
Evaluation of the powder of *Piper guineense* and pirimiphos-Methly F for the control of cowpea beetle *Callosobruchus maculatus* (F.).

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Cowpea is major source of dietary protein in tropical region of the world and damage caused by *Callosobruchus maculatus* impact negatively on its economic and nutritional values. In Nigeria today, the use of synthetic insecticides has been the major means of protecting stored grains from insect pest infestation. The drawback associated with the use of insecticides has necessitated the search for humanly safe, ecologically sound and cheap control measures. The effect of the combination of *Piper guineense* and Pirimiphos methyl as treatment in comparison with sole treatments of these powders was carried out in this study. Contact toxicity was tested for *Piper guineense* at 0.02g, 0.04g, 0.06g, 0.08g and 0.1g per 20g of cowpea seeds in separate petri-dish, while it was tested at 0.01g per 20g of cowpea seeds for Pirimiphos methyl in separate petri-dish. The combination (0.02g + 0.01g; 0.04g + 0.01g; 0.06g + 0.01g; 0.08g + 0.01g and 0.1g + 0.01g *Piper guineense* and Pirimiphos methyl respectively/20g cowpea seeds) of each of the concentrations was also tested in separate dish. Control with no treatment was also set up. *Piper guineense* at 0.10g sole concentration and 0.1g *Piper guineense* + 0.01g Pirimiphos methyl combined concentration significantly reduced the oviposition potential, egg hatching rate and emergence of adults *Callosobruchus maculatus*. The powders caused chronic toxicity and inhibit development. The inhibitory effects may due to the active ingredients that enter the digestive tract and be absorbed through it. The efficacy in respect of toxicity exhibited significant effect at 0.02g sole treatment of *Piper guineense* and 0.02g *Piper guineense* + 0.01g Pirimiphos methyl combined treatments at 12, 24 and 48 hours after infestation (HAI). All the responses were found the concentration dependent during the course of the study.

Key words: concentration, efficacy, inhibitory effects, toxicity

Introduction

Cowpea amounts to 60% daily dietary protein intake of most Nigerians (Oparaeke and Dike, 1998), and 100% damage has been reported on

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unprotected cowpea after 3-5 months storage (Singh, 1997), by *Callosobruchus maculatus* (F.). At present pest control measures in storage rely heavily on the use of synthetic insecticides and fumigants. Although effective fumigants (e.g. methyl bromide and phosphine) are available, there is global concern about their negative effects, such as ozone depletion, environmental pollution, toxicity to non-target organisms, pest resistance, and pesticide residues (Hansen and Jensen, 2002; Benhalima *et al.*, 2004; Bughio and Wilkins, 2004). Thus, there is an urgent need to develop safe alternative fumigants for stored-grain pest management. One modest way of increasing food availability to cope with the Nigerian ever-increasing population at less cost is to protect what has been produced and to achieve this, plant materials that are inexpensive, safe to the environment, users and consumers alike, need to be exploited as suitable alternatives to the expensive, toxic and environmentally unsafe synthetic insecticides (Magaji *et al.*, 2005). Herbal products are one potentially important source. The use of plant derived insecticides played important role in traditional method of storage pest control in Africa and Asia (Hassanali *et al.* 1990; Niber 1994; Bekele and Hassanali 2001). In the present paper, laboratory studies are reported on the contact toxicity of sole *Piper guineensis* and efficacy of mixing powder of *P. guineensis* and pirimiphos-methyl for the control of cowpea seeds against *C. maculatus* (F.) in storage.

Materials and methods

Experimental location

The experiment was conducted at the Entomology Laboratory of Crop, soil and Pest Management Department of Federal University of Technology, Akure, Nigeria.

Insect culture and experimental condition

The *C. maculatus* used for the study was derived from colony originating from infested cowpea seeds obtained from Erekesan Market, Akure, Ondo State, Nigeria. The emerged adults were sub-cultured in the laboratory, and the sub-culture was maintained in kilner jars in the laboratory (28± 3°C, 70± 5% RH and 12L: 12D photo regime) till new insects emerge. Ife brown (a well-known susceptible cultivar) was used as substrate. The seeds of Ife brown cowpea variety used as bioassay were obtained from seed store of Ondo State Agricultural Development Project Akure. These were properly sieved and handpicked thus ensured that only wholly and uninfested seeds were used (Adesina *et al* 2011). These were nevertheless the cowpea seeds were sterilized

in oven at 50°C for 4 hours to kill any immature stage of the insect (if any) and allowed to cool for 1 hr before use.

