**Efficacy of *Piper guineense* SCHUMACH. seed powder in the control of
Callosobruchus maculatus (FABRICIUS, 1775) (Coleoptera:
Chrysomelidae: Bruchinae) and on the nutritional and organoleptic
characteristics of stored cowpea *Vigna unguiculata* (L.) WALP.**

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ABSTRACT. Brown pepper (*Piper guineense*) seed powder, used for culinary and medicinal purposes, was evaluated in the laboratory with a conventional storage chemical, actellic 2% dust (Pirimiphos-methyl), as standard, to protect stored cowpea against the cowpea bruchid, *Callosobruchus maculatus*. Four rates of the seed powder were used (2.5, 5.0 and 10.0 g/100 g seed with a control, 0.0 g) while actellic dust was applied at 1.0, 2.0, 3.0 g/100 g seed and 0.0 g/100 g seed as control. Data on percentage mortality, oviposition, adult emergence and seed damage were collected over a six-month period. Weevil perforation indexes (WPI) were estimated with the seed damage data. All data were analysed for variance at $P \leq 0.05$. Actellic dust at the lowest dose inflicted adult mortality (90 – 100%) in the first 48 h after treatment and prevented oviposition (95 – 100%) in the first five months of storage compared to the control. The highest rate of *Piper guineense* application caused mortality of up to 90.0% in 48 h, reduced oviposition (70 – 80%), decreased adult emergence (15.90 to 28.40 insects) as against the control (104.7 – 272.7 insects) and reduced seed damage by 80 % with a WPI of between 33.3 and 10.0 %. Proximate analysis and germination tests indicated that the treatment materials increased the moisture and crude fibre content, decreased crude protein content but had no significant effect on the viability of stored seeds. Organoleptic tests demonstrated that treatment materials had no significant negative effect on taste, odour, texture, appearance and overall acceptability of cooked cowpea seeds after six months of storage. It can be safely concluded that the powdered form of *P. guineense* seed at the highest doses (5.0 and 10.0 g) gave significant protection of cowpea seeds against the cowpea bruchid, which compared favourably with actellic dust when applied topically.

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KEY WORDS: Cowpea, Organoleptic tests. *Piper guineense*, Proximate analysis, Seed damage, Weevil perforation index.

INTRODUCTION

One of the greatest challenges facing the world today is to produce sufficient amounts of food for the growing population, which is expected to exceed 8.5 billion by the year 2030 and 9.7 billion by 2050 (UN DESA 2015). Of this, sub-Saharan Africa is projected to reach a population of about 1.96 billion or 21 percent of the global population (ALEXANDRATOS & BRUINSMA 2012) with an expected food demand growth of 60 – 70 % by 2050 (GODFRAY et al. 2010, MOLLINS 2016). The scenario is particularly critical in Nigeria, where agricultural production is barely adequate to meet even current needs of her over 180 million population, mainly due to heavy post-harvest losses and quality deterioration caused by storage pests, especially insects.

YOUDEOWI (1980) and FAKAYODE et al. (2014) estimated the loss due to insects to be 10 – 100 % for cowpea and noted that the greatest loss to this pulse in storage is caused by the cowpea seed beetle *Callosobruchus maculatus* (FABRICIUS, 1775). This loss has been projected at between 2.44 and 3.67 million tonnes of cowpea (IDACHABA & OLAYIDE 1976). It is not enough, therefore, to increase pulse production to combat protein deficiency prevalent in developing countries: there is a real need to reduce quantitative and qualitative losses during storage (AGARWAL et al. 1988).

Cowpea *Vigna unguiculata* (L.) WALP. belongs to the family Leguminosae, subfamily Papilionoideae and genus *Vigna* (VERDCOURT 1970). It is acknowledged as “vegetable meat” (OYEWALE & BAMAIYI 2013), because of its high protein content and is the major protein source for more than 200 million people in sub-Saharan Africa (ENV/JM/MONO 2015). Nigeria is the largest producer and consumer of cowpeas, accounting for 61% of production in Africa and 58% worldwide (ITA 2012).

The cowpea bruchid, *Callosobruchus maculatus*, a member of the subfamily Bruchinae (seed beetles) in the family Chrysomelidae (KERGOAT et al. 2007), is a field-to-store pest and the most important pest of stored cowpea (CASWELL 1980). Infestation begins by adults laying eggs on mature pods in the field, each female laying up to 100 eggs (BECK & BLUMER 2014). Adult emergence usually occurs after harvest in the store.

