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Aspects of insecticidal action of *Piper guineense* Schum and Thonn fruit powders against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae).

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ABSTRACT

Under laboratory conditions (28 ± 3°C and 70 ± 5% r.h.) in Akure, Nigeria, powders of different particle sizes (212 µm, 300 µm, 500 µm and 1 mm, respectively) made from ripe *Piper guineense* Schum and Thonn fruits, were evaluated at five rates of application (0, 0.1, 0.2, 0.3, 0.4 and 0.50 g/20 g of seed), for insecticidal action against *Callosobruchus maculatus* (F.), a major storage pest of cowpea, *Vigna unguiculata* (L.) Walp. Treatments involved introducing freshly emerged beetles onto protected and unprotected cowpea seeds in airtight glass tubes or plastic plates. Irrespective of particle size of powder, all rates of application significantly reduced ($P < 0.05$) oviposition and adult emergence of *C. maculatus* compared with the control. Similarly, all introduced freshly emerged adult beetles were killed within 36 hours. The susceptibility of adult beetles to *P. guineense* powder increased significantly with the age of the beetle. Powder of particle size of 212 µm was always more potent than powder of particle size of 1 mm.

INTRODUCTION

The cowpea seed beetle, *Callosobruchus maculatus* (F.) is a major storage pest of cowpea, *Vigna unguiculata* (L.) Walp., an important dietary staple in many countries (Jackai and Daoust, 1986). Damaged seeds are riddled with adult beetle exit holes, and have reduced weight and poor viability. Severely damaged seeds are turned into a powdery mass which is unfit for human consumption. Synthetic insecticides such as pirimiphos-methyl, methacrifos and fenitrothion when applied as dilute dust formulations provide good control of *C. maculatus* in stored cowpea (Jackai and Adalla, 1997; Golob *et al.*, 1999). However, because of high costs,

availability of these conventional insecticides is poor in most developing countries (Lale, 1995). Additionally, most peasant farmers have little or no technical skill, education or competence to safely handle pesticides (Ogunwolu *et al.*, 1998). Peasant farmers in the tropics are reported to use various plants (whole parts, powder or ash) to protect cowpeas against pest damage during storage (Golob and Webley 1980; Lale. 1995; Golob *et al.*, 1999). The potential of using products (fruit or seed powder and oil extracts) from the West Africa black pepper, *Piper guineense* Schum and Thonn for the control of *C. maculatus* infestation of stored cowpea has been investigated (Ivbijaro and Agbaje, 1986; Olaifa and Erhun, 1988; Lale, 1994; Mbata *et al.*, 1995; Fayape, 1998). In this paper the effect of low rates of application of *P. guineense* fruit powder, of different particle sizes, on *C maculatus* adult mortality, oviposition and adult emergence from eggs laid on treated cowpea seeds was investigated.

MATERIALS AND METHODS

Beetle Culture and experimental conditions

The *C. maculatus* used was derived from a colony originating from infested cowpea seeds collected from a local market in Akure, Nigeria. The colony was maintained in Kilner jars in a cooled incubator at 30 ± 5° C and 50 ± 5% relative humidity for more than 40 generations, using Ife Brown cowpea as substrate. The experiments were carried out under ambient laboratory conditions (28 ± 3° C and 70 ± 5% relative humidity).

Preparation of fruit powders of *P. guineense*

Fresh ripe fruits of *P. guineense* were purchased in local herbal stores in markets in Ondo State, Nigeria. The identity was confirmed at The Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The fruits were oven dried to a constant weight at a temperature of 40° C. Thereafter, the dried fruits were separately ground in 1.5 HP kitchen mill (model - KOAHLBACH). The fruit powder was then sieved into four different particle sizes (212 µm, 300 µm, 500 µm and 1 mm, respectively) with a British laboratory test standard sieve (serial number 211712, 133032, 168945 and 182954 with respect to each particle). The plant powder of a given particle size was kept in a separate plastic container with a tightly fitted lid and placed in a cooled incubator at 0° C for use in the experiment..

Effect of fruit powder of *P. guineense* on oviposition and adult emergence

Fruit powder from *P. guineense* of the different particle sizes (212 µm, 300 µm, 500 µm and 1 mm respectively) was tested at 0.1, 0.2, 0.3, 0.4, and 0.50, g per 20 g of cowpea seeds in separate test tubes (13.0 cm long; 1.5 cm diameter).

