Asian Research Journal of Agriculture
1(1): 1-7, 2016, Article no.ARJA.26505

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Grain Protectant Potential of *Annona muricata* and *A. senegalensis* against Cowpea Seed Bruchid *Callosobruchus maculatus* Fab. (Coleoptera: Bruchidae)

M. N. Ishuwa[1], O. S. Elkanah[2] and J. A. Wahedi[3*]

[1]Department of Biological Sciences, University of Mkar, Gboko, Benue State, Nigeria.
[2]Department of Biological Sciences, Taraba State University, P.M.B. 1167, Jalingo, Nigeria.
[3]Department of Biological Sciences, Adamawa State University Mubi, P.M.B. 25, Mubi, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author MNI designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors MNI and OSE performed the laboratory bioassay. Author JAW performed the statistical analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARJA/2016/26505
Editor(s):
(1) Rusu Teodor, Department of Technical and Soil Sciences, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania.

Original Research Article

Received 21st April 2016
Accepted 21st May 2016
Published 30th May 2016

ABSTRACT

Annona muricata and *Annona senegalensis* powders were evaluated for their insecticidal potency against adult *Callosobruchus maculatus*. Toxicity test was conducted by contact bioassay at different concentrations of the treatment plants viz: 0.0 g, 0.5 g, 1.0 g, 2.0 g and 3.0 g was tested as well as their anti-ovipository activities, adult F1 progeny emergence suppression activities and anti-feedant activities which possibly result in the grain' weight loss and grain damage was also tested. The results revealed that the treatment plants at all concentrations showed a significant (P>0.05) insecticidal potency, by recording higher mortality rates, suppression of egg laying and adult emergence, and reduced weight loss as well as grain damage when compared with the untreated control. The root bark of *A. senegalensis* at 3.0 g powder per 20 g grain had the higher mortality rate (98.5). The leaf and root bark powder of *A. muricata* at 3.0 g reduced oviposition minimum (1.4 respectively); leaf powder of *A. muricata* at 3.0 g powder per 20 g grain also reduced F1 generation emergence minimum (9.3) and root bark powder of *A. muricata* at 3.0 grams w/w

**Corresponding author: E-mail: wajasini@yahoo.com;*

reduced grain damage minimum (0.7). The results revealed that *A. muricata* at 3.0 g powder per 20 g grain for the leaf and root bark powders to be more effective in controlling *C. maculatus* than *A. senegalensis.* Therefore, it is recommended that farmers should put the practice of using *Annona* species, especially, *A. muricata* as an alternative to the dangerous and highly persistent chemical insecticides in the control of *C. maculatus* on stored cowpea.

Keywords: Annona; Bruchid; Callosobruchus maculates; cowpea; insecticidal.

1. INTRODUCTION

The indiscriminate use of chemical pesticides has given rise to many well known serious problems such as genetic resistance of pest species, resurgence of target pest, outbreak of secondary pest, adverse impact on beneficial organisms, environmental pollution, toxic residue in crop produce and by products, increasing cost of application, hazards from handling [1,2].

These problems are of great concern in Nigeria and other third world countries where the majority of farmers and pesticide users are not trained in the safe handling and application of toxic compounds.

The problems caused by pesticide and their residues have increased the need to search for effective, biodegradable pesticides with greater selectivity. The strategies used have included the search for new types of insecticides and the re-evaluation and use of traditional botanical pest control agents [1].

Annonaceae is a large family of tropical and subtropical trees and shrubs comprising about 130 genera and more than 2000 species [3]. They are cultivated mostly for edible fruits [4]. Some species are also used in the folk medicine for the treatment of worm infestation, scabies, yaws, constipation, diarrhea and dysentery [2]. Some species are also used as antidiabetic and anticancer [5], anti-inflammatory [6], as well as anti oxidant [7]. In West Africa, five species of *Annona* have been reported by Burkill, [8]. He documented their names as: *Annona glauca*, *A. muricata, A. reticulata, A. senegalensis* and *A. squamosa*.

Given the wide spread occurrence of *Annona senegalensis* (custard apple) and *A. muricata* (sour sap) in Benue state and their vast potential as sources of insecticides, the study was aimed at evaluating the potency of the bioactivity of *Annona senegalensis* and *A. muricata* against cowpea seed bruchids, *Callosobruchus maculatus* in Benue State.