Management of cowpea seed storage pests relies heavily on the use of chemical insecticides (TIROESELE et al. 2015). These include chemicals such as lindane, dichlorvos, fumigants like phosphine tablets, ethylene dibromide, hydrogen cyanide and methyl bromide, as well as synthetic pyrethroids like permethrin and deltamethrin. Pirimiphos-methyl (actellic 2% dust) (O-(2-(diethylamino)-6-methyl-4-pyrimidinyl)-O, O-dimethyl

phosphorothioate), used for comparative purposes in this study, is the synthetic insecticide of choice in Nigeria. It is used in large-scale commercial agricultural applications as well as in consumer products for domestic purposes (ISMAN 2006, ACES 2017), especially where there is high vector resistance to pyrethroids and organochlorines (FUSEINI et al. 2011).

Despite the efficacy of these synthetic insecticides, several adverse effects have reportedly emerged from their misuse. These include: insect resistance, pest resurgence, health hazards, biomagnification of toxic residues in the trophic chain, increasing costs of application, widespread environmental hazards, poisonings of humans and domestic animals, destruction of natural enemies and contamination of underground waters and rivers (AYOOLA & AJANI 2008, GRZYWACZ & LEAVETT 2012). These debilitating effects have directed the need for effective but relatively safe, eco-friendlier, readily available and biodegradable botanical pesticides (TALUKDER & HOWSE 2000, DAYAN et al. 2009).

Piper guineense SCHUMACH., a spice plant from the class Magnoliopsida, order Piperales and family *Piperaceae*, is an important plant that has culinary, medicinal, cosmetic and insecticidal uses (JULIANI et al. 2013, BESONG et al. 2016). The plant is found in tropical regions of central and west Africa, where it is semi-cultivated in countries like Nigeria, especially in the southern parts (PURSEGLOVE et al. 1981). It is known in Nigeria as 'Uziza' (Igbo), 'Iyere' (Yoruba) and 'Masooro' (Hausa). Other common names are Ashanti pepper, Guinea pepper, Benin pepper and false cubeb.

Piper guineense is an important source of various nutrients and phytochemicals such as proteins, carbohydrates, vitamins, minerals, fat, alkaloids, steroids, lignins, glycosides, saponins, flavonoids, tannins and phenolic compounds. It is also known to have antibacterial, antioxidant, anti-inflammatory, hepatoprotective, fertility, aphrodisiac, anticonvulsant and larvicidal properties (ECHO et al. 2012, OKOYE & EBELEDIKE 2013, NWANKWO et al. 2014, BESONG et al. 2016, UKPAI et al. 2017).

The seeds contain piperine and chavicine, which are reported to have active insecticidal ingredients (OKONKWO & OKOYE 1996). Recent studies have shown that *Piper guineense* can be used as an alternative in the control of insect pests in vegetable production. OKUNLOLA & AKINRINNOLA (2014) reported that a *P. guineense* extract was effective in the control of insect pests of *Telferia occidentalis* HOOK. F., *Amaranthus hybridus* L. (African spinach) and *Abelmoschus esculentus* (L.) MOENCH (Okra), while IBEKWE et al. (2014) showed that the bio-insecticide was effective in the control of insect pests of *Solanum* spp. L. (garden egg). UKPAI et al. (2017) recently reported the insecticidal potential of *Piper guineense* seed powder against *Sitophilus zeamais* (MOTSCHULSKY, 1855) in stored corn grain.

Since chemical control is inappropriate for the small-scale farmer (VAN HUIS 1991) and local farmers are mostly receptive to storage protection methods that lie within their technical and financial means (TAYLOR 1975), this study aims at confirming and or

identifying insecticidal activity in *Piper guineense* and its effective rates when compared with a known insecticide. It goes further in that it establishes whether the plant product has effects on the viability, nutritional and organoleptic qualities of stored cowpea seeds. This should increase the diversity of plant biocides that can serve as models for the synthesis of new insecticidal compounds.

MATERIALS AND METHODS

Laboratory Culture of *Callosobruchus maculatus*

Adult *C. maculatus* were collected from infested cowpea seeds from 'Oja Oba' market in Ilorin, Nigeria. Insects were introduced into two breeding containers with susceptible cowpea seeds at ambient temperature ($28\pm3^{\circ}\text{C}$) and a relative humidity of $75\pm5\%$. These were used to establish a laboratory culture.

Collection and Preparation of Cowpea Seeds

Some quantities of untreated cowpea seeds *Vigna unguiculata* cv. TVU 3629 were collected from the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. The seeds were air-dried in the laboratory to prevent mould, after which they were sorted, sealed in a cellophane bag and put in a deep freezer (for two weeks) for thorough disinfestation. Prior to use, the seeds were air-dried for 4 h. One hundred grams of seeds were weighed out into 250 ml translucent plastic tubes, which were covered with clean baft cloth to allow the inflow of air and to mimic storage conditions in jute bags. There were altogether 21 translucent plastic tubes (with 100 g cowpea seeds) for the envisaged treatment replicates.

