*FULL LENGTH RESEARCH ARTICLE*

# ASSESSMENT OF INHIBITORY SUBSTANCES IN THE SEED COAT OF SOME COWPEA CULTIVARS FOR RESISTANCE AGAINST *Callosobruchus maculatus*

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**ABSTRACT**

Laboratory experiment was conducted at the Faculty of Agriculture and Veterinary Medicine, Imo State University, Nigeria to assess the inhibitory substances in the seed coat of 15 cowpea cultivars for resistance against *Callosobruchus maculatus*. Fifty (50) seeds of the cowpea cultivars were collected from the International Institute of Tropical Agriculture, (IITA) Ibadan and placed in 15 different Petri-dishes with perforated tops and infested with 10 adult *C. maculatus* pest (4 males : 6 females). The Petri-dishes containing the seeds and the insects were allowed to stay undisturbed for 4 weeks. Another set of 50 seeds of the cowpea cultivars were soaked in water to allow for easy removal of the seed coats. The seed coats were dried in the oven at 65$^{o}$C for about 25 min, and then ground into a fine powder. It was observed that the adult *C. maculatus* did not attack the cowpea. However, after 4 weeks, new *C. maculatus* adults were observed on the cowpea seeds. The number of emerged adult *C. maculatus* and the number of insect exit holes observed in the IT97K-568-18 and IT98K-131-2 cultivars were lower and significantly different from the other cultivars. The quantity of tannin and saponin in the seed coat of the cultivars IT97K-568-18 and IT98K-131-2 were significantly higher than the other cultivars. The ability of these two cowpea cultivars to resist the attack of *C. maculatus* was attributed to the presence of tannin and saponin in the seed coats. It is recommended that IT97K-568-18 and IT98K-131-2 cultivars be incorporated in the cowpea breeding programmes involving insect resistance.

***Key words:*** Cowpea, *Callosobruchus maculatus, Cultivars, Tannin, Saponin,Seed coat*

## INTRODUCTION

Cowpea (*Vigua unguiculata* (L) walp) is a crop grown throughout the tropics and the substropics, covering Africa, Asia, South America and parts of Southern Europe and the United States (Rachie & Rawal 1975). It is one of the staple grain legume crops cultivated in Nigeria. Cowpea is a heat loving, drought tolerant crop with a high protein and lower soil fertility requirements than many other crops (Coetzee 1995). Its outstanding potential for intercropping and crop rotation has enabled it to be intercropped for a long time in Nigeria with various other crops such as maize, groundnuts, millet and small grains (Johnson 1970). In Nigeria, cowpea has a potential yield of 3-3.5 tonnes per hectare (Emechebe 1985), while annual global production is over 3 million tonnes (Singh *et al.* 1997).

Chemical analysis of the cowpea seed indicated that a matured seed contains 22-26% protein, 1-2% fats and oil, 60-65% of carbohydrate, 4-5% fibre 3-4% of mineral matter and an energy value of 342 calories (Tindal 1992). According to Willberly (1992), the introduction of cowpea into the feeding regime has helped in balancing and combating protein deficiency-linked malnutrition which is frequent in many developing countries.

Problems militating against increased and sustainable cowpea production that result to high yield losses in Nigeria could be attributed to interplay of abiotic and biotic factors. However, a wide range of pests can cause total yield failure in cases of severe attack (Simmonds *et al.* 1989). Pests attack on cowpea causes not only lower yields returns, but also discourages most farmers from cultivating it.

*Callosobruchus maculatus* pest is the most serious insect threat to cowpea seeds during storage. The insect (*C. maculatus)* lays its eggs on the seeds of cowpea, which hatch and produced larvae that bore into the seed cotyledons on which they feed (Kitch *et al.* 1991; Stoll, 1996). A lot of control measures, including chemical, physical, biological and cultural, have been employed by farmers to combat many devastating pests in cowpea seed production and storage. Following the problems associated with the use of pesticides, ranging from human toxicity to development of pesticide-resistant insect strains, it is imperative that development of crop species that possess resistant properties to the major pests' problems is a sure way of checking pest menace in agriculture.

The present study was therefore aimed at the assessment of inhibitory substance in the seed coat of some cowpea cultivars for resistance against *Callusobruchus maculatus*.

## MATERIALS AND METHODS

The experiment was conducted in the laboratory of the Faculty of Agriculture and Veterinary Medicine, Imo State University, Owerri. Fifteen cowpea cultivars (IT97K-390-2, IT93K-452-1, IT00K-901-5, IT99K-530-1, IT98K-205-8, IT98K-131-2, IT97K-1042-3, IT99K-494-6, IT98D-1399, IT97K-568-18, IT98K-506-1, IT97K-499-35, IT96K-610,

IT98K-555-1 and IT98K-111-1) used in the study were obtained from the International Institute of Tropical Agriculture (IITA), Ibandan, Nigeria. The *Callosobruchus maculates* used were collected from infested cowpea seeds from a local grain marketer at Owerri main market.