Two freshly emerged (0-4 hours old) couples of *C. maculatus* were introduced into each tube. Living couples that did not initiate egg laying after 24 hr were replaced once. Each treatment was replicated five times. An untreated control was similarly set up. After 14 days when all introduced insects were dead, the number of eggs laid on the seeds in each replicate was counted and recorded. Number of adults that emerged from eggs laid on treated cowpea were recorded per replicate 40 days after infestation.

Effect of fruit powder of *P. guineense* on mortality of adult beetle

Fruit powders of *P. guineense* of the different particle sizes were tested at 0.5 g of material per 20 g of cowpea seeds in separate plastic Petri plates (8.5 cm diameter). Ten couples of freshly emerged (0-4 hours old) *C. maculatus* were introduced into each plate which was shaken. Adult mortality was monitored in 12, 24, 36, 48, 72 and 96 hours.

There was a control treatment involving no addition of plant powder onto seeds. Each treatment including the control was replicated five times. The ability of the insecticides to kill actively ovipositing couples that are three and five days old, respectively, was similarly determined.

Experimental design and analysis

All tests utilized a randomized complete block design with five replicates per treatment. Data collected were subjected to analysis of variance (ANOVA). Treatment means which differed significantly at $P = 0.05$ were separated using least significant difference (LSD) statistic

RESULTS

Effect *P. guineense* powder on *C. maculatus* oviposition and adult emergence

Table 1 shows the mean numbers of eggs laid by *C. maculatus* on cowpea seeds protected using *P. guineense* powder of different particle sizes and at different rates. All rates of application significantly reduced oviposition by *C. maculatus* compared with the control, irrespective of particle size . Irrespective of particle size, the application rate of 0.5 g/20 g of seed significantly reduced oviposition by the beetle compared with 0.1 g/ 20 g of seed, but not other rates except 0.2 g/20 g of seed involving powders of particle sizes 500 µm and 1 mm. Similarly, irrespective of rate of application, *P. guineense* powder of particle size 212 µm significantly reduced oviposition by *C. maculatus* compared to powder of particle size of 1 mm, but not powders of other particle sizes except 500 µm at 0.1 and 0.2 g/20 g of seed rates of application.

Table 1. Mean number (± S.E.) of eggs laid by *C. maculatus* on cowpea seeds protected by using *P. guineense* powder of different particle sizes and at different rates

Rate of application g/20 g of seed	Particle size of powder			
	212 µm	300 µm	500 µm	1 mm
0 (control)	139.4 ± 8 87	139.4 ± 8.87	139.4 ± 8.87	139.4 ± 8.87
0.1	5.4 ± 0.81	6.6 ± 1.03	13.8 ± 1.59	19.6 ± 2.87
0.2	4.0 ± 0.32	5.4 ± 0.68	11.0 ± 0.71	15.0 ± 1.34
0.3	2.8 ± 0.37	4.2 ± 0.86	7.8 ± 0.58	9.2 ± 1.98
0 4	1.8 ± 0.37	3.2 ± 0.37	7.6 ± 0.51	10.0 ± 1.87
0.5	1.0 ± 0.45	2.0 ± 0.55	3.8 ± 0.37	6.6 ± 0.75

LSD (0.05) values are 4.30 and 5.26 for vertical and horizontal comparisons, respectively.

The mean numbers of adult *C. maculatus* that emerged from eggs laid on cowpea seeds protected using *P. guineense* powder of different particle sizes and at different rates of application are given in Table 2. All rates of application significantly reduced the number of adults of *C. maculatus* emerging from eggs laid by the beetle on treated seeds compared with the control, irrespective of particle size. Irrespective of particle size, the application rate of 0.5 g/20 g of seed significantly reduced the number of adults of *C. maculatus* emerging from eggs laid by the beetle on treated seeds compared with 0.1 g/ 20 g of seed, except with particle size of 212 μm. Similarly, irrespective of rate of application, *P. guineense* powder of particle size 212 μm significantly reduced the number of adults of *C. maculatus* emerging from eggs laid by the beetle on treated seeds compared to powder of particle size of 1 mm, but not powders of other particle sizes except 500 μm at 0.1 and 0.2 and 0.4 g/20 g of seed rates of application.