2. MATERIALS AND METHODS

The experiment was carried out in Gboko, Benue State Nigeria between May, 2013 and October, 2013. Gboko is situated in Benue State Nigeria and it is located on latitude 7°08' and 7°31'N, and longitude 8°37' and 9°10'E. Gboko has a sub-humid tropical wet and dry climate, with annual temperature range of 23°C to 34°C [9]. The vegetative formation of Gboko is Guinea savannah with lot of grasses interspersed with trees growing alongside tall grasses [9].

2.1 Collection of Plant Material

Seeds, leaves, stem bark and root bark of two species of *Annona*, namely *A. muricata* (sour sap) and *A. senegalensis* (custard apple) were obtained from matured stands growing within Makurdi and Gboko environments. The plant parts were air dried, ground in an electric grinding machine and were sieved to obtain fine powders which were used for the experiment.

2.2 Insect Culture

The parent stock of the adult *C. maculatus* was obtained from infested cowpea seeds in the store. This was introduced on clean cowpea seeds in a 500 cm^3 culture jar for two to three days. The parent stock was later removed, and the culture jar was monitored overtime for F1 progeny emergence under favorable laboratory conditions (temperature of 30±2°C, and relative humidity of 70±5%). The culture was carried out in the Laboratory of Crop Science Department, University of Mkar, Gboko, Benue State. 2-3 days old newly emerged *C. maculatus* were used for the experiment.

2.3 Preparation of the Cowpea Seeds

The cowpea seeds used for the experiment were of a local variety from Kano (Bause-local), which has been genetically improved. The seeds were subsequently treated prior to the experiment as described by Wahedi et al. [10] as follows: The seeds were dried to a constant weight in an oven between 30-35℃ for 14 days. This was subsequently air-dried for about 1 hour and was wrapped tightly in a polythene bag and stored in a deep freezer for 14 days. The grains were allowed for five days of equilibration before bioassay.

2.4 Bioassay

Five concentrations of the plant powdered products (seeds, leaves, stems, barks and root barks) of *A. muricata* and *A. senegalensis* were prepared i.e 0.0 g, 1.0, 0.5 g, 2.0 g and 3.0 g. Each concentration was added to 20 g of seeds contained in 9 cm^3 jars and was thoroughly mixed by shaking the jars. In all experimental groups, each concentration was performed in quadruplicate using N = 5 male and 5 female *C. maculatus* for each replicate. The experiment was conducted in a randomized complete block design with factorial arrangements.

The following parameters were analyzed:

2.4.1 Mortality

Each experimental jar was monitored daily for seven days for adult *C. maculatus* mortality and the dead individuals were subsequently discarded. Mortality rates were calculated as the proportion of dead *C. maculatus* in the total number of survivors the previous day, as shown in the formula below:

$$Mortality\ rate = \frac{Number\ of\ dead\ insects}{Number\ of\ insects\ exposed} X\ 100$$

2.4.2 Oviposition

Ten (10) seeds from each of the treatment jar were randomly picked and examined for eggs laid (white gelatinous spots) using stereoscope. The number of eggs examined on the 10 seeds was extrapolated for the entire jar, using an average number of grains (146) per treatment per jar [10].

2.4.3 F1 progeny emergence

The F1 progeny emergence was noted and recorded for a period of two weeks from date of first emergence.

2.4.4 Grain damage

10 seeds were randomly selected from each of the treatment jar and were observed for grain damage (number of holes and punctures on the seeds) using stereoscope. The number of grain damage observed was also extrapolated for the entire jar using an average of 146 seeds per jar.

2.5 Statistical Analysis

Data collected were analyzed using Analysis of Variance. The treatment means were separated using the Least Significant Difference, at 5% level of significance (P = 0.05).

