Brazilian Journal of Biological Sciences, 2018, v. 5, No. 11, p. 709-724.
ISSN 2358-2731
https://doi.org/10.21472/bjbs.051109

Comparative toxicity of botanical powders, diatomaceous earth, pirimiphos methyl, rice husk (powder and ash) against *Callosobruchus maculatus* (Fab.)

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Abstract. This study was carried out to investigate the comparative toxicity of seeds of *Piper guineense* Schum & Thonn, *Capsicum annum* L., diatomaceous earth, pirimiphos-methyl dust, rice husk (powder and ash) against *Callosobruchus maculatus* (F.) in stored cowpea seeds**.** Toxicity test was carried out by exposing five pairs each of freshly emerged beetles to the substrate materials at the rates of 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5 g/20 g of grains. The experiment conducted at ambient temperature of 28 °C ± 2 °C and 75% ± 5% relative humidity was replicated three times and arranged in a completely randomized design. Adult mortality was observed for four days. The following phytochemicals were observed in the materials: alkaloids, saponins, tannin, flavonoid and glycosides. Results of the toxicity assay revealed that rice husk ash was the most toxic to adult *C. maculatus* with 100% mortality ($p \leq 0.05$) within 72 h of exposure at a concentration of 0.1 g/20 g grains. Pirimiphos methyl gave a significantly high mortality of 76.67% at a concentration of 0.2 g/20 g of grains in cowpea after 72 h of application. The least efficacy was observed with rice husk powder evoking 3.33% at a dosage of 0.3 g/20 g in cowpea. The treated grains significantly reduced the oviposition and adult emergence of *C. maculatus* and were significantly different from the control in all the different dosages. Oviposition and adult emergence were lowest in rice husk ash with 8 eggs and 24.62% adult emergence in *C. maculatus*, at 0.5 g/20 g dosage. The percentage adult emergence in the untreated grains was significantly different (higher) ($p > 0.05$) from the emergence in the treated cowpea grains across the dosages. At all levels of dosages, the powders and ash significantly reduced weight loss, damage and weevil perforation index caused by the beetle. Rice husk ash at 0.5 g dosage recorded the lowest weight loss, grain damage and weevil perforation index of 0.40%, 1.95% and 5.80%, respectively. The findings from this research showed that rice husk ash was most potent/toxic to *C. maculatus* and its use could be encouraged especially in small scale storage.

Received
November 24, 2018

Accepted
December 21, 2018

Released
December 31, 2018

Full Text Article

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Keywords: *Callosobruchus maculatus*; Phytochemicals; Toxicity; Adult emergence; Diatomaceous earth.

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the most important food legumes grown in the tropical savanna zones of Africa. Cowpea storage has been facing a lot of problems among which infestation by cowpea bruchid, *Callosobruchus maculatus* (F.) appeared prominent. The insect's notoriety is not restricted to the field but also found in storage where it has been reported to have caused more than 60% damage of stored cowpea. The multivoltine nature of the insect pest has been reported to be a factor making its infestation more pronounced among other insect pests of cowpea (Ashamo et al., 2013a; Ileke et al., 2014).

The destructive activities of insects and other storage pests have been subdued by chemical control methods comprising fumigation of stored commodity with carbon disulphide, phosphine or dusting with malathion, cabaryl, pirimiphos methyl or permethrin. These chemicals have been reported to be effective against stored products pests (Ogunwolu and Idowu, 1994; Adedire et al., 2011). In developed countries, conventional fumigation technology is currently being scrutinized for many reasons, such as ozone depletion potential of methyl bromide and carcinogenic concerns with phosphine (Adedire et al., 2011). Other problems associated with use of synthetic chemicals include effects on non-target insects, persistence and toxicity. One solution to these problems might be to totally replace synthetic chemicals with compounds, which occur naturally in plants. Therefore, researches have shifted towards the use of botanical ash, powder and extracts for insect control. This present study, therefore, compared the toxicity of botanical powders, pirimiphos methyl, diatomaceous earth and rice husk powder and ash against *C. maculatus* in stored cowpea.

Materials and methods

Insect culture

The cowpea seeds (ife brown) used for this study was sorted and cleaned of foreign matters, thereafter disinfested by keeping in the freezer of the Food Storage Research Laboratory at -5 °C for a minimum of seven days. This was to disinfest the seeds from all life stages, particularly the eggs of insects' pests which are very sensitive to cold. The disinfested seeds were then air-dried in the laboratory to prevent mouldiness before the introduction of insects. The insect culture was placed in a plastic container with exposed lid covered with a muslin cloth to allow entry of air into the container and prevent the insects from escaping. The insect culture was placed inside an insect rearing cage measuring 100 cm x 70 cm x 100 cm in the Food Storage Research Laboratory of the Department of Biology, Federal University of Technology, Akure, Ondo State, Nigeria, at an ambient temperature of 28 °C ± 2 °C and relative humidity of 70% ± 5%.

Sourcing of cowpea seeds

The ife brown variety of cowpea seeds (*Vigna unguiculata* L. Walp) used for the study was sourced from the Institute of Agricultural Research and Training, Moor Plantation, Ibadan, Oyo State. The seeds were kept in a polythene bag and placed in a safe, water free and well-ventilated container in the Food Storage Research Laboratory of the Department of Biology, Federal University of Technology, Akure, Nigeria until required.

Preparation of test materials

The materials used were diatomaceous earth (SilicoSec®) (DE) which was obtained from the Department of Biology, Federal University of Technology, Akure, Nigeria, rice husk was obtained from the Agricultural Development Programme, Akure, Ondo State, Nigeria. The rice husk was dried and pulverized. The pulverized rice husk was divided into two portions, the first portion was sieved to pass through 1 mm^2 mesh size to obtained fine powder and kept until use while the other part was made into ash with the aid of furnace at a temperature of 600 ^{0}C for 2 h. *Capsicum annum* and *Piper guineense* seeds were obtained from Oba market, Akure, Nigeria. They were air dried, pulverized and sieved into fine dust particle size, 10 µm sieve mesh. Ground and sieved samples were kept in a dark, airtight plastic container and stored in a safe place in the laboratory before use. Lastly Pirimiphos methyl was purchased from an Agro chemical store in Ogunpa, Ibadan, Nigeria.

