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Short Communication

Bioactive metabolites in improved cowpea seeds

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The International Institute of Tropical Agriculture (IITA) has developed some pest and disease resistant cowpeas. From these the seeds of 8 cowpea cultivars were extracted with ethanol, and partitioned into chloroform and water-soluble fractions, the water-soluble fraction was further extracted with ethyl acetate. Residues from ethanol, chloroform and ethyl acetate soluble fractions for each of the 8 cowpea cultivars were screened against brine shrimp larvae. The seed extracts of cowpea cultivars IT93K – 596 – 9 – 12, IT90K – 277 – 2 and IT93K – 452 – 1 were found to be most active, indicating that they contain cytotoxic compound(s).

Key words: Cowpea, bioactive compund, biotechnology, brine shrimp larvae, cytotoxicity.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is an herbaceous short term, annual leguminous plant which is grown in many tropical and subtropical countries (Singh and Sharma, 1996). Cowpea is a key staple food in many developing countries, and forms an integral part of the diet of about 120 million people around the world (IITA, 1995). All the parts of the plant that are used for food are nutritious, providing protein, vitamins and minerals. Cowpea grains contain about 25% protein, and the ability of cowpea plants to tolerate drought and poor soils makes it an important crop in the Savanna regions where these constraints restrict other crops (IITA, 2004).

However, cowpea production and storage has been hampered over the years by the menace of various pests and diseases, which attack the crop at various stages of its production (IITA, 1997). Major pests attacking cowpea plant includes flower thrips (*Megalurothrips sjostedti*), pod borer (*Maruca vitrata*) and pod sucking bugs. Storage weevil (*Callosobruchus maculatus*) also damage stored cowpeas. Diseases of cowpea include stem and root rots and leaf spots caused by fungal, while mosaic disease and mottle symptoms are caused by viruses (IITA, 2004).

Consequently, scientist has sought for ways for combating the menace of cowpea pests and diseases. Various methods have been device to minimize if not eliminate the damaging effect of these diseases and pests. One of such method adopted is the development and breeding of cowpea crops that are resistant to pests and diseases. An example of which is the "Bulk Population Breeding" method in which different species of cowpea with different traits are cross breed to improve yield (Allard, 1960). Another method of recent development is the genetic manipulation in which the required gene from wild relatives of plants related to cowpea is transferred to the plant through cross breeding or in some cases the foreign gene is inserted into cowpea plant using the soil bacterium, *Agrobacterium tumefaciens* as a vector.

However, over the past few years through the establishment of a regional pest management, International Institute of Tropical Agriculture (IITA) has been able to launch a number of efficient pest control technologies for cowpea plant, mainly based on resistant, higher-yielding varieties. IITA has thus been able to developed high-yielding varieties for both sole and intercropping, with resistance to major disease, insect

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pests, nematodes, and parasitic weeds (IITA, 2004; IITA, 1997). This research work thus aims at establishing whether or not disease and pest resistant cowpea plant produces toxic compounds which can be detected by the Brine Shrimp lethality Test (BST), so that on the long run compounds that can be used to protect crops from pests and disease can be isolated from cowpea.

MATERIALS AND METHOD

Eight different cowpea cultivars, which were supplied, by Dr. Bir B. Singh and Dr. Stephen K. Asante of the International Institute of Tropical Agriculture (IITA), Kano Station, were used in this investigation. The brine shrimp (*Artemia salina*) eggs used is of the brand "Argentenmia" packaged by Argent Chemical Laboratories, USA, while synthetic sea salt of the brand "Instant Ocean" manufactured by "Aquarium Systems, Sarrebourg, France" was used to prepare the sea salt solution.

50 g of grounded cowpea seeds was extracted with 200 ml of ethanol for four days, after which the mixture was filtered and the residue was re-extracted with a fresh 200 ml of ethanol for another four days and filtered. The combined filtrate was concentrated on "Buchi rotary evaporator (R110)" at 40°C. The residue was collected, and weighed. About 40 mg of the extract was kept for testing and the remaining was partitioned between 100 ml of $CHCl_3/H_2O$ (1:1) mixture, the two distinct layers formed overnight were separated. The $CHCl_3$ soluble fraction was evaporated to dryness and weighed, and the aqueous fraction was extracted with 50ml ethyl acetate. The ethyl acetate layer was dried with anhydrous Na_2SO_4, filtered and evaporated to dryness. The aqueous layer was discarded.

Brine shrimp lethality assay (McLaughilin and Chang, 1999) was carried out for each of the fraction of all the 8 cowpea cultivars.

Table 1. Brine shrimp lethality assay results for the ethanol extracts.

Sample Code	Dosage (g/ml)	Number of Mortality	Percentage Mortality
IT93K – 596 – 9 – 12	10	4	13.33
	100	8	26.67
	1000	28	93.33
IT90K – 277 – 2	10	0	0.00
	100	3	10.00
	1000	22	73.33
IT93K – 452 – 1	10	1	3.33
	100	2	6.67
	1000	19	63.33
IT845 – 2246 – 4	10	0	0.00
	100	3	3.33
	1000	7	23.33
IT89KD – 288	10	0	0.00
	100	0	0.00
	1000	3	3.33
IT86D – 719	10	1	3.33
	10	0	0.00
	1000	0	0.00
IT93K – 389	10	1	3.33
	100	1	3.33
	1000	9	30.00
IT90K – 76	10	0	0.00
	100	2	6.67
	1000	8	26.67

Table 2. Brine shrimp lethality assay results for the chloroform extracts.