3. RESULTS AND DISCUSSION

3.1 Mortality

There was a high significant difference in the mortality of *C. maculatus* exposed to *Annona senegalensis* and *A. muricata* treatments at different doses when compared with the untreated control (0.0 g). The insecticidal activity of the various plant parts varied with the rates. For *A. senegalensis* treatment doses, the highest percentage mortality was recorded in 3.0 g of the root bark powder (98.5), and was least in 0.5 g leaf powder (24.2). Although the difference was significant among the treatment doses, the highest mean mortality was recorded in the root bark powder (68.7), followed by stem bark powder (54.7) and the least was in the leaf powder (34.2). For *A. muricata*, the leaf powder treatment doses recorded the highest mean mortality (59.9) and were least in the seed powder treatment doses (49.9). Meanwhile, *A. muricata* had significant (P>0.05) higher insecticidal activity in terms of toxicity (mortality) than the *A. senegalensis* (Table 1).

The results agree with Vanichpakern et al. [2], who reported that the leaf and seed extracts of *Annona reticulata* showed contact toxicity against *C. maculatus*; Khalequzzaman and Sultan [11], reported that the seeds of *Annona squamosa* have insecticidal and abortifacient properties against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae); Shin et al. [12], reported the insecticidal potency of *Annona* seed against *Myzus persicae* (Sulzer) (Homoptera: Aphididae) and *Nilaparvata lugens* (Stal) (Homoptera: Delphacidae) and Rajapakse and Ratnasekera [13] recorded 91% mortality of *C. maculatus* treated with the leaf extract of *Annona* spp. In other studies, Ahad et al. [14] recorded 100% mortality of *C. maculatus* when treated with ethanol extract of *Annona* leaf, Nayak [15] reported the insecticidal activities of *A. reticulata* extract against *Culex* (Linnaeus)

(Diptera: Culicidae) mosquitoes, and recorded 100% mortality.

3.2 Oviposition

Table 2 shows the effect of treatments on the oviposition of *C. maculatus*. There was a significant difference (*P*>0.05) between the control untreated and treatments on the oviposition of *C. maculatus*. The untreated control recorded the highest number of eggs laid (47.5). Among the treatments, the stem bark powder of *Annona senegalensis* (0.5 g) recorded the highest (24.1) number of eggs laid, and the least (1.4) was recorded in leaf and root bark powder of *Annona muricata* (3.0 g) (Table 2). Meanwhile, the highest mean was recorded in the leaf powder of *Annona senegalensis* (21.9), followed by seed powder of *Annona muricata* (19.7) and the least (17.0) was recorded in the root bark powder of *Annona senegalensis* (Table 2). The result of the study agrees with Epino and Chang [16], who reported the anti-oviposition properties of *Annona squamosa* seed extracts against the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae).

3.3 F1 Progeny Emergence

In Table 3, the result of the progeny emergence of *C. maculatus* revealed that the treatment powders were able to suppress the emergence of the F1 progeny significantly (*P*>0.05) when compared with the control untreated. The control untreated recorded the highest emergence of up to 86.6%. Among the treatments, the highest emergence (61.7) was recorded in the leaf powder *Annona senegalensis* (0.5 g), but the minimum was recorded in leaf powder of *Annona muricata* (9.3) at 3.0 g concentration (Table 3). This shows that the powders of *Annona* spp were able to reduce the emergence of the F1 progeny of *C. maculatus*, confirming their insecticidal potentials against the bean weevils.

3.4 Grain Damage

The grain damage also followed the same pattern as the F1 emergence, where the treatments showed their insecticidal potency by suppressing *C. maculatus* activities on the grains. The highest rate of damage was recorded in the untreated controls (88.7), while the minimum damage was recorded in the root bark powder of *A. muricata* (0.7) at 3.0 g concentration, followed by the leaf powder of *A. senegalensis* (0.8) at again, 3.0 g concentration (Table 4). Meanwhile, the minimum mean grain damage (2.0) was recorded in the root bark powder of *A. senegalensis*, and was highest (35.4) in the leaf powder of *A. senegalensis* (Table 4). Grain damage as a result of the *C. maculatus* activities could result to subsequent weight loss. This is in consonant with the work reported by Boreddy and Chitra [17], where *Annona* seed extract protected *Spodoptera litura* against weight loss.