Contact toxicity of materials to *C. maculatus*

20 g of the cowpea seeds was weighed into 250 mL plastic containers. Diatomaceous earth weighing 0.0 g (control), 0.1, 0.2, 0.3, 0.4 and 0.5 g was thoroughly mixed with the seeds inside the plastic containers with a glass rod. The experiment was set up in a complete randomized design and each treatment was replicated thrice. The same procedure was done to the other materials used for the experiment i.e. plant materials (*Piper guineense* and *Capsicum annum* fruit), rice husk (powder and ash) and pirimiphos methyl. The containers were gently shaken to ensure thorough admixture of the cowpea seeds with the powders and ash of the materials. Five pairs (male and female) of newly emerged (0-24 h) old *C. maculatus* were introduced into the treated and untreated (control) cowpea at concentrations given above. Beetle mortality was observed daily for 4 days, i.e. 24, 48, 72 and 96 h, respectively, after introduction. After every 24 h, the number of dead beetles was counted, recorded and removed. The beetles were confirmed dead when there was no response to probing with a sharp pin at the abdomen. At the end of the fourth day, all the remaining beetles were brought out of the culture. The oviposition of *C. maculatus* on the cowpea seeds was counted and recorded after the 4th day. The culture was left in the insect cage and observed daily after 20 more days for adult emergence. The adults that emerged were counted and recorded. The percentage adult emergence was calculated using the method of Odeyemi and Daramola (2000), as follows:

$$Percentage\ adult\ emmergence = \frac{Number\ of\ adult\ emerged}{Total\ number\ of\ eggs\ laid} \times 100$$

After adult emergence, the samples were weighed again to determine the weight loss and percentage weight loss as follows:

$$Percentage\ weight\ loss = \frac{Initial\ weigt - Final\ weigt}{Initial\ weigt} \times 100$$

After reweighing, the number of damaged seeds was evaluated by counting wholesome and bored seeds with the beetles' emergence holes. Percentage of seed damage was calculated using the method of FAO (1985), for cowpea as follows:

$$Percentage\ grain\ damage = \frac{Number\ of\ damaged\ seeds}{Total\ number\ of\ seeds} \times 100$$

The weevil perforation index (WPI) was calculated for cowpea seeds using the method of Fatope et al. (1995) and Adedire and Ajayi (1996) as follows:

$$Weevil\ perforation\ index = \frac{\%\ treated\ cowpea\ seed\ perforated}{\%\ control\ cowpea\ seed\ perforated} \times 100$$

Phytochemical analysis

The phytochemical analysis which included alkaloid, saponins, tannin was determined using methods described by (Harborne, 1973; Makkar et al. (1993); Obadoni and Ochuko, 2001).

Statistical analysis

Data obtained from mortality, oviposition, weight loss, grain damage, weevil perforation index were subjected to One-way Analysis of Variance (ANOVA) and means were separated using Duncan's New Multiple Range Test at a significance level of α = 0.05 to estimate the differences. Data obtained from mortality were also subjected to Probit analysis to estimate the LD_{50} and LD_{95}.

Results

Phytochemicals in the materials

The phytochemicals in the materials used for the study is presented in Figure 1. The phytochemical analysis of the tested materials which were *P.guineense*, *C. annum*, diatomaceous earth, rice husk (powder and ash) revealed that certain phytochemicals are present in this materials in varying amount. Saponins, Tanin, Alkaloid, Flavonoids and Glycosides were present in the botanicals. The presence of these phytochemicals could be said to be responsible for their insecticidal activities.

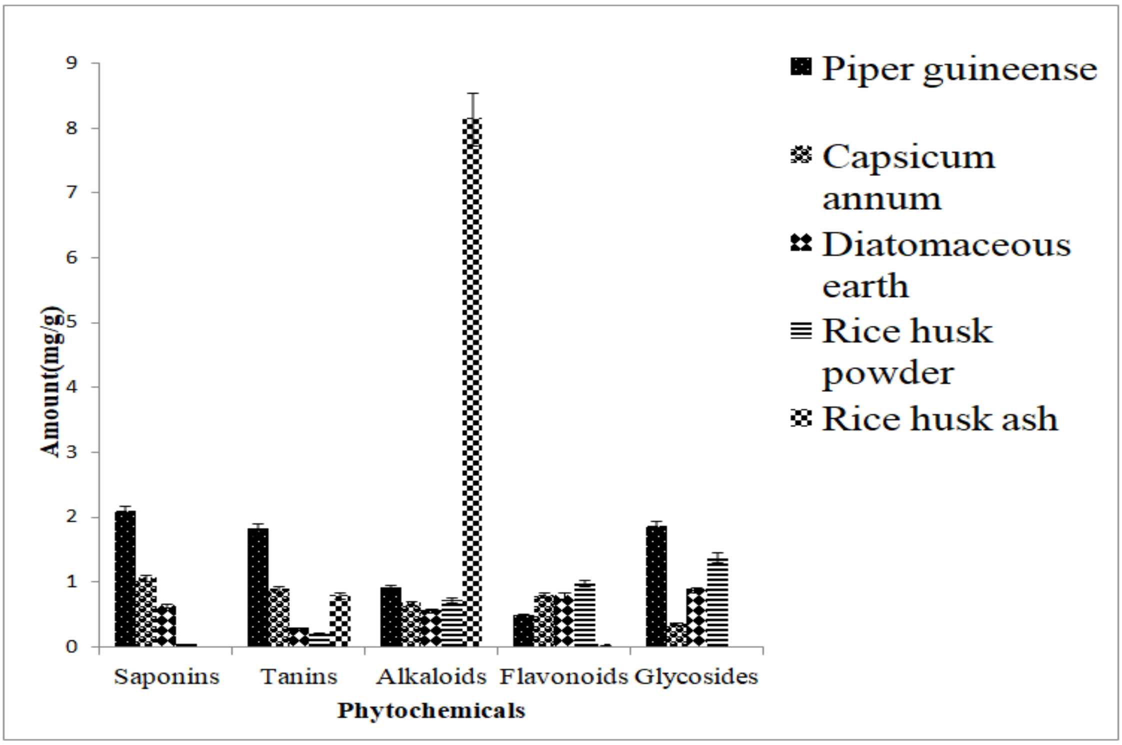

Figure 1. Phytochemicals present in rice husk (powder and ash), *Piper guineense*, *Capsicum annum* and Diatomaceous earth.