Table 2. Mean number (± S.E.) of adults that emerged from eggs laid by *C. maculatus* on cowpea seeds protected by using *P. guineense* powder of different particle sizes and at different rates

Rate of application	Particle size of powder			
g/20 g of seed	212 μm	300 μm	500 μm	1 mm
0 (control)	80.8 ± 4.28	80.8 ± 4.28	80.8 ± 4.28	80.8 ± 4.28
0.1	2.4 ± 0.51	3.2 ± 0.80	6.4 ± 1.21	10.0 ± 2.12
0.2	1.6 ± 0.24	3.0 ± 0.45	4.0 ± 0.71	7.2 ± 0.86
0.3	1.2 ± 0.37	1.8 ± 0.48	3.2 ± 0.37	4.4 ± 1.36
0.4	0.6 ± 0.24	1.4 ± 0.24	3.6 ± 0.40	3.4 ± 0.68
0.5	0.4 ± 0.24	0.8 ± 0.20	2.0 ± 0.32	2.8 ± 0.35

LSD (0.05) values are 2.15 and 2.64 for vertical and horizontal comparisons, respectively.

Effect *P. guineense* powder on mortality of freshly emerged adult *C. maculatus*

The mean numbers of freshly emerged adult *C. maculatus* killed at different times among cowpea seeds protected using *P. guineense* powder of different particle sizes and applied at 0.5 g/ 20 g of seed are given in Table 3. Irrespective of particle size of powder, the numbers of adult beetles killed significantly increased from 12 to 36 hours when all introduced freshly emerged adults were killed. At all times of observation, *P. guineense* powders of differently sized particles caused significantly higher mortality of freshly emerged adult *C. maculatus* compared to the control. At the 12- and 24-hour periods of observation respectively, mortality of freshly emerged adult *C. maculatus* increased significantly with decreasing particle size of *P. guineense* powder.

Table 3. Mortality of freshly emerged adults of *C. maculatus* after introduction onto cowpea seeds treated with *P. guineense* powder of different particle sizes, and applied at 0.5 g per 20 g of seed.

Particle size of powder	Mean number (± S.E) dead beetles at different times (hr)		
	12	24	36
Control	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
212 µm	7.6 ± 0.51	19.2 ± 0.37	20.0 ± 0.00
300 µm	6.2 ± 0.37	17.8 ± 0.37	20.0 ± 0.00
500 µm	3.6 ± 0.68	16.4 ± 0.24	20.0 ± 0.00
1 mm	3.0 ± 0.55	14.8 ± 0.37	20.0 ± 0.00

LSD (0.05) values are 0.41 and 0.54 for vertical and horizontal comparisons, respectively.

Effect *P. guineense* powder on mortality of differently aged adult *C. maculatus*

The mean numbers of differently aged adult *C. maculatus* killed after 12 hours among cowpea seeds protected using *P. guineense* powder of different particle sizes and applied at 0.5 g/ 20 g of seed are given in Table 4. Irrespective of particle size of powder, the numbers of adult beetles killed significantly increased with age of adult. For freshly emerged and 3-day old adults respectively, mortality increased significantly with decreasing particle size of *P. guineense* powder.

Table 4. Mortality of adult *C. maculatus* of different ages within 12 hours after introduction onto cowpea seeds treated with *P. guineense* powder of different particle sizes, and applied at 0.5 g per 20 g of seed.

Particle size of powder	Mean number (± S.E) of dead adult beetles in 24 hours amongst:		
	One-day old	Three-day old	Five-day old
Control	0.0 ± 0.00	1.2 ± 0.37	5.8 ± 0.88
212 µm	7.6 ± 0.50	14.0 ± 0.32	20.0 ± 0.00
300 µm	6.2 ± 0.37	13.0 ± 0.32	20.0 ± 0.00
500 µm	3.6 ± 0.68	11.2 ± 0.73	20.0 ± 0.00
1 mm	3.0 ± 0.55	8.4 ± 0.51	20.0 ± 0.00

LSD (0.05) values are 0.54 and 0.69 for vertical and horizontal comparisons, respectively.