Procurement and Preparation of Treatment Materials

Ripe seeds of *Piper guineense* were collected from a bush near the University of Ilorin, Nigeria. The collected seeds were air-dried, pulverized into fine powder, properly sieved with a 10-micron sieve and sealed in cellophane bags. Actellic 2% dust (Pirimiphos-methyl) was purchased from Syngenta, Agege Road, Lagos, Nigeria.

Treatment and Storage of Cowpea Seeds

Weights of *Piper guineense* seed powder (2.5 g, 5.0 g, 10.0 g) and actellic 2% dust (1.0 g, 2.0 g, 3.0 g) were measured out, respectively, including a control (0.00 g). The seven treatments were replicated thrice, making a total of 21 replicates, and put into the 250 ml plastic tubes with 100 g of clean cowpea seeds in each tube. The cowpea seeds were

thoroughly mixed with the test materials and laid out in a completely randomized design in the laboratory. The controls (0.00 g) had neither plant product nor actellic dust added.

Bioassay

Twenty-four hours after test material application, 50 seeds from each of the treated and untreated (control) cowpea seeds were placed in new 100 ml plastic tubes. Five pairs of male and female *Callosobruchus maculatus* were introduced into each of the plastic tubes, which were covered with baft cloth and tied firmly with a rubber band. For sex differentiation, the elytral pattern of female *C. maculatus* is usually maculate whereas that of the male is plain. The females are dark coloured and possess four elytral spots, while males are brown and less distinctly spotted. The females of most strains are larger than the males and have large, dark-coloured plates covering the end of their abdomen (HALSTEAD 1963, BECK & BLUMER 2014).

Effect on Mortality: The effect of the plant materials on the mortality of the adults was assessed 24 h, 48 h and seven days after treatment. At 24 and 48 h, the dead insects were discarded. The percentage mortality was calculated using the formula:

$$\text{Percentage Mortality (\%)} = \frac{\text{No. of dead insects}}{\text{Total No. of insects}} \times 100$$

Effect on Oviposition: The number of eggs laid on the cowpeas was recorded on the 7th day on a sub-sample of 10 randomly selected seeds. The distribution of the eggs was also recorded.

Effect on Adult Emergence: The total number of emerged F₁ adults (dead and alive) were counted on the first day that emergence was noticed (23±1 days after introduction of insects). On this day, all the insects, dead and alive, were removed from the jars in order to distinguish them from those that would emerge later.

Effect on Seed Damage: Damage was assessed by the total number and distribution of holes per cowpea seed. The number of holes per sample of 10 randomly selected seeds and the number of these seeds with holes was recorded for damage assessment. These were checked and recorded on the same date and time that the first emerging adults were noticed.

The percentage of perforated grains and the Weevil Perforation Index (WPI) (FATOPE et al. 1995) was calculated thus:

$$\text{Percentage Grains (\%)} = \frac{\text{No. of perforated grains}}{\text{Total number of grains}} \times 100$$

A weevil perforation index value exceeding 50 is regarded as enhancement of infestation by the weevil or the negative protective ability of the plant material or insecticide tested.

Residual Tests

To assess the residual effect of *Piper guineense* seed powder and actellic dust on stored seeds, the above procedures were repeated after the first, second, third, fourth, fifth and sixth months of storage of the original 100 g seeds. Fifty seeds were taken each month from the stock, placed in clean 100 ml plastic tubes and five pairs of insects introduced, as at the start of the experiment.

Sample analyses

Proximate Analysis: The first analysis was done with 3 replicates of untreated cowpeas after disinfestation and air drying of the procured seeds at the outset of the experiment. The aim was to determine base values for the seeds to be compared with the values obtained after storage with the treatment materials. The standard methods of the Association of Official Analytical Chemists (AOAC 2005) were used to determine moisture, crude fat, crude protein, total ash and crude fibre content. All the analyses were carried out in triplicate.

The second sample analysis was done six months after the cowpea seeds had been stored with the different rates of plant materials and actellic dust. The test plant material and actellic dust were very carefully sieved off the seeds before the analyses. Similar procedures as in the previous case were used.

