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Full Length Research Paper

The use of two indigenous medicinal plant leaf powders (*Cymbopogon citratus* and *Ocimum suave*) applied as mixed and individual powders to evaluate the reproductive fitness of F_1 generation (eggs laid and F_2 adult) of *Callosobruchus maculatus* (cowpea bruchid)

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It has been estimated that over 90% cowpea is lost to cowpea bruchids especially *Callosobruchus maculatus.* A mixture of plant containing *Cymbopogon citratus* (Lemon grass) (L) and *Ocimum suave* (Wild basil) (W) powders in the ratios (Lemon grass: Wild basil): 100:0, 80:20, 60:40, 50:50, 40:60, 20:80, 0:100 and 0:0 were used under ambient laboratory conditions with the aim of evaluating the effects of these plant powders on the reproductive fitness of F_1 generation of *C. maculatus* (F.) (Cowpea bruchids). The mixed powders were each applied at 1, 2, and 3 g concentration to 20 g of cowpea seeds. Number of eggs laid by F_1 and subsequent F_2 adult that emerged from the untreated combination were compared with the untreated control (0L: 0W). The plant mixture 60L: 40W had the least mean number of egg counts and significantly ($P<0.05$) suppressed F_2 adult emergence. This plant material mixture had the most knock on effect from the original parent and this shows that it can serve as grain protectants against *C. maculatus.*

Key words: *Cymbopogon citratus, Ocimum suave, Callosobruchus maculatus*, F_1, F_2 generation adults.

INTRODUCTION

It is estimated that between 60 and 80% of all grains produced in the tropics is stored at farm level. The main purpose of storing grains is to ensure house hold food supplies to cover future cash needs though sales by taking advantage of seasonal price increase. The largest quantity of food in the tropics is stored in traditional farmer's granaries and in most cases under one roof (Stathers et al., 2002). Developing countries are hit by great losses during storage of cereals and durable commodities such as pulses and oil seeds by storage insect pests. *Callosobruchus* species are major pest of stored grains and grain products in the tropics (Ofuya, 2003). Over 90% of the insect damage to cowpea seeds is caused by *Callosobruchus maculatus* (F) (Caswell and Akibu 1981). The insect lays its eggs on the seeds of cowpea, which hatch and produce larvae that bore hole into the seed cotyledons on which they feed (Onuh and Onyenekwe, 2008). Infestation may reach 100% within 3 to 5 months of storage. Control of *C. maculatus* relies heavily on the use of synthetic insecticides. Owing to the problems of synthetic organic chemicals, there is renewed interest on plants as alternative materials for use as stored grain protectants because they have been found to have broad spectrum insecticidal properties with reduced persistence compared to the organochlorines and organophosphates, carbamates and pyrethroids.

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They are easily available and can be produced within the farmers' vicinity, thus providing a more sustainable approach to pest control (FAO, 1985). They are also cheap to purchase and have no negative impact on the environment because they are easily biodegradable. Therefore, many scientists have been conducting researches over the last three decades aimed at identifying botanicals that would replace synthetic organic chemicals but the efficacies of the plant mixtures have been less investigated (Emeasor et al., 2007). This work is to evaluate the effects of *Cymbopogon citratus* (Lemon grass) and *Ocimum suave* (Wild basil) applied as mixed and individual powders to access the reproductive fitness of F_1 adults of *C. maculatus* (eggs laid and F_2 generation).

MATERIALS AND METHODS

Insect stock culture

Adult bruchids were obtained from already infested cowpea and identified as *C. maculatus* by the assistance of the Nigerian Stored Product Research Institute (NSPRI) Sapele, Delta State Nigeria. The *C. maculatus* adults obtained were introduced into undamaged cowpea (*Vigna unguiculata*) L seeds of the Kano white variety 1696 and maintained in large specimen bottles with fine mesh gauze covering the opened end, for them to mate and oviposit under laboratory conditions. Adult emergence was checked daily and the newly emerged adults were then used for the experiment.

Experimental cowpea

Undamaged and clean cowpea seeds that were used were purchased from Abraka market, Delta state. Each seed was examined under microscope to make sure there were no damages, eggs laid and exit holes on them. They were then kept by deep freezing for one week and left for 24 h under ambient conditions (Ofuya et al., 2007) with slight modifications (deep freezed for 2 weeks).

Preparation of insecticidal plant powder

Two researched plants identified as *C. citratus* and *O. suave* by Botany Department of Delta state University were used for the experiments. The plants were obtained from Issele-Azagba (Aniocha North) Local Government Area of Delta State. Fresh leaves from each plant were slowly dried for 3 weeks in an open wooden cabinet (1.0 × 0.5 × 1.0 m) under room temperature before pulverization in a motorized high speed grinder. The powder was passed through a sieve of 0.1 mm mesh size. The particles were then put in an air tight container to prevent active components from evaporating. This method was adopted by Denloye et al. (2007), with slight modification and constructed the cabinet with a 100 watt bulb.

