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Potential synergism of diatomaceous earth and Piper guineense for management of *Callosobruchus maculatus* in stored cowpea

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Abstract
Some biological effects of SILICO SEC powder [diatomaceous earth (DE)] formulation and *Piper guineense* seed powder against the cowpea seed beetle, *Callosobruchus maculatus* F. were investigated under laboratory conditions at University of Port Harcourt, Nigeria. SILICO SEC and *P. guineense* powder were used to treat infested cowpea seeds in a small transparent plastic container at 0.02, 0.04, 0.06, 0.08 g/20g of seed. Number of eggs, adult progeny development, and adult mortality were the biological effects measured. All the application rates of the diatomaceous earth produced significant mortality of *C. maculatus* 24 hours (85.6-90.0%); and 48 hours (100.0%) post-treatment compared with the control. All the application rates of seed powder of *P. guineense* produced significant mortality of *C. maculatus* 24 hours (65.0-70.0%); and 48 hours (99.6%) post-treatment with the control. Binary mixture of *P. guineense* seed powder and DE significantly reduced oviposition and progeny by the beetle in comparison with the control. The result further indicated the potential of combining *P. guineense* seed powder and SILICO SEC for increased biological effects against *C. maculatus* infesting cowpea seeds in storage.

Keywords: *Callosobruchus maculatus, Piper guineense,* Diatomaceous earth, binary mixture.

Introduction

Cowpeas (*Vigna unguiculata* L. Walp) are grown for their leaves and grains both of which are used as relish or side dishes together with the staple food [1]. The mature dry cowpeas are important in the diets especially among poor resource farmers [2]. It is readily available, inexpensive and a popular part of the traditional food system [3]. Cowpea seeds contain a high amount of protein and B-vitamins [2, 4]. A substantial part of world cowpea production comes from Nigeria, cultivated on over 4 million hectares of land with a production of 1.7 million tons of beans annually [5]. Cowpea attracts a wide range of pests both in the field starting from emergence through to the store. Insect pest constitute the most important constraint to increased cowpea production wherever the crop is grown [2]. In Nigeria where cowpea is mostly cultivated, insect pests are reported to be the most important constraint to its production [6].

Cowpea bruchid [(*Callosobruchus maculatus*) Coleoptera Chrysomelidae] [7] is a cosmopolitan pest of stored grain legumes especially cowpeas *(V. unguiculata)* in the tropics and subtropics [8] by damaging seeds such as disfiguring with egg plugs and riddling with adult exit holes [9]. Consequently seeds have reduced weight and poor germinability [9]. It is common therefore, after six months in storage to record 100% seed infestation [10]. Many conventional insecticidal dusts such as pirimiphos-methyl, permethrin and fumigants such as aluminium phosphide have been reported to be effective against *C. maculatus* in storage. However rising prices and infrastructural problems impose considerable restrictions on their use, moreover, synthetic insecticides involve risk for human health and the environment especially when improperly used which is common among uneducated rural farmers in Africa and Asia [5] the fact that cowpea are used as human food and feed for livestock, render the use of toxic synthetic insecticides unacceptable since it may lead to serious problems of toxic residues, health and environmental hazards [11]. Given the disadvantages of synthetic pesticides, small scale farmers are more inclined to use traditional approaches to protect their grains. This underscores the importance of seeking alternative anti-weevil measures such as the use of plant derived natural pesticides for grain storage since these would be readily available, Affordable, relatively less toxic and detrimental to humans and the environment [12].

Furthermore the current renewed interest in reducing global warming has served as an added impetus for the re-evaluation of non-synthetic insecticides. Over the years, plant products such as vegetable oils, essential oils, volatile oils and crude extracts [13, 14] have been used as grain protectants in storage. Dry powder made from seeds, *Azadirachta indica*, *Eugenia aromatica, Piper guineense, Dennettia tripetala* and root bark of tooth ache plant *Zanthoxylum zanthoxyloides* have also been reported to be effective in controlling cowpea bruchid in store [15, 16, 12]. Diatomaceous earth derived from mineralized rocks is also known to be effective in the control of stored grain pest since they have silica in their composition which absorbs lipids from the waxy outer layer of insect's exoskeletons causing dehydration [17]. The study therefore was to examine the potential synergism of diatomaceous earth and *P. guineense* in the management of *C. maculatus* in stored cowpea.