Mortality (%)

The percentage mortality of adult *Callosobruchus maculatus* on cowpea seeds treated with botanical powders, diatomaceous earth, rice husk (powder and ash) and pirimiphos methyl is presented in Tables 1-5. The beetle mortality increased with increase in dosage and period of exposure. The ash recorded the highest mortality of the beetle (90%) within 48 h at a dosage of 0.1 g/20 g of grains and was significantly ($p < 0.05$) different from other treatments (Table 1).

The rice husk ash recorded 100% mortality at 0.2 g dosage within 48 h of exposure, this was followed by pirimiphos methyl at same dosage evoking 53.33% beetle mortality (Table 2). In addition, there was significant difference between the treatments and the control except *C. annum*, diatomaceous earth and rice husk powder. 100% beetle mortality was also achieved by pirimiphos methyl at 0.3 g dosage within 96 h of exposure and was significantly ($p < 0.05$) different from treatments at same dosage except rice husk ash (Table 3). There was a significant difference between the treatments and the control, in terms of mortality. At the dosage 0.5 g/20 g of cowpea, only rice husk ash and pirimiphos methyl were able to cause 100% beetle mortality at 96 h. The least effective was *C. annum* that produced 6.67% beetle mortality after 96 h. Rice husk ash was also able to cause 93.33% beetle mortality after 24 h at 0.5 g dosage (Table 5). The rice husk ash performed better than synthetic insecticide (pirimiphos methyl) at all dosages and period of exposure.

Table 1. Mortality of *C. maculatus* exposed to 0.1 g dosage of botanical powders, diatomaceous earth, rice husk (powder and ash) and pirimiphos methyl.

Treatment	% Mortality in hours			
	24	48	72	96
Piper guineense	6.67±3.33[a]	16.67±3.33[b]	56.67±8.81[b]	70.00±0.00[c]
Capsicum annum	0.00±0.00[a]	0.00±0.00[a]	3.33±3.33a	3.33±3.33[a]
Diatomaceous earth	0.00±0.00[a]	0.00±0.00[a]	3.33±3.33[a]	20.00±10.00[b]
Rice husk powder	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Rice husk ash	53.00±12.01[b]	90.00±5.77[d]	100.00±0.00[c]	100.00±0.00[d]
Pirimiphos methyl	0.00±0.00[a]	46.67±8.82[c]	73.33±14.53[b]	93.33±6.67[d]
Control	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	3.33±3.33[a]

Each value is mean ± standard error of three replicates. Values followed by the same letter are not significantly ($p > 0.05$) different from each using Duncan's New Multiple Range Test.

Table 2. Mortality of *C. maculatus* exposed to 0.2 g dosage of botanical powders, diatomaceous earth, rice husk (powder and ash) and pirimiphos methyl.

Treatment	% Mortality in hours			
	24	48	72	96
Piper guineense	6.67±3.33[b]	23.33±3.33[b]	60.00±5.78[b]	76.67±3.33[c]
Capsicum annum	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	3.33±3.33[a]
Diatomaceous earth	0.00±0.00[a]	0.00±0.00[a]	3.33±3.33[a]	20.00±5.77[b]
Rice husk powder	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	3.33±3.33[a]
Rice husk ash	80.00±0.00[c]	100.00±0.00[d]	100.00±0.00[d]	100.00±0.00[d]
Pirimiphos methyl	0.00±0.00[a]	53.33±6.67[c]	76.67±12.02[c]	96.67±3.33[d]
Control	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	3.33±3.33[a]

Each value is mean ± standard error of three replicates. Values followed by the same letter are not significantly ($p > 0.05$) different from each using Duncan's New Multiple Range Test.

Table 3. Mortality of *C. maculatus* exposed to 0.3 g dosage of botanical powders, diatomaceous earth, rice husk (powder and ash) and pirimiphos methyl.

Treatment	% Mortality in hours			
	24	48	72	96
Piper guineense	10.00±5.77[b]	33.33±8.82[b]	63.33±3.33[b]	80.00±0.00[c]
Capsicum annum	0.00±0.00[a]	33.33±8.82[b]	33.33±8.82[b]	33.33±8.82[b]
Diatomaceous earth	0.00±0.00[a]	0.00±0.00[a]	6.67±3.33[a]	20.00±5.77[b]
Rice husk powder	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	33.33±8.82[b]
Rice husk ash	83.33±8.82[c]	100.00±0.00[d]	100.00±0.00[d]	100.00±0.00[d]
Pirimiphos methyl	0.00±0.00[a]	60±0.00[c]	80.00±11.55[c]	100.00±0.00[d]
Control	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	33.33±8.82[b]

Each value is mean ± standard error of three replicates. Values followed by the same letter are not significantly ($p > 0.05$) different from each using Duncan's New Multiple Range Test.

Table 4. Mortality of *C. maculatus* exposed to 0.4 g dosage of botanical powders, diatomaceous earth, rice husk (powder and ash) and pirimiphos methyl.

Treatment	% Mortality in hours			
	24	48	72	96
Piper guineense	13.33±3.33[b]	33.33±3.33[b]	68.67±3.33[b]	83.33±3.33[c]
Capsicum annum	0.00±0.00[a]	3.33±3.33[a]	3.33±3.33[a]	3.33±3.33[a]
Diatomaceous earth	0.00±0.00[a]	0.00±0.00[a]	6.67±3.33a	20.00±5.77[b]
Rice husk powder	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	3.33±3.33[a]
Rice husk ash	83.33±8.82[c]	100.00±0.00[d]	100.00±0.00[d]	100.00±0.00[d]
Pirimiphos methyl	3.33±3.33[ab]	63.33±3.33[c]	86.67±6.67[c]	100.00±0.00[d]
Control	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	3.33±3.33[a]

Each value is mean ± standard error of three replicates. Values followed by the same letter are not significantly (p > 0.05) different from each using Duncan's New Multiple Range Test.

Table 5. Mortality of *C. maculatus* exposed to 0.5 g dosage of botanical powders, diatomaceous earth, rice husk (powder and ash) and pirimiphos methyl.