Fifty seeds of each of the cowpea cultivars were put in 15 different Petri-dishes. The set-up was replicated 3 times, given a total of 45 independent experimental units, arranged in a completely randomized design. The insects were carefully introduced into each Petri-dish containing the 50 cowpea seeds at a ratio of 4 males: 6 females, using forceps and magnifying lens. The set-up was left undisturbed for 4 weeks, after which the seeds were inspected for possible insect attack, and emergence of *C. maculatus.*

**DETERMINATION OF POSSIBLE DEFENSIVE CHEMICAL SUBSTANCES IN THE SEED COAT OF THE COWPEA CULTIVARS**

Seeds of the cowpea cultivars were soaked in water and allowed to stay for about 20 min. for easy de-coating of the seeds. The seed coats were dried in the oven at 56$^{o}$C for about 25 min, and then ground into a fine powder used in the assay of the following substances.

**Assay of Tannins:** The Follins – Dennis spectrophotometric method (Pearson 1976) was used. 5gm of dried powder of the seed coat of each of the cowpea cultivars was placed in 10ml of distilled water in a test tube and shaken for 30 mins with a shaker. The mixture was filtered and 5ml of the filtrate were put into 50ml volumetric flask and diluted with 30ml of distilled water. Similarly 5ml of standard tannic acid solution diluted with 30ml of distilled water, and 35ml of distilled water put into separate flasks, served as standard and blank, respectively. 1ml of Follin-Dennis reagent was added to each of the flasks followed by 2.5ml of saturated sodium carbonate solution. The content of each flask was made up to 50ml mark and allowed to stand for 90 minutes at room temperature. The absorbance of the developed colour was measured at 760 nm wavelength with the reagent blank at zero. The experiment was repeated two more times to get an average.

$$\text{Tanin} = \frac{D}{W}\ x\ \frac{\text{Au}}{AS}\ x\ \frac{\text{C}}{1000}\ x\ \frac{Vf}{Va}$$

Where; W = weight of the sample analysed, AU = absorbance of the test sample, AS = absorbance of the standard tannic solution, C = concentration of standard in mg/ml, Va = volume of filtrate analysed, Vf = total filtrate volume, D = dilution factor.

**Assay of Saponin:** Saponin was determined by double solvent extraction gravimetric method (Harbone 1973). 5gm of dried powder of the seed coat of the cowpea cultivars were mixed with 150ml of 20% aqueous ethanol solution. The mixture was heated with periodic agitation in water bath for 90 minutes at 55$^{o}$C, and then filtered through a Whatmann fitter paper. The residue was extracted with 50ml of the 20% ethanol and both extract were pooled together. The combined extract was reduced to about 40ml at 90$^{o}$C and transferred to a separating funnel where 40ml of diethyl ether was added and shaken vigorously. Separation was by partition during which the ether layer was discarded and the aqueous layer reserved.

Re-extraction by partition was done repeatedly until the aqueous layer became clear in colour. The saponin was then extracted with 60ml of normal butanol.

The extract was washed with 5% aqueous sodium chloride solution and evaporated to dryness in a pre-weighed evaporating dish. The extract was dried at 60$^{0}$C in the oven and reweighed. The experiment was repeated two more times to get an average.

The saponin content was estimated as a ratio of the original sample; thus,

$$\text{Saponin} = \frac{W2-W1}{Wt\ of\ sample!}$$

Where, $W_1$ = weight of evaporating dish
$W_2$ = weight of dish + sample

**Data analysis:** Data collected in the experiment were analysed by the analysis of variance (ANOVA), while means were separated by the Duncan Multiple Range Test

**RESULTS**

The results showed a significant difference in the mean number of *C. maculatus* that emerged from the different cowpea cultivars, after 4 weeks of initial infestation of the cowpea seeds with the insect. Table 1 revealed that cultivar IT97K-568-18 recorded the least mean number (5.33) of emerged *C. maculatus,* which was significantly different from the mean number (73.33) of emerged *C. maculatus* recorded for the cultivar IT00K-901-5. Also, cultivar IT98K-131-2 recorded second lowest mean number (8.67) of emerged *C. maculatus,* though not significantly different from the records of cultivar IT97K-568-18. There was no significance difference in the mean number of *C. maculatus* that emerged from the following cultivars, IT97K-390-2, IT99K-530-1, 1797K-1042-3, IT97K-499-35, IT96D-610 and IT98K-555-1.