Sample Code	Dosage (g/ml)	Number of Mortality	Percentage Mortality
IT93K – 596 – 9 – 12	10	2	6.67
	100	16	53.33
	1000	29	96.67
IT90K – 277 – 2	10	1	3.33
	100	1	3.33
	1000	9	30.00
IT93K – 452 – 1	10	0	0.00
	100	0	0.00
	1000	2	6.67
IT845 – 2246 – 4	10	1	3.33
	100	1	3.33
	1000	2	6.67
IT89KD – 288	10	1	3.33
	100	0	0.00
	1000	0	0.00
IT86D – 719	10	0	0.00
	100	1	3.33
	1000	6	20.00
IT93K – 389	10	0	0.00
	100	1	3.33
	1000	0	0.00
IT90K – 76	10	0	0.00
	100	2	6.67
	1000	8	26.67

Table 3. Brine shrimp lethality assay results for the ethyl acetate extracts.

Sample Code	Dosage (g/ml)	Number of Mortality	Percentage Mortality
IT93K – 596 – 9 – 12	10	0	0.00
	100	20	66.67
	1000	30	100.00
IT90K – 277 – 2	10	0	0.00
	100	1	3.33
	1000	22	73.33
IT93K – 452 – 1	10	0	0.00
	100	0	0.00
	1000	10	33.33
IT845 – 2246 – 4	10	0	0.00
	100	0	0.00
	1000	24	80.00
IT89KD – 288	10	0	0.00
	100	0	0.00
	1000	29	96.67

Table 3. contd.

IT86D – 719	10	1	3.33
	100	1	3.33
	1000	10	33.33
IT93K – 389	10	0	0.00
	100	0	0.00
	1000	3	10.00
IT90K – 76	10	0	0.00
	100	0	0.00
	1000	1	3.33

RESULTS AND DISCUSSION

A compound is considered to be cytotoxic if it inhibits vital metabolic processes or it causes disorder in living organisms resulting in perversion of behavior or death (Fatope, 1995). Several plant extracts are known to contain cytotoxic compounds. For example, *Azadirachta indica* extracts affects about 195 species of insects at concentration ranging from 0.1 to 1000 ppm. More importantly, insects that have become resistant to synthetic pesticides can be controlled with some plant extracts (Lindquist et al., 1990; Menn, 1990).

Results presented in Tables 1 – 3 shows the cytotoxic properties of the ethanolic, chloroform and ethyl acetate extracts of improved cowpea seeds at 10, 100 and 1000 g/ml concentration. Generally the cytotoxic activity increases with increase in concentration in most of the active extracts. The extracts of IT93K – 596 – 9 – 12 are the most active. For most of the cowpea cultivars, the ethyl acetate soluble fractions are most active. The ethanolic extract of IT93K – 452 – 1 recorded the highest mortality rate.

Jones and coworkers (1994) established that azadirachtin an extract of neem tree *A. indica* block the development of the motile male gamete *in vitro*. similarly, some other plants including *Anona squamosa* (atis or sugar apple), *Eucalyptus globus* (bagras or active gum eucalyptus), *Lansium domesticum* (lansones), and *Cundiacum variegatum* (san Francisco or croton) have been found to posses insecticidal properties from their activities against *Aedes aedes aegypti* (Linnaens) and *Culex quinque fasciators* (say) Mazon et al. (1994). These plant extracts which posses insecticidal and pesticidal properties can be developed into insecticide and pesticides that will be environmentally safe alternatives to synthetic pesticides.
The results of the Brine Shrimp lethality Bioassay experiments indicates the presence of active cytotoxic compounds in the extracts of the seeds of the manipulated cowpea cultivars IT93K – 596 – 9 – 12. IT90K – 277 – 2 and IT93K – 452 – 1. These cultivars need to be investigated further so as to isolate and identify the compounds responsible for the activity.

REFERENCES

Allard RW (1960). Principle of plant Breeding, John Wiley and Sons, New York. pp. 129 – 149.

Fatope OM (1995). Phytocompounds: Their Bioassay and Diversity, Discovery and Innovation, 7(3): 229 – 236.

International Institute of Tropical Agriculture (IITA) (1995). Annual Report. pp. 15 – 19.

International Institute of Tropical Agriculture (IITA) (2004). Information available at http://www.iita.org/crop/cowpea.htm.

International Institute of Tropical Agriculture (IITA) Annual report (1997). Also available at: http://www.iita.org/info/ar97/36-37.htm.

Jones IW, Denholm AA, Ley SV, Lovel H, Wood A, Snden RF (1994). Sexual development of Malaria parasites is inhibited invitro by the neem extract azadirachtin and its semi-synthetic analogues. FEMS Microbiol. Lett. 120(3): 267 – 273.

Lindquist RK, Adams AJ, Hall FR, Adams IHH (1990). Laboratory and greenhouse evaluations of Mangosan- o against bifenthrin – resistant and - susceptible greenhouse white flies, *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). In: Locke JC, Lawson RH (eds). Proceedings of a workshop on neem's potential in pest management programs. USDA – ASR, Beltsville, MD. ARS. 86: 23 – 28.

Mazon RB, Alvior JP, Luezon LL, Morales AS, Mutuc FE (1994). South east Asian J. Trop. Med. Public Health 25(4): 7545 – 759.

McLaughlin JL, Chang CJ (1999) Simple (bench – top) bioassays and the isolation of new chemically diverse antitumor and pesticidal agents from higher plants. In: Romeo (eds). Phytochemicals in Human Health Protection, Nutrition and Plant Defense, Kluwer Acedemic/Plenum Publishers, New York, pp. 89 – 124.

Menn JJ (1990). USDA interest in neem research, in: Locke JC, Lawson RH (eds). Proceedings of a workshop on neem's potential in pest management programs. USDA – ASR, Beltsville, MD. ARS – 86, pp. 1 – 3.

Singh BB, Sharma B (1996). Restructuring cowpea for higher yield, Ind. J. Genet. 56(14): 389 – 390.