Treatment	% Mortality in hours			
	24	48	72	96
Piper guineense	16.67±8.82[b]	36.67±8.82[bc]	73.33±3.33[bc]	86.67±3.33[c]
Capsicum annum	0.00±0.00[a]	3.33±3.33[a]	6.67±3.33[a]	6.67±3.33[ab]
Diatomaceous earth	0.00±0.00[a]	0.00±0.00[a]	16.67±8.82[b]	26.67±14.53[b]
Rice husk powder	6.67±3.33[ab]	13.33±6.67[b]	16.67±8.82[a]	16.67±8.82[ab]
Rice husk ash	93.33±3.33[c]	100.00±0.00[d]	100.00±0.00[c]	100.00±0.00[c]
Pirimiphos methyl	3.33±3.33[a]	63.33±3.33[c]	86.67±6.67[bc]	100.00±0.00[c]
Control	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	3.33±3.33[a]

Each value is mean ± standard error of three replicates. Values followed by the same letter are not significantly (p > 0.05) different from each using Duncan's New Multiple Range Test.

The lethal dosage

Table 6 shows the required to achieve 50% and 95% mortality of *Callosobruchus maculatus* by botanical powders, diatomaceous earth, rice husk (powder and ash) and pirimiphos methyl within 72 h post treatment. It was noted that lower amount of rice husk ash was needed to achieve 50% and 95% mortality of *C. maculatus* as reflected by their fiducial limits. The rice husk ash was found to have the highest lethal effect as it required the lowest dosage to achieve 50% and 95% mortality of *C. maculatus.* This means that the rice husk ash treatment 0.28 (0.20-0.37), 0.34 (0.36-0.39) at LD_{50} (50% FL) and LD_{95} (95%FL), respectively, was more toxic to *C. maculatus* than other treatment. The order of effectiveness of the treatment can be arranged thus: rice husk ash > pirimiphos methyl > *Piper guineense* > diatomaceous Earth > rice husk powder > *C. annum.* The fiducial limit reflected that *Capsicum annum* 4.63 (4.17-7.45), 10.54 (10.32-16.38) at LD_{50} (50%FL) and LD_{95} (95%FL) respectively was the least toxic of the treatment.

Table 6. The lethal dosage required to achieve 50 and 95% mortality of *Callosobruchus maculatus* by botanical powders, diatomaceous earth, rice husk (powder and ash) and pirimiphos methyl within 72 h post treatment.

Treatment	Slope ± SE	Intercept ± SE	X^2	LD_{50} (50%FL)	LD_{95} (95% FL)	Sig.
Piper guineense	-1.08±0.12	0.96±0.07	550.339	0.55 (0.53-0.63)	0.75 (0.71-0.87)	0.000
Capsicum annum	0.22±0.21	-1.87±0.12	114.463	4.63 (4.17-7.45)	10.54 (10.32-16.38)	0.0001
Diatomaceous earth	-0.25±0.13	-0.79±0.07	316.615	3.73 (3.52-5.44)	7.09 (6.98-8.96)	0.000
Rice husk powder	1.51±0.75	-2.48±0.17	188.853	3.74 (3.54-5.46)	7.00 (6.96-8.95)	0.000
Rice husk ash	-10.07±0.68	7.48±0.49	316.386	0.28 (0.20-0.37)	0.34 (0.36-0.39)	0.000
Pirimiphos methyl	-3.52±0.22	2.93±0.14	2,988.795	0.47 (0.49-0.51)	0.50 (0.51-0.53)	0.000

X^2: Chi-square; SE: Standard error; FL: Fiducial limits; LD: Lethal dosage.

Oviposition and adult emergence

Figures 2-6 present the oviposition and adult emergence of *C. maculatus* exposed to different dosages of botanical powders, diatomaceous earth, rice husk (powder and ash) and pirimiphos methyl. The oviposition and adult emergence of the beetles decreased with increase in the dosages of the treatment. The treated seeds significantly reduced the oviposition and adult emergence of *C. maculatus* and were significantly different from the control across the different dosages.

The number of eggs laid by *C. maculatus* on treated cowpea seeds was significantly ($p > 0.05$) lower than the number of eggs laid on the untreated seeds. Rice husk ash has the least oviposition by *C. maculatus* of 15.67 eggs followed by rice husk powder which had 21.33 eggs. The highest oviposition of 86.33 eggs was recorded in *Piper guineense* at 0.1 g dosage. The percentage adult emergence in the untreated seeds was significantly different (higher) ($p > 0.05$) from the emergence in the treated cowpea seeds across the dosages. *Piper guineense* has the least adult emergence of 37.28% followed by rice husk ash with 40.44% beetle emergence. At the dosage of 0.2 g/20 g of cowpea seeds, Pirimiphos methyl had the highest oviposition of 68.67 eggs followed by *Piper guineense* which had 64.67 eggs. The least oviposition was observed in rice husk ash which had 13.67 eggs (Figure 4). Rice husk powder had the highest percentage emergence of 70.61% followed by *C. annum* which had 66.31% emergence. The least emergence was from *P. guineense* with 35.33% emergence (Figure 3). Pirimiphos methyl and *P. guineense* maintained the same trend across the dosages. At 0.3, 0.4, and 0.5 g/20 g of cowpea seeds, Pirimiphos methyl had the highest oviposition of 62.33, 56.67 and 54.67 eggs, respectively, while rice husk ash has the least oviposition of 11.67, 9.67 and 7.67 eggs, respectively. Rice husk powder had the highest percentage emergence of 68.33%, 60.32% and 52.78%, respectively, while rice husk ash had the least percentage emergence of 35.05%, 30.70% and 24.62% in the dosages from 0.3 g to 0.5 g (Figures 4-6). Values for oviposition and adult emergence from the treated cowpea seeds were significantly different ($p > 0.05$) from the control.