Viability Tests: For the first viability test, 30 cowpea seeds were selected from the reserve of untreated seeds. Ten seeds each were placed in three Petri dishes, whose bases were lined with Whatman No.44 filter paper moistened with water. The experiment was left for seven days to ensure all the viable seeds germinated. Numbers of emerged seedlings per Petri dish were recorded seven days after set-up. The next germination test was carried out six months after the onset of the experiment. Ten seeds were randomly taken from the stored samples and placed in Petri dishes laid out as described above. This gave a total of 21 Petri dishes. As before, numbers of emerged seedlings per Petri dish were recorded seven days after set-up. The percentage germination was calculated using the following formula:

$$\text{Percentage germination (\%)} = \frac{\text{No. of germinated seeds}}{\text{No. of seeds planted}} \times 100$$

Organoleptic Evaluation: Only the highest rates (10.0 g/100 g seed for *Piper guineense* and 3.0 g/100 g seed for pirimiphos-methyl) were used. This gave a total of three samples (including a control). The highest rate was chosen to bring out the 'best' or 'worst' organoleptic property, as the case may be.

One hundred grams (100 g) each of the samples were washed and cooked with clean water in clean pots over a gas cooker with four cooking points and boiled for 65 min. No extraneous flavours were added to the cooking and the samples were presented to panellists at the same temperature (LARMOND 1977). To eliminate subconscious bias in the panellists, cryptic labelling was employed. Samples were labelled A to C. The cooked seeds were served to a panel of ten judges in a tasting room devoid of environmental interferences of extraneous odour and noise. Questionnaires were drawn up to determine the sought-after parameters, that is, taste, odour, texture, appearance and overall acceptability. A five-point hedonic scale method (AMERINE et al. 1965) with scores ranging from 0 to 4, where 0 is bad and 4 excellent, was used to rate the cooked seeds.

RESULTS

Effect of Actellic Dust

Very high mortality of adult *Callosobruchus maculatus* (73.3 – 100.0 %) was recorded within 24 h, in the first four months, in cowpea seeds treated with actellic dust (Table 1). The untreated seeds recorded between 0.0 – 6.7 % mortality within the same period. Seeds treated with actellic dust also had a very low number and distribution of eggs throughout the six months of the experiment (Table 2). The highest number of eggs laid per seed, for the first four months of the experiment, was 0.7 eggs, while a maximum of about 33.0% seeds were oviposited on as against the 5.6 eggs, and 100% of the seeds in the untreated control, respectively. The synthetic inhibited the emergence of adult *C. maculatus* in treated samples during the six months of storage (Table 3) with the highest emergence of 14.7 insects as against 272.7 insects in the control. The least number of damaged cowpea seeds stored over the six-month period of storage (Table 4) was also recorded in seeds treated with the synthetic. The mean number of holes per seed and the number of seeds with holes ranged from 0.0 to 0.3 and 0.0 – 3.3, respectively, whereas that of the untreated control, ranged from 1.4 to 3.8 holes/seed and from 8.7 to 10.0 seeds with holes, respectively. A Weevil Protection Index of between 0.00 – 33.3% was recorded, indicating that actellic had better protection ability. There were no significant differences between the rates of application. However, it appears that the medium and highest rates, 2.0 and 3.0 g/100

Table 1. Efficacy of *Piper guineense* seed powders and Actellic 2 % dust on adult mortality of *Callosobruchus maculatus*. (c) – control.

Materials	Rate (g/ 100g)	24hr	48hr	7 days
Commencement				
Actellic dust	1.0	96.7 a	100.0 a	100.0 a
	2.0	100.0 a	100.0 a	100.0 a
	3.0	96.7 a	100.0 a	100.0 a
<i>P. guineense</i>	2.5	13.3 de	66.7 c	100.0 a
	5.0	20.0 cd	63.3 c	100.0 a
	10.0	36.7 b	76.7 b	100.0 a
	0.0 (c)	0.0 f	3.3 ef	36.7 c
S. E. M.		2.8	3.3	4.4
Month 2				
Actellic dust	1.0	100.0 a	100.0 a	100.0 a
	2.0	100.0 a	100.0 a	100.0 a
	3.0	100.0 a	100.0 a	100.0 a
<i>P. guineense</i>	2.5	43.3 b	56.7 de	96.7 ab
	5.0	40.0 bc	76.7 bc	96.7 ab
	10.0	36.7 bc	90.0 ab	100.0 a
	0.0 (c)	6.7 gh	30.0 ef	63.3 cd
S. E. M.		4.5	6.2	7.6
Month 4				
Actellic dust	1.0	73.3 b	90.0 a	100.0 a
	2.0	100.0 a	100.0 a	100.0 a
	3.0	100.0 a	100.0 a	100.0 a
<i>P. guineense</i>	2.5	10.0 e	43.3 c	96.7 a
	5.0	10.0 de	40.0 c	96.7 a
	10.0	16.7 cd	60.0 b	100.0 a
	0.0 (c)	0.0 f		
S. E. M.		2.1	3.4	4.0
Month 6				
Actellic dust	1.0	30.0 b	56.7 b	96.7 ab
	2.0	33.3 b	63.3 b	100.0 a
	3.0	43.3 a	76.7 a	100.0 a
<i>P. guineense</i>	2.5	0.0 f	30.0 ef	100.0 a
	5.0	0.0 f	20.0 f	100.0 a
	10.0	10.0	20.0 f	100.0 a
	0.0 (c)	0.0 f	3.3 g	86.7 c
S. E. M.		1.62	3.23	2.82

Means followed by the same letter(s) in each section of the same column are not significantly different at $P \leq 0.05$ using the New Duncan Multiple range test.