Materials and Methods

Study Site and experimental material

The experiment was carried out in the Faculty of Agriculture, Crop and Soil Science Laboratory at room temperature from October to December. Ripe dried seeds of black pepper [*P. guineense,* (Shum and Thonn)] were purchased from an open market in Ondo State, Nigeria and oven dried at 28ºC for seven days before pulverizing using an electric milling machine. The powder was sieved using a mesh of 4.25mm in diameter to obtain very fine particles and stored in an air tight polyethylene bag until needed. The diatomaceous earth (DE) was obtained from Federal Research centre for cultivated plants, Julius Keuhn-Institute (KJI), institute for Ecological Chemistry and Plant Analysis and stored products protection, Berlin Germany originally supplied by AGRINOVA/BIOFA, Germany with the trade name SILICO-SEC.

Rearing of *C. maculatus*

A culture of *C. maculatus* was obtained from infested cowpea products in a local open market in Choba, Rivers State, Nigeria and introduced on 200g Ife-brown cowpea variety obtained in Akure, Ondo State, Nigeria and kept in a Kilner jars with their tops opened and covered with a perforated net for aeration and left undisturbed and the adults were sieved out after 7 days. The set-up was left on the shelf for the emergence of F_1 progeny of similar age. Freshly emerged one to two days adults of *C. maculatus* were then used for the experiment.

Experimental set up and sexing of *C. maculatus*

Twenty gram of Ife-brown cowpea was weighed using sensitive electronic balance (MP 2001 model) and introduced into a small transparent plastic container. The insects were sexed based on their elytra patterns or description by [18] that the females are dark coloured and have four elytra spots in contrast to the pale brown and less distinctly spotted male. Six pairs of adult *C. maculatus* were introduced in to the experimental jars containing 20g of cowpea seeds that served as both feeding substrates and oviposition medium.

Bio assay

Powder of *P. guineense* and Diatomite were thoroughly blended using laboratory pestle and mortar to obtain a homogenous material and different binary mixtures of 1:1, 1:2, 1:3, 1:4, 1:5 and 2:1, 3:1, 4:1 and 5:1 of *P. guineense* and Diatomite were prepared. Four quantities (0.02g, 0.04g, 0.06g, and 0.08g) of each ratio were weighed using a sensitive Metler balance (JA 2003 model) and admixed with 20g of Ife brown cowpea seeds. Each plastic container treated was shaken gently until the materials were distributed all over the cowpea seeds. Each treatment was replicated three times and a control was included. Newly emerged *C. maculatus* were introduced into each treatment and left on the open shelf for 13 days to allow egg laying.

Mortality, Progeny and Damage assessment and toxicity assays.

To determine the efficacy of *P. guineense* and SILICO SEC or their binary mixture as post-treatment effect on *C. maculatus*, a bio-assayed at 6 hours intervals was conducted by carefully emptying the content of each jar on a separate filter paper and any insect that remained motionless after pricking it with sharp needle was considered dead. The number of eggs laid by the female beetles on the seeds was counted on the 13th day after infestation; number of F_1 progeny and grain weight loss attributed to *C. maculatus* activity was measured as described by [19].

$$\% \text{ weight loss} = \frac{\underline{UNd - DNu}}{U\,(Nd+Nu)} \times 100$$

Where U and Dare the weights of undamaged and damaged grains respectively, while Nu and Nd are the number of undamaged and insect damaged grains respectively.

Data Analysis

Data were subjected to analysis of variance and significant means were separated using LSD at 5% level of probability.

Results

Table 1 shows an average mortality rate of *C. maculatus* at 6 hours interval on different doses and binary mixtures of *P. guineense* and diatomaceous earth on stored cowpea. From the table, mortality after 6 hours was higher in cowpea seeds treated with a binary mixture of 2:1 at 0.04g/20g cowpea seeds and the least was in control though not significantly different from binary mixture in the ratios of 1:3, 1:4, 3;1, 4:1 and 5:1 at 0.02g/20g cowpea seeds. Mortality rate at 12 hours was higher in cowpea seeds treated at 0.02g in a binary mixture of 1:4 while sole cowpea seeds treated with *P. guineense* at 0.02g had the higher mortality at 18 hours post infestation. The overall mortality declined with increase in time of post infestation across the binary mixtures of the bio-pesticide except in treatment combination after 12 hours of post infestation where higher mortality rates peaks were observed.