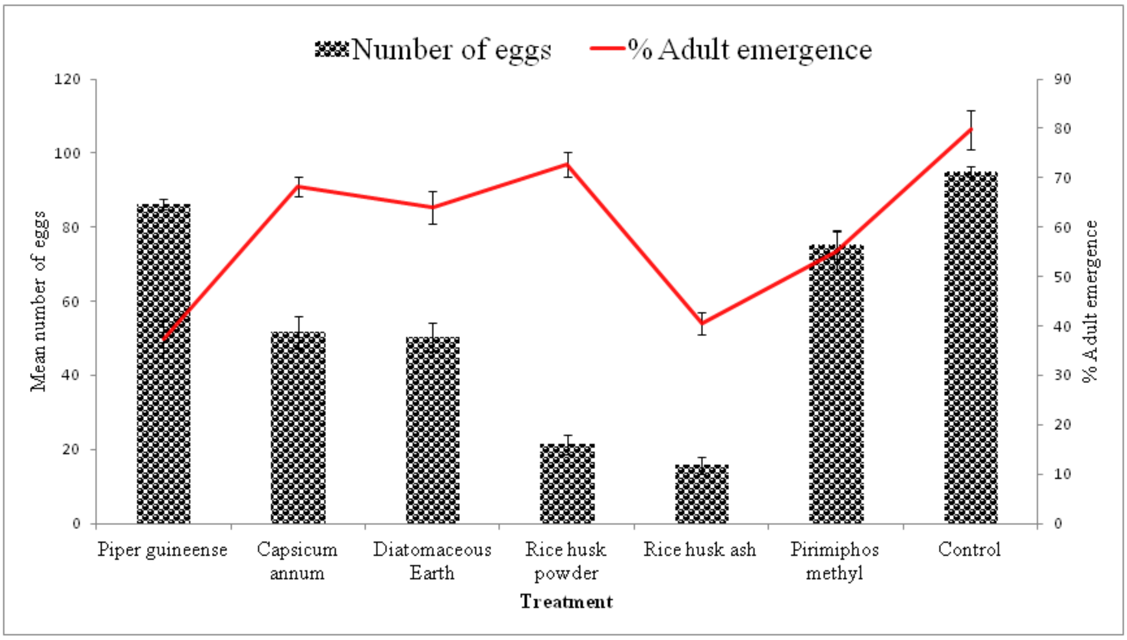

Figure 2. Number of eggs laid (oviposition) and percentage emergence of adult *C. maculatus* exposed to 0.1 g dosage of botanical powders, diatomaceous earth, rice husk (powder and ash) and pirimiphos methyl.

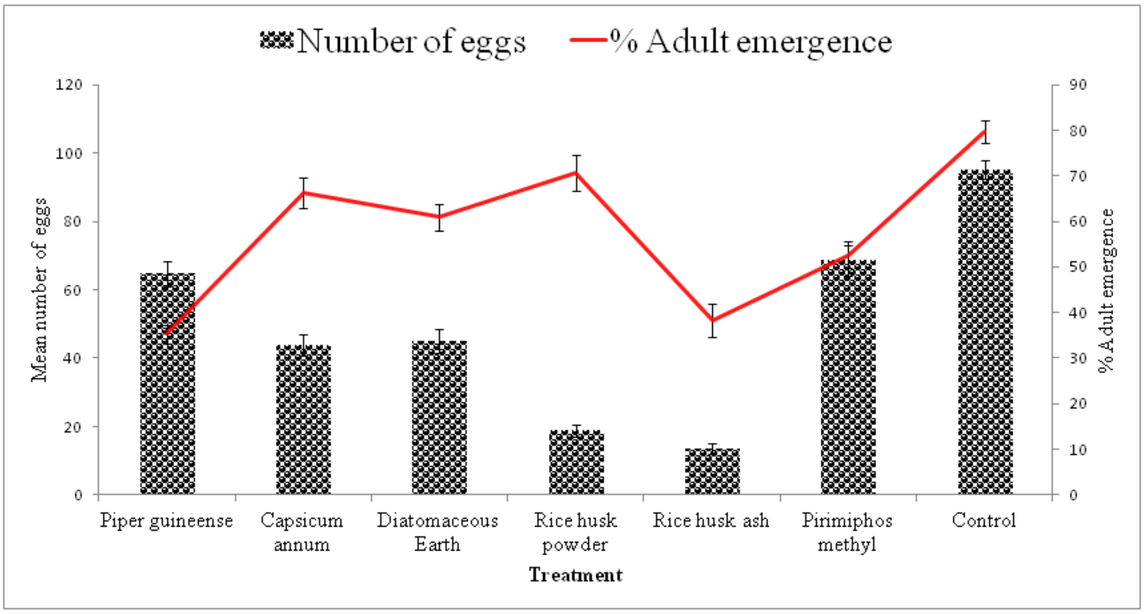

Figure 3. Number of eggs laid (oviposition) and percentage emergence of adult *C. maculatus* exposed to 0.2g dosage of botanical powders, diatomaceous earth, rice husk (powder and ash) and pirimiphos methyl.

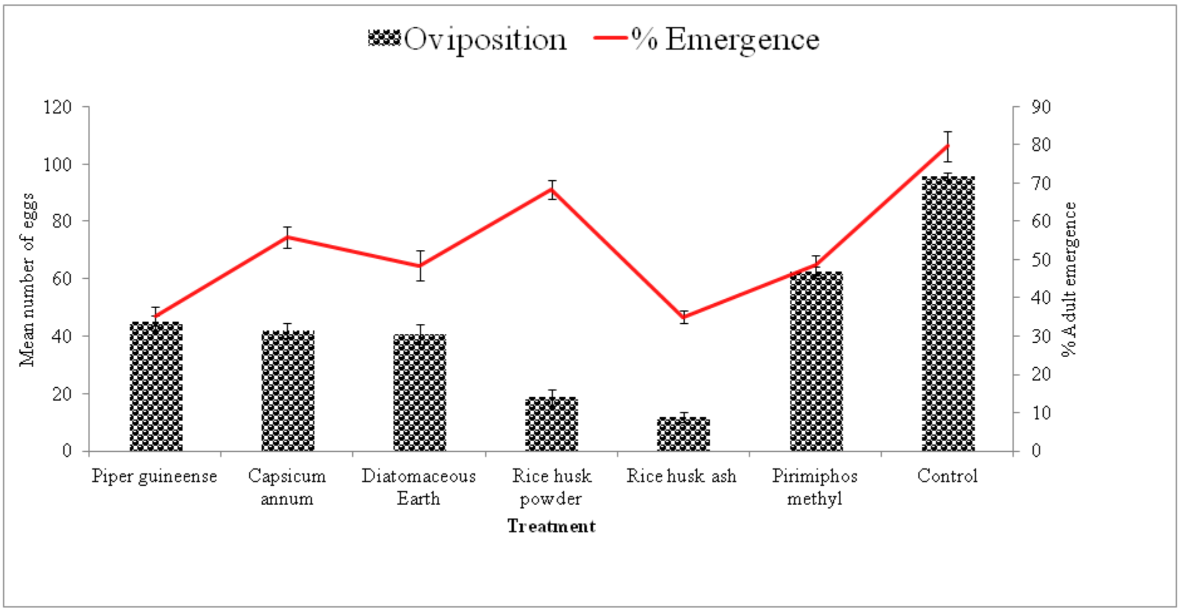

Figure 4. Number of eggs laid and percentage emergence of adult C. maculatus exposed to 0.3g dosage of botanical powders, diatomaceous earth, rice husk (powder and ash) and pirimiphos methyl.