Table 2. Efficacy of *Piper guineense* seed powders and Actellic 2 % dust on oviposition of adult *Callosobruchus maculatus*. (c) – control.

Materials	Rate (g/ 100g)	No. of eggs/seed	No. of seeds with egg
Commencement			
Actellic dust	1.0	0.7 ab	3.3 b
	2.0	0.5 a	1.7 a
	3.0	0.5 a	3.0 a
<i>P. guineense</i>	2.5	1.1 ab	3.3 ab
	5.0	0.5 a	2.0 ab
	10.0	0.7 ab	4.0 b
	0.0 (c)	5.6 cd	10.0 de
S. E. M.		0.64	0.66
Month 2			
Actellic dust	1.0	0.2 a	1.0 a
	2.0	0.1 a	0.7 a
	3.0	0.1 a	0.3 a
<i>P. guineense</i>	2.5	0.3 ab	6.7 cd
	5.0	0.9 bc	4.3 b
	10.0	1.0 bc	1.7 ab
	0.0 (c)	2.9 c	9.7 d
S. E. M.		0.51	0.69
Month 4			
Actellic dust	1.0	0.2 a	2.0 a
	2.0	0.2 a	1.0 a
	3.0	0.1 a	1.0 a
<i>P. guineense</i>	2.5	1.7 b	8.0 ef
	5.0	1.5 ab	7.7 de
	10.0	1.4 ab	7.7 de
	0.0 (c)	4.8 cd	10.0 e
S. E. M.		1.01	0.36
Month 6			
Actellic dust	1.0	30.7 a	5.7 c
	2.0	0.4 a	4.0 b
	3.0	0.2 a	20. a
<i>P. guineense</i>	2.5	1.6 ab	7.0 d
	5.0	1.1 a	5.3 a
	10.0	1.0 a	4.7 bc
	0.0 (c)	8.2 d	10.0 ef
S. E. M.		0.68	0.40

Means followed by the same letter(s) in each section of the same column are not significantly different at $P \leq 0.05$ using the New Duncan Multiple range test.

Table 3. Efficacy of *Piper guineense* seed powders and Actellic 2 % dust on emergence of adult *Callosobruchus maculatus* (F₁ Generation). (c) – control, FE – first emergence, 48 hr – emergence at 48 hr, 96 hr – emergence at 96 hr, TCE – total cumulative emergence.

Materials	Rate (g/ 100g)	FE	48 hr	96 hr	TCE
Commencement					
Actellic dust	1.0	1.0 a	0.3 a	0.0 a	1.3 a
	2.0	0.7 a	0.3 a	0.0 a	1.0 a
	3.0	0.0 a	0.0 a	0.0 a	0.0 a
<i>P. guineense</i>	2.5	21.0 bc	9.7 b	2.3 ab	33.0
	5.0	12.0 ab	8.0 b	1.0 ab	21.0 ab
	10.0	6.3 ab	4.7 ab	2.3 ab	13.3 ab
	0.0 (c)	125.0 c	24.3 c	7.7 c	157.0 d
S. E. M.		13.37	6.60	4.4	19.27
Month 2					
Actellic dust	1.0	0.0 a	0.0 a	0.0 a	0.0 a
	2.0	0.0 a	0.0 a	0.0 a	0.0 a
	3.0	0.0 a	0.0 a	0.0 a	0.0 a
<i>P. guineense</i>	2.5	14.3 ab	1.7 ab	2.3 ab	18.3 ab
	5.0	13.7 ab	4.3 bc	5.0 cd	23.0 ab
	10.0	9.0 a	3.7 b	3.3 bc	16.0 ab
	0.0 (c)	67.3 b	21.3 c	16.0 d	152.7 c
S. E. M.		19.57	6.83	4.22	28.4
Month 4					
Actellic dust	1.0	0.7 a	0.0 a	0.0 a	0.7 a
	2.0	0.3 a	0.0 a	0.0 a	0.3 a
	3.0	0.3 a	0.0 a	0.0 a	0.3 a
<i>P. guineense</i>	2.5	13.7 a	2.7 a	0.7 a	17.0 ab
	5.0	10.7 a	2.7 a	1.7 ab	15.0 ab
	10.0	18.3 a	2.0 a	2.7 bc	13.0 ab
	0.0 (c)	99.0 c	37.3 b	16.3 d	152.7 c
S. E. M.		19.57	6.83	4.22	28.4
Month 6					
Actellic dust	1.0	9.7 a	3.0 a	2.0 a	14.7 a
	2.0	5.7 a	0.7 a	1.0 a	7.3 a
	3.0	4.0 a	1.3 a	0.0 a	5.3 a
<i>P. guineense</i>	2.5	19.0 a	13.3 a	6.0 ab	38.3 a
	5.0	15.3 a	5.7 a	3.3 a	24.3 a
	10.0	7.3 a	3.3 a	1.3 a	12.0 a
	0.0 (c)	189.7 c	56.3 b	26.7 c	272.7 b
S. E. M.		13.24	5.87	3.11	15.9