Table 2 shows the number of eggs laid by pristine *C. maculatus* and teneral adult progeny emergence from cowpea seeds treated with different binary mixtures of *P. guineense* fruit powder and diatomaceous earth at different doses. Cowpea seeds treated at 0.02g/20g and the control (untreated) had a significantly higher number of eggs laid followed by sole *P. guineense.* Pristine *C. maculatus* did not lay eggs on cowpea seeds treated at 0.08g/20g with sole *P. guineense* and diatomaceous earth and in all the binary mixtures evaluated cowpeas seeds treated at same rate in 1:1 and 1:2 *P. guineense*:

DE had the least number of eggs laid and cowpea seeds treated with a binary mixture of 1: 5 had the least number of eggs laid. Larvicidal potential of the bio-pesticides increased with increase in dosage at the various binary mixtures. The result for adult progeny number followed a similar trend where higher eggs were laid, more adults emerged and fewer adult progenies emerged from cowpeas that had fewer eggs laid.

Adult mortality and percentage grain weight loss is shown in table 3. The result shows that significantly higher adult mortality was recorded in cowpea seeds treated with sole *P. guineense.* Untreated cowpea seeds (control) had the least adult mortality. Cowpea seeds treated with a binary ratio of 4:1 at 0.02/20g (*P. guineense*: DE) had higher adult mortality count. The least adult mortality was recorded in binary mixture 1:2 at 0.08/20g cowpea. Mean percentage adult mortality was higher in cowpea seeds treated at a dose of 0.02/20g in all the binary mixtures and the least in cowpea seeds treated at 0.08/20g and adult mortality decreased with increase in dosage in the different binary mixtures except in cowpea treated with 0.06/20g. Percentage grain weight loss followed similar trend with the exception of cowpea seeds treated at 0.06g/20g where binary ratios 1:5 and 2:1 recorded significantly lower % grain weight loss.

Discussion

Continuous need to protect stored product against quantitative and qualitative loss during storage has become necessary [20]. Synthetic pesticide is known for its residual toxicity, environmental pollution and adverse effect on humans [21] coupled with its uninterrupted and indiscriminate use has led to the development of resistant strains and health hazards [22] which therefore, calls for promotion and utilization of botanicals to protect stored products [20, 23]. Idoko and Adesina (2012) reported the use of dry fruit of *P. guineense* as a one of the bio-pesticide candidate with good potential for protection against insect depredation in developing countries of the world. The result of this study has provided additional data to substantiate these claims. Powder obtained from dry fruits of *P. guineense* and DE were found to cause significant mortality in adult *C. maculatus* infesting stored cowpea seeds within 48 hours.

Oviposition and adult progeny development in *C. maculatus* were also significantly depressed by *P. guineense* powder. Adult mortality after 6 hr of infestation was higher in binary mixture of *P. guineense*: DE in 2:1 at 0.04g/20g cowpea seeds. These biological effects of *P. guineense* powder on *C. maculatus* attacking cowpea seeds in storage have been reported by other workers [24, 25]. Mortality of adults, inhibition of oviposition and reduced progeny development caused *P. guineense* on *C. maculatus* will culminate in reduced damaged to cowpea by the beetle as reported by [26] that ovicidal activity of plant extracts is an important measure in controlling pest which could avert damage by other stages in the long run. Also the results of this study demonstrated the insecticidal value of diatomaceous earth as it was observed to cause significant mortality of *C. maculatus* within 6 hours of treatment. In fact all adults introduced on cowpea seeds treated with DE were killed within 18 hours. Quick kill of adults is important in *C. maculatus* control since they lay most of their eggs within their first three days of life [27]. The effectiveness of diatomite against many other stored product pests has been reported by other workers [28-33]. Diatomite kills by compromising the oily or waxy outer cuticular layer which protects insects from dehydration and when the thin waterproof layer of the epicuticle is lost, the insect loses water and dies from desiccation [34].

Perhaps more importantly, the result of this study has indicated the potential of enhancing the effectiveness of plant-derived insecticidal products by combining it with SILICO SEC. For instance, admixture of 0.06g *P. guineense* fruit powder and DE at 0.02g reduced egg laying and progeny development in *C. maculatus* by at least half compared to the levels of the same parameters in cowpeas treated with only 0.02g *P. guineense* powder. Additionally, admixture of *P. guineense* powder and diatomite at 0.04g/20g cowpea seeds reduced oviposition and the least number of eggs laid by *C. maculatus* was recorded in a binary mixture of 1:3 and 1:1 of *P. guineense*: DE. Although 100% adult mortality were recorded in some of the levels of doses of both *P. guineense* and SILICO SEC, the overall adult mortality was higher in the binary mixtures compared to sole *P. guineense* and diatomite. Similar results was observed by [35] who reported improved efficacy in the control of the bostrichid, *Prostephanus truncatus* (Horn) by combining diatomite with a Chinese plant extract and [36] who reported the effectiveness of binary combination of vermiwash with different biopesticide in reducing infestation ability of *Helicoverpa armigera* in pigeon pea.