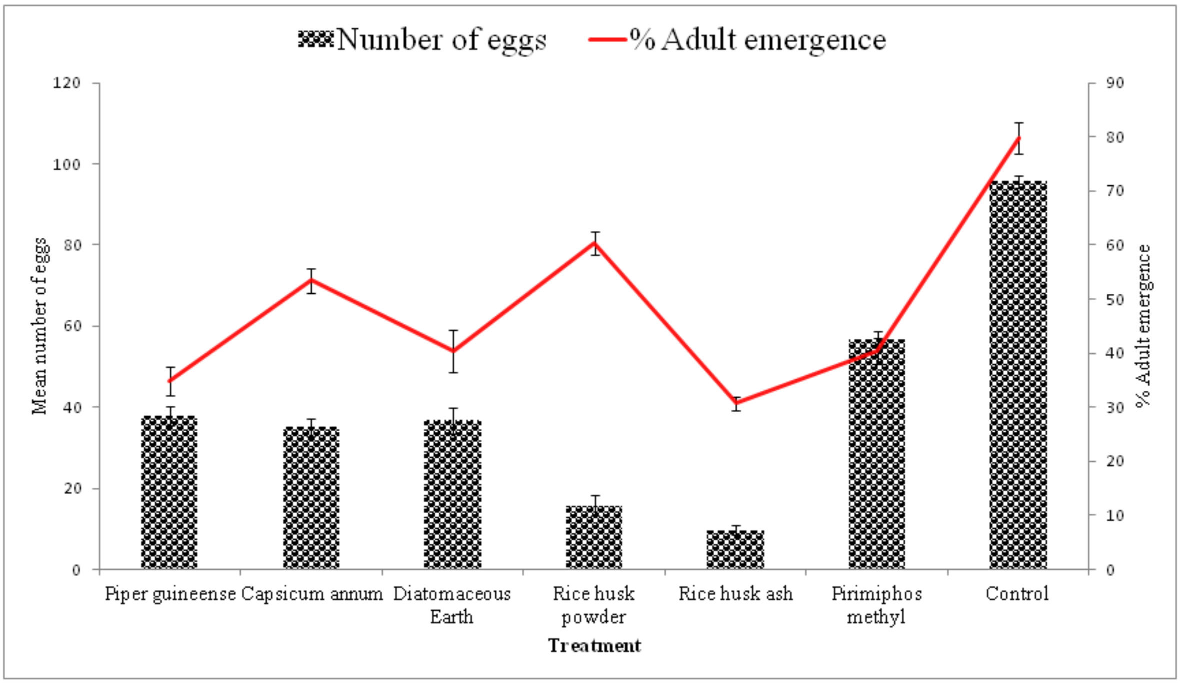

Figure 5. Number of eggs laid (oviposition) and percentage emergence of adult C. maculatus exposed to 0.4 g dosage of botanical powders, diatomaceous earth, rice husk (powder and ash) and pirimiphos methyl.

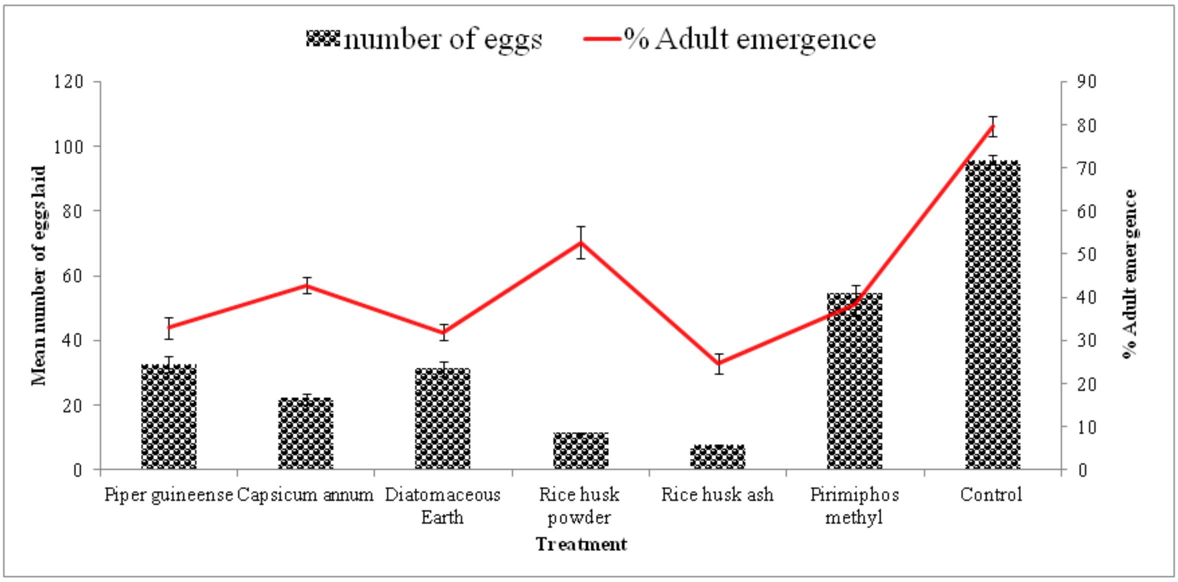

Figure 6. Number of eggs laid (oviposition) and percentage emergence of adult *C. maculatus* exposed to 0.5g dosage of botanical powders, diatomaceous earth, rice husk (powder and ash) and pirimiphos methyl.

