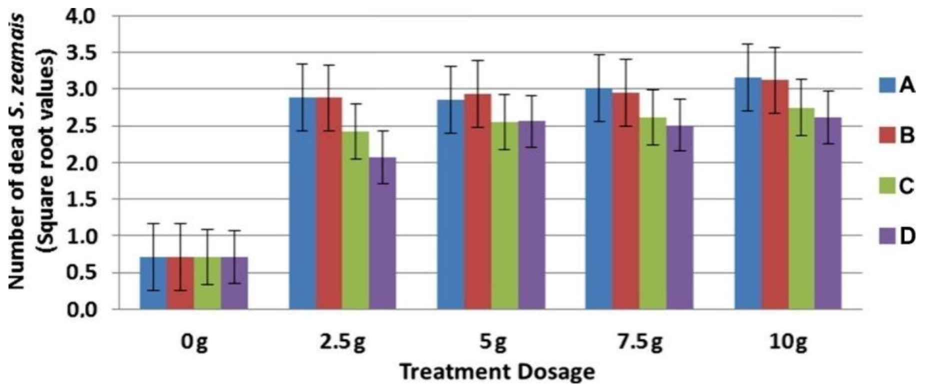

Fig. 1. Mean mortality of *Sitophilus zeamais* treated with different doses of botanicals. A – Permethrin, B – Piper guineense, C – *Jatropha* CURCAS, D – *Thevetia peruviana*.

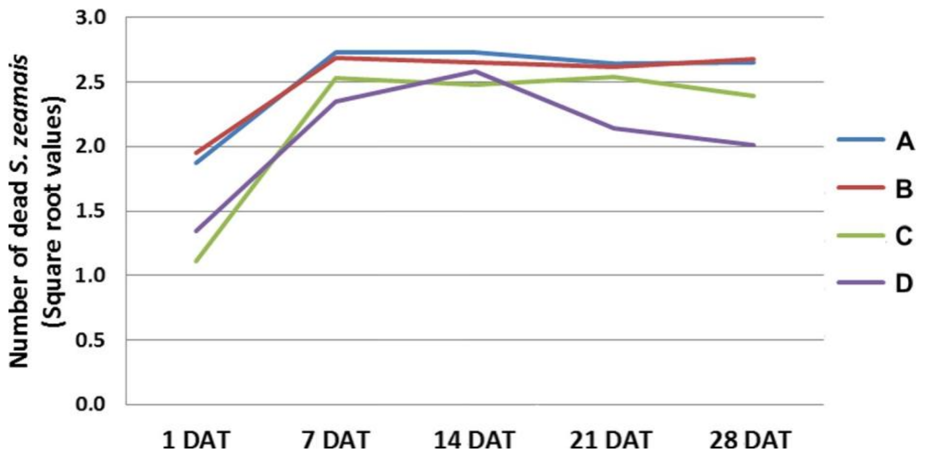

Fig. 2. Mean mortality of *Sitophilus zeamais* treated with botanicals at different days after treatment (DAT). A – Permethrin, B – Piper guineense, C – *Jatropha curcas*, D – *Thevetia peruviana*.

Mean mortality of *Sitophilus zeamais* treated with *Jathropha curcas* powder in stored maize

The result of maize grains treated with *Jatropha curcas* powder for the control of *Sitophilus zeamais* is shown in Table 2. Analysis of variance of the data showed that mortality levels of adult *S. zeamais* in grains treated with *Jatropha curcas* dust differed significantly from each other. Irrespective of storage duration, batches treated with 10.0 g caused significantly higher total mortality of adult *Sitophilus zeamais* (2.75); this was followed by batches with 7.5 g (2.55). The control recorded no mortality. The effect of storage duration on the mortality of weevils increased significantly with increased storage duration, but there were no significant differences between adult *S. zeamais* mortality recorded at 7, 14, 21 and 28 DAT, except at 1 DAT.

Table 2. Mean mortality of *Sitophilus zeamais* treated with *Jathropha curcas* dust in stored maize.