Conclusion

Synergetic effect of the potential of powdered *P. guineense* and diatomaceous earth in imparting acute toxicity, discouraging oviposition and damage to cowpea seeds by *C. maculatus* has been demonstrated in this study. Its adoption for use as a grain protectant is likely to be easy because the plant already forms part of their diet and it is easy to prepare and apply. The technology should be encouraged as it would serve as a suitable alternative to the synthetic insecticides. There is therefore the potential of combining *P. guineense* seed powder and diatomaceous earth for increased biological effects against *C. maculatus* infesting cowpea seed in storage.

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Table 1: Average mortality rate of *C. maculatus* at 6 hours interval on different doses and binary mixtures of diatomaceous earth and *P. guineense* on stored cowpea.

	6hr				12hrs				18hrs				24hrs			
Treatment	**0.02(g)**	**0.04(g)**	**0.06(g)**	**0.08(g)**	**0.02(g)**	**0.04(g)**	**0.06(g)**	**0.08(g)**	**0.02(g)**	**0.04(g)**	**0.06(g)**	**0.08(g)**	**0.02(g)**	**0.04(g)**	**0.06(g)**	**0.08(g)**
PG	1.00	1.89	1.41	1.61	1.40	1.61	0.91	0.69	2.00	0.91	0.81	1.01	0.09	0.00	0.00	0.12
DE	1.01	1.31	2.13	1.72	1.39	0.94	1.41	1.02	0.93	1.01	1.06	0.96	0.01	0.00	0.00	0.00
PGDE1:1	0.01	0.2	1.01	1.67	1.21	1.12	0.76	0.67	1.00	1.93	2.00	2.14	0.00	0.00	0.00	0.00
1:2	0.03	0.41	1.21	1.43	1.01	0.76	1.00	1.45	0.69	0.58	0.92	0.61	0.00	0.01	0.00	0.00
1:3	0.00	0.31	0.91	1.29	1.26	0.92	1.01	1.09	0.16	1.06	1.21	0.92	0.01	0.00	0.06	0.00
1:4	0.00	0.01	0.48	0.62	2.01	0.16	0.36	1.06	0.19	0.16	0.36	0.92	0.00	0.01	0.09	0.12
1:5	0.06	0.51	0.61	0.72	1.96	1.01	1.01	1.00	1.06	1.00	0.14	0.12	0.16	0.00	0.00	0.10
2:1	0.03	1.91	0.21	0.36	1.31	0.34	0.81	0.63	0.34	0.41	0.21	0.09	0.00	0.00	0.00	0.00
3:1	0.00	0.42	0.15	1.56	0.92	0.61	0.39	0.14	0.01	0.21	0.13	0.02	0.00	0.00	0.00	0.00
4:1	0.00	0.31	0.12	1.43	1.43	0.69	1.00	1.00	0.69	0.10	0.03	0.14	0.00	0.00	0.00	0.03
5:1	0.00	0.21	0.14	1.51	0.73	1.09	2.10	1.01	0.28	0.16	0.38	0.19	0.00	0.01	0.01	0.06
Control	0.00	0.10	0.09	1.03	0.26	0.31	0.32	0.04	0.01	0.02	0.02	0.01	0.00	0.00	0.00	0.00
Mean	0.18	0.64	0.71	1.25	1.25	0.80	0.92	0.82	0.61	0.62	0.61	0.60	0.02	0.00	0.01	0.04
LSD	0.13	0.31	0.11	0.31	0.30	0.41	0.21	0.35	0.31	0.30	0.51	0.91	0.21	0.10	0.11	0.27
SED	0.27	0.24	0.31	0.71	0.67	1.01	0.46	0.76	0.34	0.61	1.02	1.21	0.51	0.31	0.24	0.54

DE = Diatomaceous earth; PG = *P. guineense;* PGDE = *P. guineense* and Diatomaceous earth.