Weight loss, seed damage in cowpea and weevil Perforation Index

Tables 6-10 showed the effect of botanical powders, diatomaceous earth, rice husk (powder and ash) and pirimiphos methyl on the ability of *C. maculatus* in causing seed weight loss, seed damage of cowpea and weevil perforation index. At all levels of dosages, the powders significantly reduced the weight loss, damage and weevil perforation index caused by the beetle. However, none of the powders achieved 0% seed weight loss regardless of the dosages. Rice husk ash at 0.5 g dosage recorded the lowest weight loss, seed damage and weevil perforation index of 0.40%, 1.95% and 5.80% respectively (Table 10). In addition, the result from rice husk ash at this dosage (0.5 g) was not significantly ($p > 0.05$) different from rice husk ash at dosages 0.1 g to 0.4 g. Nevertheless, rice husk ash was not significant ($p > 0.05$) different from *P. guineense* and Diatomaceous earth but different from *C. annum*, rice husk powder and pirimiphos methyl at 0.5 g dosage (Table 10). The cowpea seeds treated with the rice husk ash had the least weight loss, seed damage and weevil perforation index across the different dosages applied while the least effective powder was *C. annum* which had the highest seed damage and weevil perforation index. The rice husk ash showed more effectiveness than the other powders at all levels of dosages applied and the control was higher for each of the parameters considered.

Table 6. Weight loss, Damage and Weevil Perforation Index of C. maculatus exposed to 0.1 g dosage of botanical powders, diatomaceous earth, rice husk (powder and ash) and pirimiphos methyl.

Treatment	Weight loss (g)	Damage (g)	WPI (g)
Piper guineense	7.35±0.28^{bc}	24.25±2.82^{bc}	43.09±4.65^{bc}
Capsicum annum	12.00±2.72^{c}	25.31±1.35^{c}	44.28±0.46^{bc}
Diatomaceous earth	9.55±0.68^{c}	23.28±3.46^{bc}	41.93±4.92^{bc}
Rice husk powder	13.97±1.54^{cd}	17.40±1.53^{b}	35.25±1.91^{b}
Rice husk ash	2.45±0.02^{a}	7.75±1.06^{a}	19.61±2.86^{a}
Pirimiphos methyl	13.20±0.69^{cd}	20.79±2.14^{bc}	39.32±0.74^{b}
Control	17.08±1.50^{e}	31.93±2.30^{d}	50.00±0.00^{d}

Table 7. Weight loss, Damage and Weevil Perforation Index of *C. maculatus* exposed to 0.2g dosage of botanical powders, diatomaceous earth, rice husk (powder and ash) and pirimiphos methyl

Treatment	Weight loss (g)	Damage (g)	WPI (g)
Piper guineense	6.40±0.36^{b}	21.71±2.70^{bc}	40.40±4.75^{bcd}
Capsicum annum	10.88±1.83^{c}	23.46±2.69^{c}	42.19±2.52^{cd}
Diatomaceous Earth	8.15±1.50^{bc}	21.51±3.43^{bc}	39.99±4.79^{bcd}
Rice husk powder	8.27±1.12^{bc}	14.39±0.90^{b}	31.19±2.43^{b}
Rice husk ash	1.62±0.10^{a}	7.18±1.47^{a}	18.37±3.78^{a}
Pirimiphos methyl	7.92±0.56^{bc}	16.75±1.55^{bc}	34.36±1.49^{bc}
Control	17.08±1.50^{d}	31.93±2.30^{d}	50.00±0.00^{d}

Table 8. Weight loss, Damage and Weevil Perforation Index of C. maculatus exposed to 0.3 g dosage of botanical powders, diatomaceous earth, rice husk (powder and ash) and pirimiphos methyl.

Treatment	Weight loss (g)	Damage (g)	WPI (g)
Piper guineense	5.65±0.75^{b}	20.47±2.94^{ab}	38.95±3.18^{ab}
Capsicum annum	8.32±1.04^{b}	22.22±3.21^{ab}	40.71±2.90^{ab}
Diatomaceous earth	6.51±1.64^{b}	20.83±2.63^{ab}	31.34±2.14^{ab}
Rice husk powder	7.13±1.44^{b}	12.94±2.91^{ab}	28.31±2.34^{a}
Rice husk ash	1.25±0.14^{a}	5.53±0.67^{a}	14.93±2.34^{a}
Pirimiphos methyl	6.90±1.16^{b}	13.56±3.37^{ab}	29.37±6.08^{a}
Control	17.08±1.50^{c}	31.92±2.30^{c}	50.00±0.00^{c}

Table 9. Weight loss, Damage and Weevil Perforation Index of *C. maculatus* exposed to 0.4 g dosage of botanical powders, diatomaceous earth, rice husk (powder and ash) and pirimiphos methyl.

Treatment	Weight loss (g)	Damage (g)	WPI (g)
Piper guineense	4.75±0.14^{ab}	19.26±2.31^{b}	37.63±3.48^{b}
Capsicum annum	7.33±1.01^{b}	21.22±2.21^{b}	40.00±2.30^{bc}
Diatomaceous earth	5.50±1.60^{b}	18.83±2.63^{ab}	29.24±2.14^{ab}
Rice husk powder	6.11±1.34^{b}	10.54±2.51^{ab}	28.33±2.30^{ab}
Rice husk ash	1.10±0.10^{a}	3.23±0.37^{a}	14.93±2.24^{a}
Pirimiphos methyl	5.87±1.13^{b}	13.24±2.92^{ab}	29.01±4.08^{ab}
Control	17.08±1.50^{c}	31.92±2.30^{c}	50.00±0.00^{c}

Table 10. Weight loss, Damage and Weevil Perforation Index of C. maculatus exposed to 0.5 g dosage of botanical powders, diatomaceous earth, rice husk (powder and ash) and pirimiphos methyl.