Preparation of P. guineense powder

Dry fruits of *P. guineense* were purchased from local herbs seller at Erekesan Market, Akure, Ondo State, Nigeria. Unwanted materials were removed, thereafter, the dried fruits were grounded using electric laboratory hammer mill. The finely grounded powder was kept in separate air tight container and placed a wooden cupboard in the laboratory for future use. Pirimiphos-methyl dust was obtained from Ondo State Agricultural Input Supply Company, Store, Akure, Ondo State. It is well packed and sealed at purchase.

Contact toxicity of sole P. guineense powder to C. maculatus

The powder of *P. guineense* was tested at 0.02g, 0.04g, 0.06g, 0.08g and 0.1g per 20g of clean (uninfested) Ife brown cowpea in separate petri-dish glass (9cm). Each of the Petri plate was tumbled several times to ensure homogenous mixing of powder with grains (Adesina and Ofuya, 2011). Five pairs of *C. maculatus* were introduced into each petri-dish. Adult mortality was monitored and counted 12, 24 and 48 hours after infestation (HAI) and thereafter all insect were removed 2 weeks after. The number of eggs laid by the female beetles on the seeds was then counted. Adult emergences from egg hatched were also counted as from 21 days after infestation (DAI). There was also control treatment involving no addition of *P. guineense* powder onto the seeds. The technique described by Bandara and Saxena (1995) for sexing and handling of bruchids was used in the experiment.

Contact toxicity of sole pirimiphos-methyl dust to C. maculatus

The dust of pirimiphos-methyl was tested at 0.01g per 20g of clean (uninfested) Ife brown cowpea in separate petri-dish glass (9cm). Each of the Petri plate was tumbled several times to ensure homogenous mixing of powder with grains (Adesina and Ofuya, 2011). Five pairs of *C. maculatus* were introduced into each petri-dish. Adult mortality was monitored and counted 12, 24 and 48 hours after infestation (HAI) and thereafter all insect were removed. The number of eggs laid by the female beetles on the seeds was then counted. Adult emergences from egg hatched were also counted as from 21 days after infestation (DAI). There was also control treatment involving no addition of *P. guineense* powder nor pirimiphos-methyl dust onto the seeds.

Contact toxicity of combined *P. guineense* powder and pirimiphos-methyl dust to *C. maculatus*

The same procedure adopted for sole treatment of *P. guineense* powder and pirimiphos-methyl dust was equally followed. The plant powder and pirimiphos-methyl were admixed with the cowpea grains at the following concentrations: 0.02g *P. guineense* + 0.01g Pirimiphos-methyl, 0.04g *P. guineense* + 0.01g Pirimiphos-methyl, 0.06g *P. guineense* + 0.01g Pirimiphos-methyl, 0.08g *P. guineense* + 0.01g Pirimiphos-methyl, 0.1g *P. guineense* + 0.01g Pirimiphos-methyl.

Experimental Design and Data Analysis

The experimental design adopted for the experiment was Complete Randomized Design (CRD) and each treatment was replicated three times. Data collected were subjected to analysis of variance (ANOVA), using SPSS software package. Prior to analysis egg counts were subjected to square root transformation, percentage adult mortality and emergence were arcsine transformed. Treatment means were separated using Turkey test at $P < 0.05$.

Results

Effects of treatment on adult *C. maculatus* mortality

The adult mortality of *C. maculatus*, differ significantly ($P < 0.05$) when treated with sole and combination of *P. guineense* and Pirimiphos methyl (Table 1). There was a significant different in adult mortality of *C. maculatus* when treated with sole powder of *P. guineense* and Pirimiphos-methyl dust respectively compared to control. In sole *P. guineense* application, highest mortality was recorded on cowpea seeds treat with 0.1g dosage rate at 12, 24 and 48 hours after infestation (HAI) and lowest in seeds treated with 0.02g *P. guineense*. Mortality was also significantly ($P < 0.05$) higher in treatment involving 0.08g and 0.1g compared to other application rates and control. However, irrespective of the dosage rates of *P. guineense* powder used in the study, adult mortality was significantly ($P < 0.05$) in all the treatment at 48 HAI, compared to adult mortality recorded at 12 and 24 HAI.

The effect of treatment combination of *P. guineense* and Pirimiphos-methyl at different concentration on *C. maculatus* adult mortality differs significantly ($P < 0.05$) compared to control. 100% adult mortality was

recorded in treatment combination involving 0.1g *P. guineense* powder and 0.01g Pirimiphos-methyl dust, but was not significantly ($P < 0.05$) differ when Pirimiphos-methyl dust sole was used and the lowest adult mortality was recorded in treatment involving combination of 0.02g *P. guineense* and 0.01g Pirimiphos-methyl. However, the highest adult mortality was recorded in all the treatment at 48 HAI. Irrespective of the treatment used adult mortality of *C. maculatus* increased with the increase in exposure to various treatment used.