DAT	Treatment Dosage (g)					
	0	2.5	5.0	7.5	10.0	Mean
1	0.0 (0.71)	0.25 (0.84)	1.25 (1.31)	0.50 (1.31)	2.50 (1.73)	0.90 (1.11)[b]
7	0.0 (0.71)	8.25 (2.1)	8.50 (2.99)	9.50 (2.99)	7.25 (2.78)	6.75 (2.53)[a]
14	0.0 (0.71)	8.50 (2.95)	8.25 (2.95)	9.25 (2.49)	9.25 (3.12)	6.60 (2.48)[a]
21	0.0 (0.71)	7.25 (2.78)	6.25 (2.78)	8.50 (3.04)	9.75 (3.20)	6.85 (2.54)[a]
28	0.0 (0.71)	6.00 (2.55)	8.75 (2.55)	7.75 (2.91)	8.00 (2.90)	5.95 (2.39)[a]
Mean	0.0 (0.71)[b]	6.05 (2.42)[a]	8.00 (2.55)[a]	7.10 (2.62)[a]	7.35 (2.75)[a]	–

DAT = Days After Treatment
Figures in parenthesis are square root (√x +0.5) values.
Means within a column/row followed by the same letter do not differ significantly from each other (P > 0.05; SAS, PROC GLM, SNK).

Mean mortality of *Sitophilus zeamais* treated with *Piper guineense* in stored maize

Table 3 shows the evaluation of *Piper guineense* against *Sitophilus zeamais* in stored maize. Batches of grains treated with 10.0 g *P. guineense* recorded the highest total adult *S. zeamais* mortality (3.12), which was significantly different from the other treatments. Batches treated with 7.5, 5.0, and 2.5 g did not differ significantly, but were significantly higher than the untreated control.

There were no significant differences between adult *S. zeamais* mortality recorded at 7, 14, 21 and 28 DAT, except at 1 DAT.

Table 3. Mean mortality of *Sitophilus zeamais* treated with *Piper guineense* dust in stored maize.

DAT	**Treatment Dosage (g)**					
	0	**2.5**	**5.0**	**7.5**	**10.0**	**Mean**
1	0.0 (0.71)	4.00 (2.12)	1.25 (1.99)	3.50 (2.17)	7.25 (2.78)	3.80 (1.95)[b]
7	0.0 (0.71)	9.50 (3.16)	8.50 (3.20)	9.75 (3.12)	10.00 (3.24)	7.70 (2.69)[a]
14	0.0 (0.71)	9.25 (3.12)	8.25 (3.20)	9.75 (3.12)	9.25 (3.12)	7.50 (2.65)[a]
21	0.0 (0.71)	8.00 (2.91)	6.25 (3.08)	9.00 (3.08)	10.00 (3.24)	7.30 (2.62)[a]
28	0.0 (0.71)	9.00 (3.08)	8.75 (3.20)	9.75 (3.20)	9.75 (3.20)	7.65 (2.68)[a]
Mean	0.0 (0.71)[c]	7.95 (2.88)[b]	8.35 (2.94)[b]	8.40 (2.95)[b]	9.25 (3.12)[a]	–

DAT = Days After Treatment
Figures in parenthesis are square root (√x +0.5) values.
Means within a column/row followed by the same letter do not differ significantly from each other (P > 0.05; SAS, PROC GLM, SNK).

Mean mortality of *Sitophilus zeamais* treated with *Thevetia peruviana* in stored maize

Significantly higher adult *Sitophilus zeamais* mortality was recorded on samples treated with higher dosages (5.0, 7.5 and 10.0 g) than with the lower doses (2.5 and 0.0g) (Table 4). Also treatments containing 2.5 g *Thevetia peruviana* seed dust caused significantly higher mortality than the untreated control.

Thevetia peruviana treated grains recorded significantly different *Sitophilus zeamais* mortality at the various storage periods (DAT). Significantly higher mortality was recorded at 14 DAT, followed by 7 DAT; the lowest was at 1 DAT (Table 4).

Table 4. Mean mortality of *Sitophilus zeamais* treated with *Thevetia peruviana* dust in stored maize.