Treatment	Weight loss (g)	Damage (g)	WPI (g)
Piper guineense	3.83±0.71[ab]	17.26±2.25[abc]	32.56±2.66[bc]
Capsicum annum	6.35±1.77[b]	20.99±2.53[bc]	39.48±1.49[bc]
Diatomaceous earth	3.97±1.53[ab]	15.64±1.83[abc]	24.59±2.30[ab]
Rice husk powder	4.40±0.39[b]	7.08±1.19[ab]	18.15±2.99[ab]
Rice husk ash	0.40±0.12[a]	1.95±0.01[a]	5.80±0.38[a]
Pirimiphos methyl	5.00±1.10[b]	13.00±2.92[ab]	28.01±5.47[ab]
Control	17.08±1.50[c]	31.92±2.30[c]	50.00±0.00[c]

Discussion

Phytochemicals are natural plant products that belong to the so called secondary metabolites, which included alkaloids, terpenoids, phenolics, and minor secondary chemicals. In general, their action disturbs the cellular and physiological processes responsible for maintaining homeostasis, and they can provoke sublethal changes within various tissues and organs, which can ultimately lead to death. This present study has revealed the efficacy of plant powders, diatomaceous earth, rice husk (powder and ash) and pirimiphos methyl in controlling *C. maculatus.* The results obtained from this research shows that some of the control materials were effective in controlling *C. maculatus*. But rice husk ash had a notable effect on the mortality of *C. maculatus*. This effect increased with increasing concentration and the period of exposure. The rice husk ash also achieved a higher beetle mortality even at lower dosage as revealed by their LD_{50} and LD_{95} values. The rice husk ash and pirimiphos methyl achieved 100% beetle mortality within 96 h of application at a dosage of 0.3 g/20 g of cowpea seeds. This is in agreement with the findings of Ashamo et al. (2013b) who evaluated the protectant ability of *Newbouldia laevis* (Seem.) extracts against infestation by *C. maculatus* in cowpea, *Vigna unguiculata* L. (Walp.). They tested the extracts from wood ash, leaf, stem and root bark at different concentrations of 0%, 1%, 2%, 3%, 4% and 5% and observed one hundred per cent mortality of adult beetles was achieved at all concentrations within 72 h of treatment with extracts except in wood ash at 1% concentration, but they were significantly different ($p < 0.05$) from the controls. The result of this research also agreed with previous works in which extracts of botanicals were used in the control of *C. maculatus* and other stored product pests (Adedire et al., 2011; Ileke and Olotuah, 2012). The high mortality caused by these extracts may be due to inability of the beetles to feed on the treated cowpea seeds which had been coated with the extracts thereby leading to starvation. Also, the extract might have disrupted the normal respiratory activity of the beetle leading to asphyxiation and subsequent death. The powders probably have also blocked the spiracles of these beetles therefore leading to suffocation. Diatomaceous earth is a non-toxic safe substance made up from fossils of freshwater organisms and crushed to fine powder, which has been reported to be effective against storage insect pests by many researchers (Attanassiou et al., 2005; Badii et al., 2014; Ofuya and Adler, 2015). This has great potential as grain protectants for small-holder farmers in sub Saharan Africa. Diatomaceous earth absorbs the wax layers of the insect's cuticle, causing desiccation and mortality due to water loss. The insecticidal efficacy of DEs is highly influenced by concentration rate, time of exposure, temperature and type of DE formulation. Similar results were obtained by previous researchers (Korunic, 1997; Arnaud et al., 2005; Yang et al., 2010). These studies showed increased mortality of stored-product beetles exposed to inert dusts for increasing time intervals and this was in agreement with the work of Golnaz et al. (2011) who carried out laboratory experiments in order to evaluate the insecticidal effect of diatomaceous earth against adults of *C. maculatus* and *Sitophilus granarius* (L.). Wheat and cowpea grains were treated with the diatomaceous earth formulation Silicosec® (Biofa, Germany), at five concentrations determined with preliminary tests and compared with untreated wheat and cowpea as control. Results showed that increasing concentration of Silicosec® and days after treatment (DAT) significantly increased the mortality rates of adults to above 90% in both experiments. However, in this present study DE was found to be not as effective as in previous studies cited even though its action was better than some of the plant powders. Ofuya and Adler (2015) observed that one day post treatment with DE produced 100% mortality in *Lasioderma serricorne* and *C. maculatus* which was significantly higher than mortality recorded in all other treatments. One of the main mechanisms of action of plant extracts is their ability to penetrate the chorion of insects' eggs *via* the micropyle, thereby leading to the death of developing embryos through asphyxiation (Ileke and Olotuah, 2012; Ashamo et al., 2013b). The inability of these beetles to emerge might be due to the death of the larvae which may occur due to inability of the larvae to fully cast off their exoskeleton which remained linked to the posterior part of their abdomen. This is in agreement with the observation made by Oigiangbe et al. (2010) who worked on insecticidal properties of an alkaloid from *Alstonia boonei*. He administered the material at the rate of 0.00125%, 0.001%; 0.0005%, 0.00025%, and 0.00% (Control). He reported that the *A. boonei* stem bark alkaloid was insecticidal against *Sesamia calamistis* and he observed that the insecticidal effects included reduced larval survival and pupation as well as growth distruption. The ability of insect pests to cause damage to stored grains did not depend on the number of eggs they laid but the ability of their eggs to hatch and develop to other destructive stages in their life cycle.

The result of this work revealed that the powders significantly reduced the oviposition rate of *C. maculatus* compared with the control experiment. The reduction in oviposition rate of the beetles could be due to inability of beetles to mate many times before death. The result also showed that the powders have obvious effect on post embryonic survival of the beetles. The result obtained in this research was in accordance with previous researches in which plant extracts had been used to deter oviposition of *C. maculatus* and other stored product pests (Adedire et al. 2011; Ileke and Olotuah, 2012). The prevention or reduction of damage and weight loss of the protected cowpea grains achieved by the control materials most especially rice husk ash might be due to inability of the insect larvae to feed on the protected cowpea seeds. The secondary metabolites present in these plants could be responsible for the inability of the adult insect to emerge as opined by Yang et al (2006) that secondary metabolites in botanicals are found to disrupt growth and reduced larva survival as well as disruption of life cycle of insects. The tested phytochemicals are saponins, tanins, alkaloids, flavonoids and glycosides. The findings from this research showed that rice husk ash was most potent/toxic to *C. maculatus* and its use could be encouraged especially in small scale storage.

Acknowledgement

The first author is grateful for the support of the Tertiary Education Trust Fund (TETFund) of the Federal Republic of Nigeria, Year 2015-2016 Merged TETFund Research Project Intervention towards the research.

Conflicts of interest

Authors declare that they have no conflict of interests.

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