Effects of sole and combined treatments on oviposition by female C. maculatus

The mean number of eggs laid by female *C. maculatus* on cowpea seeds treated with sole and combined *P. guineense* and Pirimiphos-methyl is shown in Table 2. Result obtained shows that irrespective of treatment combination and sole treatment of *P. guineense* and Pirimiphos-methyl, eggs laid by female *C. maculatus* on treated cowpea seeds is significantly ($P < 0.05$) suppressed compared to control. Sole application of *P. guineense* at 0.02g recorded the highest oviposition and lowest oviposition was recorded at 0.1g sole *P. guineense* application.

Pirimiphos-methyl sole application at 0.01g significantly inhibited egg laying by the female beetles compared to control treatment. However, the number of eggs laid by *C. maculatus* when treated with sole Pirimiphos-methyl recorded significant ($P < 0.05$) higher number of egg laid compared to sole and combined application of *P. guineense* and Pirimiphos-methyl concentrations. The result also shows that application involving different treatment combinations of *P. guineense* and Pirimiphos-methyl significantly ($P < 0.05$) suppressed eggs laying by *C. maculatus* compared to sole application of *P. guineense* and Pirimiphos-methyl. The minimum egg laying was observed in treatment combination of 0.02g *P. guineense* + 0.01g Pirimiphos-methyl and maximum at 0.1g *P. guineense* + 0.01g Pirimiphos-methyl.

Table 1. Effect of different concentration of *P. guineense* and Pirimiphos-methyl in sole and combinations on adult mortality of *C. maculatus*

Treatments	% Adult mortality		
	12 hours	24 hours	48 hours
g/20g cowpea sole treatments			
0.02g <i>P. guineense</i>	23.33 ^g	40.00 ^f	56.67 ^e
0.04g <i>P. guineense</i>	26.67 ^{fg}	53.33 ^{ef}	63.33 ^d
0.06g <i>P. guineense</i>	36.67 ^f	60.00 ^{dc}	73.33 ^b
0.08g <i>P. guineense</i>	43.33 ^{dc}	70.00 ^{bcd}	83.33 ^b
0.1g <i>P. guineense</i>	53.33 ^{bcd}	70.00 ^{bcd}	84.00 ^b
0.01g Pirimiphos-methyl	43.33 ^{dc}	80.00 ^{ab}	96.67 ^a
Combined treatments			
0.02g <i>P. guineense</i> + 0.01g Pirimiphos methyl	36.67 ^{cf}	63.33 ^{cde}	66.67 ^{cd}
0.04g <i>P. guineense</i> + 0.01g Pirimiphos methyl	50.00 ^{cd}	70.00 ^{bcd}	76.67 ^{bc}
0.06g <i>P. guineense</i> + 0.01g Pirimiphos methyl	56.67 ^{bc}	76.67 ^{abc}	83.33 ^b
0.08g <i>P. guineense</i> + 0.01g Pirimiphos methyl	63.33 ^{ab}	76.67 ^{abc}	83.33 ^b
0.1g <i>P. guineense</i> + 0.01g Pirimiphos methyl	73.33 ^a	86.67 ^a	100.00 ^a
Control	3.33 ^h	13.33 ^g	23.33 ^f

Means followed by the same superscripts letter are not significantly different at 5% probability level of significance.

Percentage adult emergence of *C. maculatus*

The percentage adult emergence of *C. maculatus* from cowpea seeds treated with sole and combined 0.02g *P. guineense* and Pirimiphos-methyl is presented in Table 3. The result shows that treatment combination of *P. guineense* and Pirimiphos-methyl at different combinations and sole significantly ($P < 0.05$) inhibit adult emergence from treated cowpea seeds compared to control. Sole application of 0.02g *P. guineense* and 0.01g Pirimiphos-methyl recorded same number of adult emergence (44.00%) being the highest adult emergence observed in sole application. In treatment combinations 0.1g *P. guineense* + 0.01g Pirimiphos-methyl recorded the lowest percentage adult emergence (21.00%) and highest adult emergence was obtained from 0.02g *P. guineense* + 0.01g Pirimiphos-methyl (35.00%).

Table 2. Mean number of eggs laid by *C. maculatus* when treated with different concentration of *P. guineense* and Pirimiphos-methyl in sole and combinations.

Treatments	mean no of eggs laid
g/20g cowpea Sole treatments	(Oviposition)
0.02g <i>P. guineense</i>	50.00 ^e
0.04g <i>P. guineense</i>	46.67 ^d
0.06g <i>P. guineense</i>	38.00 ^f
0.08g <i>P. guineense</i>	36.00 ^f
0.1g <i>P. guineense</i>	28.00 ^g
0.01g Pirimiphos-methyl	53.00 ^b
Combined treatments	
0.02g <i>P. guineense</i> + 0.01g Pirimiphos methyl	42.00 ^e
0.04g <i>P. guineense</i> + 0.01g Pirimiphos methyl	36.00 ^f
0.06g <i>P. guineense</i> + 0.01g Pirimiphos methyl	28.00 ^g
0.08g <i>P. guineense</i> + 0.01g Pirimiphos methyl	26.00 ^g
0.1g <i>P. guineense</i> + 0.01g Pirimiphos methyl	24.00 ^h
Control	68.00 ^a

Means followed by the same superscripts letter are not significantly different at 5% probability level of significance.