Table 1. Effect of *Annona* powdered products on adult mortality of *Callosobruchus maculatus*

Treatment	Treatment (g)					Mean
	0.0	**0.5**	**1.0**	**2.0**	**3.0**	
A. senegalensis						
Leaf powder	7.5	24.2	38.7	52.8	55.5	34.2
Seed powder	7.5	24.8	38.4	58.5	81.3	42.1
Stem bark powder	1.0	36.1	61.6	78.4	87.5	54.7
Root bark powder	7.5	61.2	87.5	88.2	98.5	68.7
	P<0.05 LSD = 0.17					
A. muricata						
Leaf powder	7.5	50.0	61.2	83.7	97.1	59.9
Seed powder	10.0	38.4	50.0	61.2	89.8	49.9
Stem bark powder	10.0	47.0	64.1	83.7	89.4	58.8
Root bark powder	10.0	41.5	61.4	86.3	88.3	57.5
	P<0.05 LSD = 0.04					

Table 2. Effect of *Annona* powdered products on oviposition of *Callosobruchus maculatus*

Treatment	Treatment (g)					Mean
	0.0	**0.5**	**1.0**	**2.0**	**3.0**	
A. senegalensis						
Leaf powder	46.2	24.0	21.2	10.9	7.3	21.9
Seed powder	46.2	19.4	16.0	10.2	3.2	19.0
Stem bark powder	47.5	24.1	10.2	4.4	2.9	18.0
Root bark powder	47.1	21.2	8.4	6.3	2.0	17.0
	P>0.05					
	LSD = 0.17					
A. muricata						
Leaf powder	46.2	20.3	12.3	6.3	1.4	17.3
Seed powder	46.2	22.0	16.1	10.9	3.2	19.7
Stem bark powder	46.2	23.0	9.0	6.8	1.7	17.3
Root bark powder	46.2	22.1	10.0	5.8	1.4	17.1
	P>0.05					
	LSD = 0.17					

Table 3. Effect of *Annona* powdered products on F1 progeny emergence of *Callosobruchus maculatus*

Treatment	Treatment (g)					Mean
	0.0	**0.5**	**1.0**	**2.0**	**3.0**	
A. senegalensis						
Leaf powder	80.6	61.7	50.0	39.4	21.9	51.9
Seed powder	80.6	28.4	20.0	17.2	10.7	32.6
Stem bark powder	84.2	38.6	28.4	26.5	23.7	40.3
Root bark powder	84.9	27.4	23.8	15.9	10.9	30.4
	P>0.05					
	LSD = 0.04					
A. muricata						
Leaf powder	86.6	35.5	16.3	9.5	9.3	31.4
Seed powder	86.6	23.2	18.0	15.5	9.6	30.6
Stem bark powder	86.6	31.3	22.2	19.3	18.4	35.6
Root bark powder	86.6	33.9	29.2	24.1	20.8	38.9
	P>0.05					
	LSD = 0.06					

The insecticidal potentials of *Annona* plant species may be due to the constituents present in the plants [2]. The leaves of *Annona* spp contain the following: acetogenins, alkaloids, carbohydrates, essential oils, flavonoids, glycosides, phenolic compounds, proteins, saponins, sterols and tannins [18,4]. Annonaceous acetogenins have been reported to possess insecticidal activities [19,20].

Table 4. Effect of *Annona* powdered products in the protection of grain from damage by *Callosobruchus maculatus*

Treatment	Treatment concentration (g)					Mean
	0.0	**0.5**	**1.0**	**2.0**	**3.0**	
A. senegalensis						
Leaf powder	76.9	50.5	25.6	15.5	0.8	35.4
Seed powder	76.9	10.3	5.3	1.4	1.1	18.9
Stem bark powder	75.1	25.5	15.1	10.1	1.0	25.4
Root bark powder	80.2	15.6	6.5	1.1	1.4	2.0
	P>0.05 LSD = 0.03					
A. muricata						
Leaf powder	88.7	14.5	5.6	1.1	1.0	23.1
Seed powder	88.7	16.5	5.4	2.6	1.0	22.8
Stem bark powder	88.7	20.8	6.2	1.6	1.0	23.7
Root bark powder	88.7	18.5	12.1	4.2	0.7	24.8
	P>0.05 LSD = 0.03					

4. CONCLUSION

In conclusion, results from powders of *Annona muricata* and *Annona senegalensis* showed significant alterations on biology of *C. maculatus* demonstrating its potential insecticidal activity. Therefore, it is recommended that farmers should put the practice of using *Annona* species, especially, *A. muricata* as an alternative to the dangerous and highly persistent chemical insecticides to control *C. maculatus* on stored cowpea.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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