Means followed by the same letter(s) in each section of the same column are not significantly different at $P \leq 0.05$ using the New Duncan Multiple range test.

Table 4. Efficacy of *Piper guineense* seed powders and Actellic 2 % dust on the damage assessment of stored cowpea seeds. (c) – control.

Materials	Rate (g/ 100g)	No. of holes/seed	No. of seeds with holes	Weevil perforation index (WPI)
Commencement				
Actellic dust	1.0	0.0 a	0.3 a	3.3 a
	2.0	0.0 a	0.0 a	0.0 a
	3.0	0.0 a	0.0 a	0.0 a
<i>P. guineense</i>	2.5	0.3 a	2.0 ab	20.0 bc
	5.0	0.1 a	1.0 a	10.0 ab
	10.0	0.1 a	1.0 a	10.0 ab
	0.0 (c)	2.6 d	10.0 d	–
S. E. M.		0.37	0.72	8.29
Month 2				
Actellic dust	1.0	0.0 a	0.0 a	0.0 a
	2.0	0.0 a	0.0 a	0.0 a
	3.0	0.0 a	0.0 a	0.0 a
<i>P. guineense</i>	2.5	0.5 ab	3.3 bc	40.5 cd
	5.0	0.5 ab	3.3 bc	37.7 bc
	10.0	0.3 a	2.0 ab	23.7 ab
	0.0 (c)	1.4 d	8.7 d	–
S. E. M.		0.28	0.92	11.85
Month 4				
Actellic dust	1.0	0.0 a	0.0 a	0.0 a
	2.0	0.0 a	0.3 a	3.3 a
	3.0	0.0 a	0.0 a	0.0 a
<i>P. guineense</i>	2.5	0.5 ab	2.0 c	21.2. bc
	5.0	0.4 ab	2.7 c	27.8 c
	10.0	0.3 ab	9.7 d	24.1 c
	0.0 (c)	2.3 d	9.7 d	–
S. E. M.		0.47	0.53	6.10
Month 6				
Actellic dust	1.0	0.3 a	3.3 b	33.3 b
	2.0	0.2 a	3.0 b	30.0 b
	3.0	0.1 a	1.7 a	16.7 a
<i>P. guineense</i>	2.5	0.4 a	4.0 bc	40.5 bc
	5.0	0.4 a	3.7 bc	40.0 bc
	10.0	0.3 a	3.3 b	33.3 b
	0.0 (c)	3.8 b	10.0 e	–
S. E. M.		0.38	0.33	3.76

Means followed by the same letter(s) in each section of the same column are not significantly different at $P \leq 0.05$ using the New Duncan Multiple range test.

g seed, respectively, were better than the lowest rate (1.0 g/100 g seed). The medium rate, therefore, seems to be the most reasonable rate to be recommended. The seeds treated with actellic dust were clean, hard and mostly undamaged.

Effect of *Piper guineense*

The mortality of insects in seeds treated with *P. guineense* reached 90.0 % in 48 h in the second month of storage (Table 1). When compared with the control, the bioinsecticide performed well in reducing the number of eggs laid throughout the six months of the experiment (Table 2). The effect of *P. guineense* in suppressing oviposition was statistically the same as in seeds treated with actellic dust. However, the eggs laid were more evenly distributed in the seeds treated with *P. guineense* than those treated with actellic dust.

Total cumulative emergence from seeds treated with *P. guineense* was consistently very low. The highest application rate (10.0 g) recorded a very low number of emerged insects, with the highest being 16.0 insects (as against 272.7 insects in the control), throughout the six months of the experiment (Table 3).