Table 3. Mean percentage adult emergence of *C. maculatus* treated with different concentration of *P. guineense* and Pirimiphos-methyl in sole and combinations

Treatments	% adult emergence
g/20g cowpea Sole treatments	
0.02g <i>P. guineense</i>	44.00 ^b
0.04g <i>P. guineense</i>	34.67 ^c
0.06g <i>P. guineense</i>	34.00 ^c
0.08g <i>P. guineense</i>	31.00 ^d
0.1g <i>P. guineense</i>	25.00 ^e
0.01g Pirimiphos-methyl	44.00 ^b
Combined treatments	
0.02g <i>P. guineense</i> + 0.01g Pirimiphos methyl	35.00 ^c
0.04g <i>P. guineense</i> + 0.01g Pirimiphos methyl	34.00 ^c
0.06g <i>P. guineense</i> + 0.01g Pirimiphos methyl	31.00 ^d
0.08g <i>P. guineense</i> + 0.01g Pirimiphos methyl	25.00 ^e
0.1g <i>P. guineense</i> + 0.01g Pirimiphos methyl	21.00 ^f
Control	58.00 ^a

Means followed by the same superscripts letter are not significantly different at 5% probability level of significance.

Discussion

The results from this study are previously reported by many researchers (Olaiya, *et al* 1987; Ofuya, 2001, Lale, 2002; Asawalam and Emosairue, 2006; Asawalamm, 2007; Kabeh and Jalingo, 2007) that *Piper guineense* powder, pirimiphos - methyl dust and its mixture has a significant contact toxicity action against stored product insect pests. The ability of the sole plant powders application to cause adults mortality of *C. maculatus*, inhibited oviposition by female beetles on cowpea grains and eventual suppression of F₁ progeny emergence can be attributed to contact toxicity of the powders on the weevil.

The plant family Piperaceae to which *P. guineense* belongs has been reported to possess some forms of insecticidal properties against eggs of cowpea storage bruchid (Adedire and Lajide, 1999) which are capable of suppressing various developmental instars of *Callosobruchus maculatus*. Fasakin and Aberejo (2002) have reported that pulverized plant material from *P. guineense* inhibited egg hatchability and adult emergence of *Dermestes maculatus* Degeer in smoked catfish (*Clarias gariepinus*) during storage. Similar effects of plant materials as insect protectants have been observed in the treatment of cowpea and maize weevils (Ofuya and Dawodu, 2002; Adedire and Ajayi, 1996).

Insecticidal property of any plant material would depend on the active constituents of the plant material. Okonkwo and Okoye (1996) reported that *P. guineense* contains piperine and chavicine, which are insecticidal while Lale (1995), included piperidine and alkaloids as the major active components in *P. guineense* seeds.

The reduction in eggs laid and adult emergence of the treatment with *P. guineense* powder suggest that *C. maculatus* development was adversely affected on grains treated with the powder than the control. This was in agreement with the findings of Asawalam and Emosairue, 2006; Asawalam *et al* 2007. The active constituent in this plant material appears to be responsible for its insecticidal properties against *C. maculatus*. Olaiya *et al* (1987) reported that the mode of action of the phytochemical present in *P. guineense* to be contact toxicity, he further postulated that the powder may also cause physical abrasion to the cuticle of bruchids with a resultant loss of body fluids or blockage of spiracles. The observed efficacy of insecticidal activity of combination of *P. guineense* powder and pirimiphos-methyl dust as a single treatment more than the sole application of *P. guineense* can be attributed to the joint effects of the combined treatments on the insects. In all the treatment application (sole and combined), the increased in adult mortality, oviposition inhibition and suppression of F₁ progeny emergence are all concentration dependent.

The effectiveness of sole and combined treatment of *P. guineense* powder and pirimiphos-methyl dust in controlling *C. maculatus* infestation in cowpea grains during storage could be encouraging and a possible means of ensuring a steady supply of good quality cowpea grains.

The multiple effects of the powders and potential for its local availability make it attractive potentials plant derived insecticides in upgrading traditional post-harvest protection practices. More investigation needs to be carried out on the use of mixing more botanicals and conventional synthetic insecticides because this has proved effective in the control of *C. maculatus* evaluated in this study.

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