Table 2: Egg lay and adult progeny development of *C. maculatus* (F.) on different doses and binary mixtures of diatomaceous earth and *P. guineense* on stored cowpea.

Treatment	**Egg lay**					**Adult progeny**				
	0.02(g)	**0.04(g)**	**0.06(g)**	**0.08(g)**	**Mean**	**0.02(g)**	**0.04(g)**	**0.06(g)**	**0.08(g)**	**Mean**
P. guineense	48.22	22.00	29.44	0.00[j]	24.92	30.00	11.44	28.00	0.00	17.36
DE	15.00	39.00	14.00	0.00	17.00	3.00	37.23	4.00	0.00	11.06
PGDE 1:1	21.00	30.00	10.44	3.22	16.17	18.00	25.00	9.00	2.67	13.67
1:2	27.21	27.00	29.00	3.44	21.66	23.00	14.00	23.00	2.67	15.67
1:3	23.00	15.22	17.44	9.22	16.22	12.22	14.00	15.00	5.00	11.56
1:4	14.44	20.44	15.22	14.43	16.13	10.00	14.44	13.23	11.44	12.28
1:5	29.43	2.44	12.44	6.44	12.69	17.22	2.00	11.44	3.44	8.53
2:1	24.00	18.44	17.44	11.13	17.75	13.00	17.23	14.44	8.44	13.28
3:1	38.44	20.22	32.44	12.00	25.78	22.33	12.44	24.46	8.22	16.86
4:1	24.00	18.22	27.00	18.13	21.84	22.23	16.44	19.44	11.44	17.39
5:1	26.44	11.00	14.44	19.13	17.75	20.00	11.00	13.44	11.00	13.86
Control	101.44	118.00	87.22	118.00	106.17	101.67	114.23	99.23	114.33	107.37
Mean	32.72	28.49	25.54	17.94		24.36	24.13	22.89	14.84	
LSD ($P \geq 0.05$)	0.436	0.360	0.511	0.370	0.41	0.261	0.395	0.437	0.475	0.39
SED	2.81	1.79	2.37	2.20	1.32	2.09	2.79	2.04	2.11	1.56

DE = Diatomaceous earth; PG = *P. guineense;* PGDE = *P. guineense* and Diatomaceous earth.

Table 3: Adult mortality of *C. maculatus* (F.) and grain weight loss of cowpea treated with diatomaceous earth and *P. guineense*

Treatment	Adult mortality (%)					% grain weight loss				
	0.02 (g)	**0.04(g)**	**0.06(g)**	**0.08(g)**	**Mean**	**0.02(g)**	**0.04(g)**	**0.06(g)**	**0.08(g)**	**Mean**
P. guineense	24.22	1.44	22.42	0.00	12.02	8.27	5.02	8.25	0.00	5.39
DE	2.44	2.44	4.00	0.00	2.22	2.53	8.45	2.54	0.00	3.38
PGDE 1:1	16.00	18.46	7.42	2.42	11.08	6.27	9.10	0.00	3.16	4.63
1:2	5.00	11.44	17.23	0.75	8.61	6.33	3.07	9.43	0.00	4.71
1:3	11.22	9.21	10.00	3.42	8.46	6.16	0.00	3.13	6.36	3.91
1:4	8.00	10.23	9.42	8.23	8.97	3.16	9.43	3.16	3.14	4.72
1:5	16.00	1.23	10.42	6.42	8.52	6.24	3.15	0.00	3.16	3.14
2:1	9.00	16.00	14.00	2.21	10.31	3.16	6.15	0.00	6.28	3.90
3:1	18.22	12.23	13.00	6.42	12.47	6.33	9.65	9.41	6.34	7.93
4:1	20.22	13.21	14.00	11.00	14.61	6.27	9.55	6.28	3.15	6.31
5:1	12.00	8.00	12.00	9.23	10.31	6.35	0.00	0.00	3.12	2.37
Control	1.33	5.00	3.21	5.00	3.64	6.41	2.56	6.41	2.56	4.49
Mean	11.97	9.07	11.43	4.59		5.63	5.51	4.06	3.11	
LSD ($P \geq 0.05$)	0.26	0.42	0.38	0.40	0.37	0.47	0.50	0.43	0.37	0.42
SE	1.16	0.91	0.86	0.60	0.82	0.28	0.60	0.60	0.38	0.45

DE = Diatomaceous earth; PG = *P. guineense;* PGDE = *P. guineense* and Diatomaceous earth.