International Journal of Multidisciplinary Research and Growth Evaluation.

Chemical composition and evaluation of *Annona muricata* linnaeus leaf on *Callosobruchus maculatus* fabricius (Coleoptera: Chrysomelidae) infesting *Vigna unguiculata* seeds in the store

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Article Info

ISSN (online): 2582-7138
Volume: 06
Issue: 01
January-February 2025
Received: 10-12-2024
Accepted: 12-01-2025
Page No: 1749-1754

Abstract
A laboratory study was carried out on the chemical composition and evaluation of *Annona muricata* leaf extract on *Callosobruchus maculatus* infesting *Vigna unguiculata* seeds in the store. Air dried leaves of *Annona muricata* were pulverized into fine powder using an electric grinder (Model: Binatone electric grinder BL 400). The leaves were extracted with soxhlet extractor at 60^{o}C in the laboratory, using ethanol as the solvent. The effects of the extract was investigated on adult mortality, ovipostion, emergence of F1 progeny, germinability and seed damage under ambient laboratory conditions of 28 ^{o}C and 70% Relative Humidity. The results obtained showed that, adult mortality generally increased with increased in dosages applied and the length of time the weevils were exposed to the extracts. There was 100% mortality of *C. maculatus* in the cowpea seed treated with 0.9 mL/20 g and 1.2 mL/20 g of the extract within 96 h and 72 h respectively. Oviposition and adult emergence decreased with increased in the amount of dosages used. Oviposition and adult emergence were significantly lowered ($p \leq 0.05$) in the cowpea seeds treated with the extract than the untreated seeds (control). Oviposition was significantly low (9.20) and adult emergence were totally prevented in cowpea seeds with 0.9 mL/20g of *A. muricata* leaf extract, while oviposition and adult emergence were totally prevented in cowpea seeds treated with 1.2 mL/20g extract. Almost all the treated seeds germinated regardless of the dosages of the leaf extracts. The untreated cowpea seeds had the highest germination of 100%, followed by seed Treated with 0.3, 0.6 and 0.9 mL which are not significantly different ($p \leq 0.05$). *A. muricata* leaf extract at dosages of 0.6, 0.9, and 1.2 mL, completely prevented, seed damage and weight loss of the treated cowpea seeds. The qualitative phytochemical screening of *A. muricata showed the presence of glycoside, flavonoids, saponins and steroids, while alkaloid, phenols and tannins were not detected.* The results obtained from this study revealed that the extracts of *A. muricata* are effective in managing *C. maculatus* and could serve as an alternative to synthetic insecticides for the protection of stored cowpeas against weevils.

Keywords: Adult emergence, germinability Mortality, oviposition, phytochemical, plants extracts, synthetic insecticides

1. Introduction

Cowpea (*Vigna unguiculata*) has become increasingly popular as a food crop due to its high protein content (Cheng and Bruno 2013) [10]. Cowpea is an important commercial crop in many developing nations due to its high protein content, adaptability to many types of soil and intercropping systems, drought resistance, and ability to improve soil fertility and prevent erosion. On a dry weight basis, cowpea seed is found to contain 25% protein and numerous vitamins and minerals.

It can be consumed in a variety of ways and all parts of the cowpea crop are used since they have higher nutrients and fibre contents (Nkomo *et al.*, 2021) [24].

Various parts of cowpea plant serve as food/feed, such as forage, hay, and silage for livestock. It also provides green manure and cover crop which maintain the productivity of soils (Alemu *et al.*, 2016) [8]. As a leguminous plant, it compensates for the loss of nitrogen absorbed by cereals, thus, it has a positive impact by improving soil fertility. This is due to the ability of cowpea to fix atmospheric nitrogen and performs well even in poor soils (Rosenblueth *et al*., 2018) [33].

Production of cowpea faces enormous problems; notable among them is insect pest infestation. Post-harvest losses to storage insect pests limit cowpea production in Sub-Saharan Africa; a region which otherwise was responsible for about 70% of total world production (IITA, 2010) [15]. Cowpea seeds are damaged by insect pests in most cowpea producing nations, which lead to economic losses (Spochacz, *et al*., 2018) [35].

Callosobruchus maculatus infestations have been reported to cause substantial reduction in quality and quantity of cowpea seeds within three to five months of storage (Ileke *et al*., 2012) [16]. It has been recognized that postharvest loss to storage coleopterans pests such as *C. maculatus* is a major constraint to food security in developing nations such as Nigeria (Udo, 2011) [39].

It has become necessary to protect agricultural products from pests and diseases in order to meet the world's demand for food. Management of insect pests to secure agricultural products has been found to be of utmost importance all over the world so as to achieve continuous and safe food supply (Abd and Salam, 2010) [1].

It has been reported by several researchers that farmers used too much synthetic chemical insecticides in other to control many insect pests to protect our crops and to produce high quality food (Adedire *et al.*, 2011; Ileke, 2012; Adekunle *et al*., 2017) [3, 16, 4]. Synthetic chemical insecticides have a high purchasing cost, risks to human health, environmental pollution and leads to development of a new resistance of pests (Thiaw and Sembène, 2010) [36]. Currently, synthetic chemical insecticides is the major means of managing beetles infestations in stored cowpea seeds (Onekutu *et al*., 2015) [26]. However, consequent upon reported ozone depletion by methyl bromide and carcinogenic concerns with phosphine, conventional fumigation technology is under scrutiny in the developed countries (Adedire *et al*., 2011; Ileke *et al*., 2012) [3, 16].