DISCUSSION

The results of this study are consistent with previous reports (Ivbijaro and Agbaje, 1986; Olaifa and Erhun, 1988; Lale, 1994; Mbata *et al.*, 1995; Fayape, 1998) that

P. guineense seed powder is effective in protecting stored seeds of cowpea from infestation and damage by *C. maculatus*. The efficacy of high dosage rates of powder of 0.5 g and greater per 20 g of cowpea seed had mostly been previously verified, but in this study the efficacy of lower dosage rates of between 0.1 and 0.5 g per 20 g of cowpea seed was determined. It was observed that with 0.1 g of *P. guineense* powder per 20 g of cowpea seed significant reduction in *C. maculatus* oviposition and adult emergence was achieved. *P. guineense* was also observed to cause significant mortality of adult beetles.

Ivbijaro and Agbaje (1986) had earlier observed that when applied as ground powder, *P. guineense* was more active insecticidally than when applied as whole fruits. In this study, particle size of powder has further been observed to influence the insecticidal action of *P. guineense* against *C. maculatus*. For instance, the most finely ground powder (of particle size 212 μm) was more active insecticidally than the most coarse powder (of particle size 1 mm). Similar observations have been recorded with root bark powder of *Zanthoxylum zanthoxyloides* (Lam.) Waterm. and seed powder of *Azadirachta indica* A. Juss. against *C. maculatus* (Ogunwolu and Idowu, 1994). Particle size affects dispersion, and the finer the particles, the more uniformly the powders will coat treated seeds and storage container thus enhancing contact with the target pest. The observations on effect of particle size will be important when this material is to be formulated for use in stored products protection. From the results, there is the potential of using particle size of powder to enhance the effectiveness of lower rates of application of *P. guineense* seed powder.

Irrespective of particle size of powder, *P. guineense* was more lethal to older adult beetles (5-day and 3-day old beetles) than to freshly emerged ones. The developmental stage and age of insect treated is well known to influence the susceptibility of insects to insecticides (Buraimoh *et al.*, 2001). Since *C. maculatus* is semelparous, a lot of energy would already have been expended on mating and oviposition which may have rendered the older beetles more susceptible to the powder. It is therefore important to state the ages of insects treated, in reporting experiments involving use of plant derived insecticides, as this can be a source of variability.

Extraction of *P. guineense* seed powder with hexane or acetone yields oils which appear more potent in protecting stored seeds of cowpea against infestation and damage by *C. maculatus* (Olaifa and Erhun, 1988; Ivbijaro, 1990; Mbata *et al.*, 1995). Adults of the beetle are rapidly killed and oviposition and subsequent adult emergence may be completely prevented. However, the seed powder may be easier and cheaper to apply by resource-poor small scale farmers of the tropics. Using cost-benefit analysis, Ivbijaro and Agbaje (1986) demonstrated that it was economically advantageous to apply *P. guineense* seed powder at an effective

application rate of 1 g per 20 g of cowpea. The economic advantage should obviously be greater with lower effective dosage rates. Even though the use of relatively low rates of application of *P. guineense* powder will not completely protect stored cowpea from bruchid damage, it can still be recommended for use by small scale farmers because of the high tolerance of poor quality produce in the tropics (Taylor, 1974). Apart from enhancement through appropriate particle size, low rates of application of plant derived insecticides may work well in combination with insect resistant cultivars. This requires empirical verification. Surface treatment of cowpea with *P. guineense* seed powder does not adversely affect viability and cooking properties of the treated seeds (Ivbijaro and Agbaje, 1986).

P. guineense is reputable for its effective medicinal activities in Africa (Okogun and Ekong, 1974; Olivier-Bever, 1986). Fruits contain the amides piperine, *N*-isobutyloctadeca-trans-2-trans-4-dienamide, stylvatine, α,β-dihydro-piperine and trichostachine (Olivier-Bever, 1986). The essential oil from the berries is composed of the terpenes phellandrene, pipene and limonene (Olivier-Bever, 1986). More recently, the toxic principle in essential oils from *P. guineense* was reported to comprise mainly of phenlypropanoid derivatives, mostly dillapiole and myristicin (Martins *et al.*, 1998).

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