The result presented in the Table 2 showed that there was significant difference in the number of insect exit holes recorded in the seeds of the various cowpea cultivars. Cultivar IT00K-901-5 had the highest mean member (78.33) of insect exit holes, which was significantly different from the mean insect exit holes recorded in the other cultivars. The least number of insect exit holes was recorded in the IT97K-568-18 cultivar, with mean value of 6.33, though not significantly different from that of cultivar IT98K-131-2, with mean value of 12.00.

Table 3 showed results of assay of tannin. It showed that the highest proportion of tannin was obtained in the IT97K-568-18 cultivar with a mean value of 0.52, however, not significantly different from the mean tannin value recorded in the IT98K-131-2, but both values were significantly different from the mean proportions of tannins recorded for the other cultivars.

Table 4 gave the results of assay of saponins, It revealed that the proportion of saponin in the IT97K-568-18 cultivar was significantly higher (1.84) than in the other cultivars (Table 4). Cultivar IT98K-131-2 recorded the second highest value (1.40) of saponin content in the seed coat, however, not significantly different from the saponin proportion in the cultivar IT99K-494-6.

**TABLE 1: MEAN NUMBER OF *C. MACULATUS* THAT EMERGED FROM THE SEEDS OF THE COWPEA CULTIVARS 4 WEEKS POST INITIAL INFESTATION.**

| Cowpea cultivars | **Mean* number of emerged *C. maculatus*** |
|---|---|
| IT97K-390-2 | 28.33$^{bcde}$ |
| IT93K-452-1 | 44.67$^{abc}$ |
| IT00K-901-5 | 73.33$^{a}$ |
| IT99K-530-1 | 35.33$^{bcde}$ |
| IT98K-205-8 | 56.00$^{ab}$ |
| IT98K-131-2 | 8.67$^{de}$ |
| IT97K-1042-3 | 34.00$^{bcde}$ |
| IT99K-494-6 | 20.00$^{cde}$ |
| 1T97K-568-18 | 5.33$^{e}$ |
| IT97K-499-35 | 53.00$^{ab}$ |
| IT968-610 | 32.67$^{bcde}$ |
| IT98K – 555-1 | 25.33$^{bcde}$ |
| IT98D-1399 | 36.00$^{bdc}$ |
| IT98K – 111 – 1 | 39.33$^{bdc}$ |
| IT98K – 506-1 | 40.33$^{bdc}$ |

*Means with the same letter(s) are not significantly different, at p=0.05, according to Duncan Multiple Range Test.

**TABLE 2: MEAN NUMBER OF INSECT EXIT HOLES OBSERVED IN THE SEEDS OF THE COWPEA CULTIVARS AT 4 WEEKS AFTER INITIAL INFESTATION**

| Cowpea cultivars | **Mean* number of exit holes created by *C. maculatus* in the cowpea seeds** |
|---|---|
| IT97K-390-2 | 28.67$^{bcd}$ |
| IT93K-452-1 | 56.33$^{abc}$ |
| IT00K-901-5 | 78.33$^{a}$ |
| IT99K-530-1 | 37.00$^{bcd}$ |
| IT98K-205-8 | 66.67$^{ab}$ |
| IT98K-131-2 | 12.00$^{d}$ |
| IT97K-1042-3 | 34.33$^{bcd}$ |
| IT99K-494-6 | 21.00$^{cd}$ |
| 1T97K-568-18 | 6.33$^{d}$ |
| IT97K-499-35 | 41.33$^{abcd}$ |
| IT968-610 | 31.33$^{bcd}$ |
| IT98K – 555-1 | 36.67$^{bcd}$ |
| IT98D-1399 | 57.00$^{abc}$ |
| IT98K – 111 – 1 | 39.33$^{abcd}$ |
| IT98K – 506-1 | 42.33$^{abcd}$ |

*Means with the same letter(s) are not significantly different, at p=0.05, according to Duncan Multiple Range Test.

**TABLE 3: MEAN PROPORTION OF TANNIN CONTAINED IN THE SEED COAT OF THE COWPEA CULTIVARS**

| Cowpea cultivars | Mean* Proportion of tannin in the seed coat of cowpea seeds |
|---|---|
| IT97K-390-2 | 0.42$^{d}$ |
| IT93K-452-1 | 0.33$^{f}$ |
| IT00K-901-5 | 0.33$^{f}$ |
| IT99K-530-1 | 0.39$^{e}$ |
| IT98K-205-8 | 0.43$^{cd}$ |
| IT98K-131-2 | 0.51$^{a}$ |
| IT97K-1042-3 | 0.46$^{b}$ |
| IT99K-494-6 | 0.43$^{cd}$ |
| 1T97K-568-18 | 0.52$^{a}$ |
| IT97K-499-35 | 0.45$^{bc}$ |
| IT968-610 | 0.46$^{b}$ |
| IT98K – 555-1 | 0.42$^{d}$ |
| IT98D-1399 | 0.46$^{b}$ |
| IT98K – 111 – 1 | 0.46$^{b}$ |
| IT98K – 506-1 | 0.43$^{cd}$ |

*Means with the same letter(s) are not significantly different, at p=0.05, according to Duncan Multiple Range Test.