DAT	**Treatment Dosage (g)**					
	0	**2.5**	**5.0**	**7.5**	**10.0**	**Mean**
1	0.0 (0.71)	0.50 (0.96)	3.75 (2.06)	1.75 (1.48)	1.75 (1.49)	1.55 (1.34)[d]
7	0.0 (0.71)	4.75 (2.29)	7.75 (2.87)	8.75 (3.04)	8.50 (2.99)	5.95 (2.38)[b]
14	0.0 (0.71)	6.75 (2.99)	9.75 (3.20)	9.25 (3.12)	9.75 (3.20)	7.10 (2.58)[a]
21	0.0 (0.71)	4.75 (3.20)	5.50 (2.44)	6.00 (2.54)	7.00 (2.74)	4.65 (2.14)[c]
28	0.0 (0.71)	4.00 (2.74)	4.50 (2.22)	5.25 (2.38)	6.50 (2.64)	4.05 (2.01)[c]
Mean	0.0 (0.71)[c]	4.15 (2.07)[b]	6.25 (2.56)[a]	6.20 (2.51)[a]	6.70 (2.61)[a]	–

DAT = Days After Treatment
Figures in parenthesis are square root (√x +0.5) values.
Means within a column/row followed by the same letter do not differ significantly from each other (P > 0.05; SAS, PROC GLM, SNK).

Mean mortality of *Sitophilus zeamais* treated with Permethrin dust in stored maize

All treatments containing Permethrin dust caused significantly higher total adult *Sitophilus zeamais* mortality than the untreated control. Mortality of adult *S. zeamais* was significantly higher in samples treated with higher dosages (7.5 and 10.0 g) compared with the lower dosages (5.0, 2.5 and 0.0 g) (Table 5).

Table 5. Mean mortality of *Sitophilus zeamais* treated with Permethrin dust in stored maize.

DAT	Treatment Dosage (g)					
	0	2.5	5.0	7.5	10.0	Mean
1	0.0 (0.71)	2.75 (1.80)	2.25 (1.65)	4.75 (2.29)	8.00 (2.92)	3.55 (1.87)[b]
7	0.0 (0.71)	10.00 (3.24)	10.00 (3.24)	10.00 (3.24)	10.00 (3.24)	8.00 (2.74)[a]
14	0.0 (0.71)	10.00 (3.24)	10.00 (3.24)	10.00 (3.24)	10.00 (3.24)	8.00 (2.74)[a]
21	0.0 (0.71)	8.25 (2.96)	8.75 (3.04)	10.00 (3.24)	10.00 (3.24)	7.40 (2.64)[a]
28	0.0 (0.71)	9.50 (3.16)	9.25 (3.12)	9.00 (3.08)	9.50 (3.16)	7.45 (2.65)[a]
Mean	0.0 (0.71)[c]	8.10 (2.88)[b]	8.05 (2.88)[b]	8.75 (3.02)[ab]	9.50 (3.16)[a]	–

DAT = Days After Treatment
Figures in parenthesis are square root (√x +0.5) values.
Means within a column/row followed by the same letter do not differ significantly from each other (P > 0.05; SAS, PROC GLM, SNK).

Table 6. Phytochemical analysis of the botanicals and Permethrin dust.

Samples (Botanicals)	Alkaloids	Tannins	Flavonoids	Saponins	phenols
Piper guineense	++	++	++	+	+
Jatropha curcas	++	++	+	+	+
Thevetia peruviana	+	-	+	-	+
Permethrin dust	++	++	++	+	+

++ (high level), + (low level), - (absent)

Determination of phytochemical composition of treatments

The phytochemical composition of the treatments indicated that alkaloids were present at high levels (++) in *Jatropha curcas*, *Piper guineense* and Permethrin dust and at low levels (+) in *Thevetia peruviana*. High levels of tannins were present (++) in *Jatropha curcas*, *Piper guineense* and Permethrin dust, whereas they were absent (-) in *Thevetia peruviana*. Flavonoids were present in high concentrations (++) in *Piper guineense* and Permethrin dust, but in low ones (+) in *Jatropha curcas* and *Thevetia peruviana*. Saponins were present in low concentrations (+) in *Jatropha curcas*, *Piper guineense* and Permethrin dust, but absent (-) in *Thevetia peruviana*. Low levels of phenols were present (+) in all the plant seed dusts and Permethrin (Table 6).