There were relatively few damaged cowpea seeds stored with *P. guineense*. The highest numbers of holes per seed and seeds with holes sampled were 0.60 holes and 6.0 seeds as against the 3.80 holes and 10.00 seeds in the untreated control, respectively. The WPI for the highest rate of *P. guineense* treated seeds (10.0 – 33.3%) compared favourably with the protection ability of actellic dust (with a WPI of between 0.0 – 33.3% for the lowest rate of application). The efficacy of *P. guineense* in the experiment is discernibly dose-related, with the highest rate (10.0 g/100 g seed) performing better than the lower rates.

Effect on Proximate Analysis

Piper guineense seed powder and actellic dust increased the moisture and crude fibre content, decreased crude protein but had no discernible effect on crude fat and total ash of cowpea seeds (Figure 1). The effects were dose-related.

Effect on Seed Viability

Actellic dust and seed powder of *Piper guineense* used in storing the cowpea seeds had no significant effect on viability (Figure 2).

Effect on Organoleptic Analysis of Cooked Cowpea Seeds

The treatment materials had no significant effect on the taste, odour, texture, appearance and overall acceptability of cooked cowpea seeds (Figure 3).

Fig. 1. Proximate analysis of cowpea seeds after treatment with *Piper guineense* seed powder (Pg.) and Actellic 2% dust (Act.) at different rates.

Fig. 2. Percentage germination after treatment with *Piper guineense* seed powder and Actellic 2% dust and storing for six months.

Fig. 3. Organoleptic ratings of cooked cowpea after treatment with *Piper guineense* seed powder (Pg.) and Actellic 2% dust (Act.) and storing for six months.

DISCUSSION

The result obtained regarding the efficacy of actellic dust is in agreement with earlier works by OGUNWOLU & ODUNLAMI (1996), DANKWA (1998) and MULUNGU et al. (2007). In their assessment of the residual activity of pirimiphos-methyl on *Callosobruchus maculatus* treatment, DASBAK et al. (2009) also affirmed that the insecticide drastically reduced the developmental potential and extent of pigeonpea seed damage by the bruchid, six months post treatment. AKINKUOLERE (2012) has, however, reported that cowpea seeds treated with 4.0 % pirimiphos-methyl elicited only 60.0% mortality in 4 days after treatment (DAT) when compared with *Monodora myristica* (GAERTN.) DUNAL (80.60%) and *Momordica charantia* (L.) (69.80%), respectively. This may well be due to the source of the pirimiphos-methyl used, as the issue of unbridled adulteration/counterfeiting of pesticides in Nigeria has been variously reported (ASOGWA & DONGO 2009, IDRIS et al. 2013).

Pirimiphos-methyl has been found to have contact and fumigant activity (ČERNÁKOVÁ et al. 1992, WHO 2004), is fast acting, effective at low concentrations and could, at 375 ml/100 litres of water, be used to control aphids (*Myzus persicae* SULZ.) and whiteflies (*Bemisia tabaci* (GENN.)) in Okra (SHOURA CHEMICALS 2012).

Piper guineense performed well when compared with the control in causing insect mortality and in reducing the number of eggs laid, but egg dispersal on seeds was greater. This could be the result of high insect mortality in actellic dust treated seeds within the first 24 h, as against those in the *P. guineense* treated seeds, which gave the insects room to move about for a more even distribution of the eggs. Since the percentage mortality of insects in *P. guineense* treated seeds was far less than that of actellic dust, it does seem that *P. guineense* has an oviposition deterrent effect.

Fewer emerged insects in the higher rates of *P. guineense* during 6 months of storage indicated that the effect of the plant materials was the same despite the length of time in storage. This is contrary to the effect of actellic dust on treated seeds, which showed that the toxic/suppressant effect of the synthetic gradually started to wear off after the first 4 months. The total numbers of emerged insects in the *P. guineense* treated seeds (for all three rates) for the fourth and sixth months (45.0 and 74.6, respectively) when compared with the number of emerged insects in the control (152.7 and 272.7 insects, respectively), clearly showed that the plant material was highly effective. When compared with the relatively large number of surviving insects during the first 48 h of the introduction of insects into treated samples, it becomes evident that *P. guineense* not only suppressed oviposition but also inhibited subsequent emergence of adults. AKAMI et al. (2017) has observed that in cowpea seeds treated with *Lippia adoensis* HOCHST ex. WALP. essential oil, most of the eggs laid could not complete their life cycle, thereby leading to a reduced adult population capable of producing subsequent generations and thus limiting seed damage.