**TABLE 4: MEAN PROPORTION OF SAPONIN CONTAINED IN THE SEED COAT OF THE COWPEA CULTIVARS**

| Cowpea cultivars | Mean* Proportion of saponin in the seed coat of cowpea seeds |
|---|---|
| IT97K-390-2 | 0.62$^{de}$ |
| IT93K-452-1 | 0.44$^{ef}$ |
| IT00K-901-5 | 0.80$^{cd}$ |
| IT99K-530-1 | 0.48$^{f}$ |
| IT98K-205-8 | 0.82$^{cd}$ |
| IT98K-131-2 | 1.40$^{b}$ |
| IT97K-1042-3 | 1.48$^{ef}$ |
| IT99K-494-6 | 1.40$^{b}$ |
| 1T97K-568-18 | 1.84$^{a}$ |
| IT97K-499-35 | 0.29$^{f}$ |
| IT968-610 | 0.42$^{ef}$ |
| IT98K-555-1 | 0.64$^{de}$ |
| IT98D-1399 | 0.28$^{f}$ |
| IT98K-111-1 | 0.32$^{f}$ |
| IT98K- 506-1 | 1.00$^{c}$ |

*Means with the same letter(s) are not significantly different, at p=0.05, according to Duncan Multiple Range Test.

**DISCUSSION**

There were no signs of insect holes on the cowpea seeds six days post infection, during which the *C. maculatus* died, an indication that the adult insects did not feed on the cowpea seeds but instead laid eggs and died. This observation was in accordance with Kitch *et al.* (1991) who reported that the adult beetles that did not feed on stored produce only live for a short time, not more than 12 days after laying eggs.

The numbers of insects that emerged from cultivars IT97K-568-18 and IT98K-131-2 were significantly lower than the numbers that emerged from the other cultivars, suggesting resistance against *C. maculatus* by these cultivars. It was also observed that the number of insect emergence did not vary much from the number of exit holes recorded, agreeing with Stoll (1996) who observed that the eggs laid by adult insects hatch into larvae which bore into the seed cotyledons on which they feed, thus making holes for adult insects to escape. The above observations in addition to the much lower numbers of insect exit holes observed on the seeds of cultivars IT97K-568-18 and IT98K-131-2 could be an indication that the two cultivars (IT97K-568-18 and I98K-131-2) were resistant to the *C. maculatus,* hence the insect larva found it difficult to penetrate the seed cotyledons.

Moraes *et al.* (2000) reported that both the common bean and cowpea are endowed with compounds called general defensive compounds that protect their seeds against different pests. Among these compounds are Alkaloids flavonoids, tannins, cyanogenic glucosides (HCN) and trypsin inhibitors. Saponin was reported to be responsible for the inability of *C. chinensis* to develop in soybeans (Applebaum *et al.* 1965)*, Vicia faba* (Kemal & Smith 1996).Results from this study showed that the seed coats of the various cowpea cultivars assayed contained tannins in different proportions. The tannins present in the IT97K-568-18 and IT98K-131-2 cultivars were significantly higher than the tannins recorded in the other cultivars. The presence of higher proportions of tannins in the IT97K-568-18 and IT98K 131-2 cultivars could be responsible for the resistance of the two cultivars against *C. maculatus.* Larranzio *et al*. 2005 hypothesised that seed coat tannins should be considered as one of the biochemical defence mechanisms that can deter, poison or starve *bruchid* larvae that feed on cowpea seeds.

The result of this experiment revealed high saponin content in the cultivar IT97K-568-18, followed by cultivar IT98K-131-2. The presence of high quantity of saponins in these two cultivars could be a source of defence against the attack by *C. maculatus,* as evidenced by the low numbers of insects emerging and insect exit holes, respectively. Earlier observation (Applebaum *et al.* 1965) stated that the inability of *C. chinensis* to develop in soybean was attributed partly to its saponin content.

It is concluded that the cowpea cultivars IT97K-568-18 and IT98K-131-2 have the ability to resist *C. maculatus* pest in storage, which could have been due to the presence of the chemical compounds tannins and saponins present in the seed coat of the seeds. It is recommended that these two cowpea cultivars be incorporated into the breeding programme involving insect resistance.

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