DISCUSSION

The results of the study revealed that the botanical treatments tested significantly reduced the number of adult *Sitophilus zeamais* in maize stored for 28 DAT. The insecticidal activities of various plant parts and products on *S. zeamais* with similar trends have been reported by IVBIJARO (1983), COBBINAH & APPIAH-KWARTENG (1989), OBENG-OFORI et al. (1997), ARANNILEWA et al. (2006), ARAYA & EMANA (2009) and EDELDUOK et al. (2012). ADEDIRE & AJAYI (1996) recorded 100% mortality of *S. zeamais* treated with *Capsicum frutescens* L. 28 DAT on maize grains. This is comparable to the synthetic Permethrin as indicated by DANJUMMA et al. (2009). Similarly, recent studies by EDELDUOK et al. 2015 revealed that cotyledon powder of melon significantly reduced natality and oviposition of *Sitophilus zeamais* in storage while significantly increasing mortality.

It has also been shown that the efficacy of treatments increases with increasing dosage. This result is similar to the work done by UKEH et al. (2008), where 5 and 10 g powdered *Piper guineense* was significantly toxic to *Sitophilus zeamais* and suppressed F_1 progeny emergence compared to 1 g of powder and the control. This result is also in agreement with the observations of UDO (2005) and ASAWALAM et al. (2007), whose studies on plant spices demonstrated that a higher quantity of *Piper guineense* on *Sitophilus zeamais* caused significant mortality and also reduced the production of progeny. Studies on the insecticidal activity of *Jatropha curcas* have been reported by HABOU et al. (2011). The use of *Thevetia peruviana* fruits for suicide attempts has been reported in Sri Lanka and southern India (EDDLESTON et al. 1999). KHANAM et al. (2008) tested *Sapium indicum* WILLD., *Thevetia neriifolia* JUSS. and *Jatropha gossypifolia* L. on *Tribolium castaneum* (HERBST, 1797) and *T. confusum* JACQUELIN DU VAL, 1861 in Bangladesh and found them to possess insecticidal properties.

Although the mode of action of these botanicals was not determined in this trial, the death of *Sitophilus zeamais* might have been caused by blockage of the spiracles leading to asphyxiation and/or the insect ingesting lethal doses of the treatment thus resulting in stomach poisoning. LAW-OGBOMO & ENOBAKHARE (2007) and SIMBARASHE et al. (2013) made similar observations. Odours from *Piper guineense* was reported to significantly repel adult *Sitophilus zeamais* (UKEH et al. 2010, AKINBULUMI et al. 2017).

The presence of alkaloids, flavonoids and phenols in these botanicals may be responsible for their bioactivity. This hypothesis had earlier been supported by LALE

& ALAGA (2001), NWEZE et al. (2004) and CHAIEB (2010). AKHTAR & ISMAN (2004) also were of the opinion that the effectiveness of different plant materials on maize weevils may depend on several factors, such as chemical composition and maize weevil species susceptibility. The presence and level of secondary chemical metabolites may have contributed to the differences observed in the results. ARAYA & EMANA (2009) reported that the insecticidal activities of the plant powders are variable and broad, and depend on different factors like the presence of bioactive chemicals in them. Although post-treated seed viability was not tested in this trial, OJIAKO & KAYODE (2014) suggested that maize treated with botanicals had no negative effect on viability, and thus should be used as seeds for planting. However, OJIAKO et al. (2014) concluded that *Jatropha curcas* treated maize seeds should not be used for food on account of their containing poisonous alkaloids. The findings from this study give further credence to the insecticidal properties of the extracts from these locally available plants and could constitute a component of a maize weevil management programme.

CONCLUSIONS

1. Seed dusts of *Piper guineense, Jatropha curcas* and *Thevetia peruviana* significantly reduce the number of adult *S. zeamais* in stored maize.
2. Alkaloids, flavonoids and phenols were present in these botanicals and might be responsible for their bioactivity.

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