Formulation of insecticidal plant powders into treatment combinations and efficacy test

The powders obtained from 3.3 were mixed in the following ratios (Lemon grass: Wild basil): 100:0, 80:20, 60:40, 50:50, 40:60, 20:80 and 0:100. Each combination was replicated two times. The untreated combination (0:0) served as the control for the experiment. Each combination was admixed with 20 g of experimental cowpea seed (3.2) at 1, 2 and 3 g concentrations or rates of application. Each formulated treatment combination were admixed with 20 g of cowpea seed of various sizes at different concentration of 1, 2 and 3 g and put into Petri dishes with lid. 3 pairs of adult *C. maculatus* (3 males and 3 females) were introduced into each Petri dishes and kept under laboratory conditions for 12 days. The adults were allowed to lay eggs for 5 days after which they were removed and eggs were counted between 5 and 8 days after infestation (DAI) with the aid of a magnifying lens. Adult emerged 21 DAI and counted for three consecutive weeks. Each adult that emerged was removed and put into a Petri dish. Three (3) pairs of F_1 adult were put into 20 g of cowpea seed. The number of eggs laid was counted with a binocular microscope and F_2 generation adults were counted after 21 days for 3 consecutive weeks.

Statistical analysis

All data collected were subjected to Analysis of Variance (ANOVA) and Multiple Comparism using Duncan's Multiple Range Test and LSD.

RESULTS

Oviposition (eggs laid) and F_1 adult emergence of *C. maculatus* in treated cowpea grain

Oviposition (number of eggs laid)

The mean number of eggs laid (oviposition) by the adult *C. maculatus* in cowpea seeds treated with different mixed proportions of *C. citratus* (Lemon grass, L) and *O. suave* (Wild basil, W) and the untreated seeds are shown in Table 1. There was significant difference (P<0.05) between the concentrations of the plant materials in the number of eggs laid. However, the Duncans' multiple range test could not differentiate where the difference lies. There was significant difference (P<0.05) between the treatment concentrations of the plant materials and the untreated in the number of eggs laid (Ojianwuna and Umoru, 2010).

Number of adult emergence (F_1 generation)

The mean number of adult *C. maculatus* that emerged from cowpea seeds treated with different concentrations of mixed (%) combinations of *C. citratus* (Lemon grass, L) and *O. suave* (Wild basil, W) and the untreated seeds are shown in Table 2. There was significant different (P<0.05) between the concentrations of the plant materials in the number of F_1 adults that emerged. However, the Duncans' multiple range test could not differentiate where the significant lies. There was significant difference (P<0.05) between treatment combinations of the plant materials in the number of F1 adult that emerged (Ojianwuna and Umoru, 2010).

Table 1. Mean number of eggs laid by the adult parents *Callosobruchus maculatus* on cowpea seeds treated with different mixed proportions of *Cymbopogon citratus* (lemon grass, L) and *Ocimum suave* (Wild basil, W) plant powders and the untreated (0:0) seeds.

Mixed proportions (%) of plant materials (L:W)	Concentration of plant material (grams)			$\bar{X}$
	1	2	3	
100:0	62.0	55.0	44.0	53.7^{b}
80:20	58.5	41.0	35.5	45.0^{bc}
60:40	41.0	37.0	21.5	33.2^{c}
50:50	45.5	42.0	27.5	38.3^{bc}
40:60	56.5	44.0	38.5	46.3^{bc}
20:80	44.5	39.5	33.5	39.2^{bc}
0:100	58.0	51.5	39.0	49.5^{b}
0:0 (Control)	95.5	97.5	92.0	95.0^{a}
$\bar{X}$	57.7^{x}	50.9^{x}	41.4^{x}	

Means with the same superscript letters do not differ significantly (P<0.05) using Duncans Multiple Range Test (Ojianwuna and Umoru, 2010).

Table 2. The mean number of adults (F_1 generation) *Callosobruchus maculatus* adult that emerged from cowpea seeds treated with different mixed proportions of *Cymbopogon citratus* (Lemon grass, L) and *Ocimum suave* (Wild basil, W) plant powder on the untreated (0:0).

Mixed proportions (%) of plant materials (L:W)	Concentration of plant material (grams)			$\bar{X}$
	1	2	3	
100:0	21.5	16.0	11.0	16.2^{b}
80:20	24.0	19.0	13.0	18.7^{b}
60:40	16.5	11.0	9.0	12.2^{b}
50:50	22.5	11.0	12.0	15.2^{b}
40:60	29.0	14.5	11.0	18.2^{b}
20:80	25.0	18.5	11.0	18.2^{b}
0:100	24.5	16.5	12.5	17.8^{b}
0:0 (Control)	56.0	57.0	55.0	56.0^{a}
$\bar{X}$	27.4^{x}	20.4^{x}	16.8^{x}	

Means with the same superscript letters do not differ significantly (P<0.05) using Duncans multiple range test (Ojianwuna and Umoru, 2010).