There were also relatively few damaged cowpea seeds stored with *Piper guineense*. The WPI of the lowest rate of actellic dust was the same as the highest rate of *P. guineense*. Extracts of *P. guineense* have been shown to have insecticidal action (IVBIJARO 1990). Later works by LALE (1992) and ASHAMO & ODEYEMI (2001) reported that an oil extract of *P. guineense* was extremely toxic to adult *Sitophilus zeamais* when compared with oils from other plant products.

The potency of *Piper guineense* has been attributed to piperine acting in synergism with guineensine (OKOGUN et al. 1977), which was reported to be lethal to the grasshopper *Zonocerus variegatus* (LINNAEUS, 1758). Piperine, the main amide active in *Piper guineense* (SCOTT et al. 2004), acts as a neurotoxin in insects (SCOTT et al. 2008), but is considered safe to mammals (OKUNLOLA et al. 2008). UKPAI et al. (2017) have recently reported a high content of flavonoids in *P. guineense* and suggested that the presence of alkaloids, flavonoids and phenols may be responsible for the bioactivity and consequent mortality of *Sitophilus zeamais* in maize stored with the botanical. The piperamides found in the genus *Piper* L. are unique due to their bi-functional nature and its formulations also have repellent activity (IVBIJARO & AGBAJE 1986).

It is, however, important to note that the susceptibility of *Callosobruchus maculatus* to *Piper guineense* could vary across location sources of the bruchid. In a study to determine the tolerance of *Callosobruchus maculatus* populations to *Piper guineense* powder within Ekiti State (in Nigeria), GBAYE et al. (2015) observed that bruchids obtained from Emure were the most tolerant (LT50: 1.19 hours and 2.45 hours, respectively) while those from Ikere were the most susceptible (LT50: 0.62 hours and 1.24 hours respectively) to this botanical. Corroborating this finding, OYENIYI et al. (2015) reported that bruchid populations from the north-eastern part of Osun State (Nigeria) showed greater tolerance towards *P. guineense* than their counterparts from the south-western part of that state. OYENIYI et al. (2015) further showed that the variety of cowpea seeds has a significant effect on the efficacy of *P. guineense*. The authors reported that bruchids emerging from cowpea variety AR48V had a greater tolerance to *P. guineense* than bruchids reared on IFBV.

The observed increase in the moisture content of treated seeds showed that the treatment materials not only retained but aided the absorption of moisture. These materials could have acted as a film over the seeds, thereby protecting the seeds from evapotranspiration. This agrees with the work of BOEKE et al. (2004), which reported that leaf powder of *Momordica charantia* L. was effective against weight loss of stored cowpea.

Actellic dust had no negative effect on seed viability. This is corroborated by earlier works of OGUNWOLU & ODUNLAMI (1996), ABDUL-RAFIU (2010) and OJIAKO et al. (2013), which reported that actellic did not affect the viability of stored cowpea or maize, respectively. Ogendo et al. (2004) and AKINKUOLERE et al. (2006) also stated that plant products generally do not affect the viability of seeds treated with them. *Jatropha curcas* L. seed extract has been reported to have no adverse effect on the viability of stored cowpea seeds (AHUCHAOGU & OJIAKO 2015). However, the highest rate of *Piper guineense* gave 81.2 % germination as against the control (86.7 %), suggesting that for seeds stored for planting purposes, the medium rate of *P. guineense* should not be exceeded.

Piper guineense and actellic dust had no significant effect on the taste, odour, texture, appearance and overall acceptability of cooked cowpea seeds. This result is consistent with earlier works with actellic dust (OPARAEKE et al. 1998) and *Piper guineense* (OKONKWO & OKOYE 2002). Other plant products: *Capsicum* spp. (ONU & ALIYU, 1995), *Allium sativum* L. (OPARAEKE & DIKE 1996), *Eucalyptus* L'HÉR. powder (DIKE & MSHELIA 1997), *Hyptis suaveolens* (L.) POIT. (OPARAEKE et al. 2002), *Denettia tripetala* BAK.F. (OKONKWO & OKOYE 2002) and *Mondora myristica* (Gaertn.) Dunal (OPARAEKE & DIKE 2005) have variously been found not to affect the organoleptic qualities of stored seeds treated with them.

CONCLUSION

Although actellic 2% dust offered the quickest control measure in the first four months of storing cowpea seeds, *Piper guineense* seed powder at higher concentrations offered comparatively good protection of the stored seeds. The powder did not adversely affect seed viability, proximate composition or the organoleptic characteristics of the stored seeds.

P. guineense, a highly nutritional and medicinal plant that is locally available, could therefore be an acceptable, cheap, safe and environmentally friendly alternative to synthetic chemicals for protecting seeds in storage. This could also increase the diversity of plant materials that could serve as models for the synthesis of new insecticidal compounds.

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