Reproductive fitness

Oviposition (eggs laid) and F_2 adult emergence of C. maculatus in untreated cowpea grains

Oviposition (number of eggs laid): The mean number of eggs laid by the adult *C. maculatus* of the first filial generation (F_1) on the treated and untreated cowpea seeds significantly (P<0.05) reduced as the concentration of plant material increased from 1 to 3 g per 20 g cowpea seeds (Table 3). However, the Duncans' multiple range test could not differentiate where the significance lies. There was also significant difference (P<0.05) between the treatment combination of the plant materials and the untreated in the number of eggs laid.

Number of adult emergence (F_2 generation): The mean number of adult *C. maculatus* (F_2) that emerged from the treated and untreated cowpea seeds significantly reduced (P<0.05) as the concentration of plant materials *C. citratus* (Lemon grass) and *O. suave* (Wild basil) increased from 1 to 3 g per 20 g cowpea seeds. However, the Duncans' multiple range test could not differentiate where the significance lies. There was also significant difference (P<0.05) between the treatment combinations of plant material and the untreated in the adult F_2 (Second Filial Generation) *C. maculatus* emergence (Table 4).

DISCUSSION

The plant materials were toxic to the insects as the concentration increased from 1 to 3 g/20 g cowpea

Table 3. Mean number of eggs laid by F_1 adult *Callosobruchus maculatus* that emerged from cowpea seeds treated with different concentrations and mixed combinations of *Cymbopogon citratus* and *Ocimum suave* compared with the untreated cowpea seeds.

Mixed proportions (%) of plant materials (L:W)	Concentration of plant material (g)			$\overline{X}$
	1	2	3	
100:0	31.5	30.5	24.0	28.7^{b}
80:20	27.5	22.0	20.0	23.2^{bc}
60:40	21.5	19.5	14.5	18.5^{c}
50:50	22.5	20.5	15.5	19.5^{bc}
40:60	31.0	27.5	21.5	26.7^{bc}
20:80	30.0	26.0	19.0	25.0^{bc}
0:100	29.5	27.5	19.0	103.3^{a}
0:0 (Control)	106.5	111.0	92.5	95.0^{a}
$\overline{X}$	37.5^{x}	35.6^{x}	28.3^{x}	

Means with the same superscript letters do not differ significantly (P<0.05) using Duncans multiple range test.

Table 4. The mean number of adults (F_2 generation) *Callosobruchus maculatus* that emerged from the treated and untreated cowpea grains.

Mixed proportions (%) of plant materials (L:W)	Concentration of plant material (g)			$\overline{X}$
	1	2	3	
100:0	13.5	9.5	6.0	9.7^{b}
80:20	11.5	10.0	5.5	9.0^{b}
60:40	9.0	7.0	3.5	6.5^{b}
50:50	10.5	8.0	4.0	7.5^{b}
40:60	13.0	9.5	5.5	9.3^{b}
20:80	13.5	10.5	5.5	9.8^{b}
0:100	13.5	8.5	5.0	9.0^{b}
0:0 (Control)	57.0	56.0	50.0	54.3^{a}
$\overline{X}$	17.7^{x}	14.9^{x}	10.6^{x}	

Means with the same superscript letters do not differ significantly (P<0.05) using Duncans multiple range test.

seeds. The toxicity of the mixtures of plant materials in their different proportions could be as a result of the volatile compounds in the plant materials which acted as fumigants with insecticidal effects on the bruchids. In evaluating the reproductive fitness of *C. maculatus*, it was observed in this study that the number of eggs laid by the F_1 adults that emerged from treated seeds was significantly (P<0.05) reduced when compared to the eggs laid by the adults F_1 parents from the untreated control. It may be that the toxic material in the plant mixtures had a knock-on effect from the original parents of the F_1 generation adults. This could have caused reduction in the number of eggs laid in a number of ways; it may be that the males were sterile, or physiological changes that occurred in the female made it to lay a few non-viable eggs. Dike and Mbah (1992) suggested that *C. citratus* may be ovicidal or larvicidal in their action. Notably in the 60L: 40W and 50L: 50W, the toxicity of the plant material was most retained in the F_1 progeny as seen from the number of eggs laid.

If the insecticidal materials in the plant mixture are systemic, they could move from the outside of the seeds into the inside to affect the feeding and developing F_1 larvae that would subsequently become F_1 adults. In this way, the mixture of plant materials in various proportions, especially in the 60L: 40W and 50L: 50W affected the number of F_2 adults that emerged. The number of F_2 adults that emerged could have further been reduced if the F_1 parents laid non-viable eggs compared to the F_1 adults from the untreated cowpea seeds. In an earier study by Ojianwuna and Umoru (2010), it was reported that the number of eggs laid by adult *C. maculatus* in the proportions of plant mixtures significantly reduced as the concentration increased from 1 to 3 g / 20 g cowpea seeds with 60L: 40W having the least number of eggs laid.

In conclusion, this study has shown that mixed proportions of *C. citratus* (lemon grass) and *O. suave* (Wild basil) especially at 60L: 40W could be most effective in the control of bruchids. However, there is need for more investigation to identify the use of other local plant material mixtures to access their effects on the bruchids- *C. maculatus.*

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