One possible alternative to overcome the shortcomings of synthetic insecticides is to substitute it with naturally-occurring plant insecticidal materials (Ileke *et al.*, 2012; Khater, 2012) [16, 19]. Laterza (2024) [21] defined botanicals as substances derived from naturally occurring materials (i.e., plants, microorganisms and minerals) characterized by low environmental effects, rapid degradation, and low toxicity for humans and beneficial insects.

Plant extracts contain biologically active compounds and hence has been the matter of interest for nearly sixty years ago (Jibrin *et al.*, 2013) [18]. The chemicals in plants are the major subject of interest due to the fact that their large-scale synthesis and production for commercial use is not yet well achieved. This commercialization can only be achieved when immense knowledge of the phytochemical components and their effects on the stored product as well as human health is acquired.

Plant extracts and plant dried- powders that have insecticidal potential and pose little or no threat to the ecosystem and the health of users have been locally employed with varying effectiveness in the management of crop pests. Botanicals in this category are medicinal plants, such as neem oil, wood ash, lemon grass, ginger and garlic among others (Prowse *et al*., 2006) [29]. Botanicals are non-toxic to mammals; and they do not persist because they rapidly breakdown and are metabolized easily by animals receiving sub-lethal doses (Ling, 2003) [22].

Botanical pesticides are however, less problematic and may give the desired results. Thus, the interest of this research is to determine the phytochemicals and investigate the insecticidal activities of the leaves of soursop, *Annona muricata* leaves against *C. maculatus*

2. Materials and methods

2.1 Collection and preparation of *Annona muricata* plants leaf

The leaves of Soursop, *Annona muricata* were collected from a farm in Olorunsogo Community, Ado Ekiti, Ekiti State, Nigeria. The leaves were washed in clean water and allowed to drain. The drained leaves were spread in a tray and air-dried for 20 days in the Laboratory. After 20 days, they were ground into powder, using a Binatone electric grinder. The powder was divided into portions and stored separately in specimen bottles until requires for the experiment.

2.2 Preparation of leaf extracts of *A. muricata*

A portion of the leaf powder (50 g) was measured into a beaker and packed into thimbles and extracted with 250 mL of 70% alcohol in a Soxhlet apparatus at 60 ^{o}C. The leaf extract was concentrated using rotary evaporator. The resulting extract was air-dried to remove traces of the solvent. The extract was poured into a specimen bottle and stored in a refrigerator until needed for the experiment.

2.3 Rearing of *Callosobruchus maculatus*

Parent stock of *C. maculatus* used for this study was obtained from naturally infested cowpea seeds bought at Mojere Market in Adebayo, Ado Ekiti, Nigeria. The weevils were reared on cowpea seeds in a transparent plastic container covered with muslin cloth held in place with rubber band to allow gaseous exchange at 28 ^{o}C and 70% Relative humidity. The muslin cloth allowed ventilation of the grains and also prevented entry and exit of weevils and other insects or pests, such as rats and reptiles. New First filial generation of *C. maculatus* was raised from the stock.

2.4 Effects of *A. muricata* leaf extract on mortality of *C. maculatus*

Twenty grams (20 g) of cowpea seed was measured into Petri dishes. The leaf extract of *A. muricata* was applied to the different Petri dishes containing the 20 g of the cowpea seeds at the doses of 0.3, 0.6, 0.9 and 1.2 mL. the cowpea seeds and the extracts were thoroughly mixed with a glass rod to enhance uniform coating of the extracts on the grains surfaces. The control experiments were set up but the cowpea seeds in the controls were not treated with extracts. Twenty (20) adult *C. maculatus* that newly emerged (0- 24 h old) were introduced into each of the Petri dishes containing treated seeds (0.3, 0.6, 0.9 and 1.2 mL leaf extract). Each treatment and the control were replicated four times. A

Complete Randomized experimental Design was adopted for the experiment. The Petri dishes were covered with Petri Plates. Thereafter, the experiments were allowed to stay for 96 h during which the number of dead insects were counted and recorded at 24 h interval. This was done by gently probing the insect with a sharp pin on the abdomen. Insect that did not respond to the probe were considered dead.

2.5 Effects of *A. muricata* leaf extracts on oviposition and adult emergence of *C. maculatus.* Twenty grams (20 g) of cowpea seed was measured into Petri dishes. The leaf extract of *A. muricata* was applied to the different Petri dishes containing the 20 g of cowpea seeds at the doses of 0.3, 0.6, 0.9 and 1.2 mL. The cowpea seeds and the leaf extract were thoroughly mixed with a glass rod enhance uniform coating of the extracts and the seeds. The control experiment was without any extract. Two males and two female adult *C. maculatus* that were newly emerged (0- 24 h old) were introduced into each of the Petri dishes containing 0.3, 0.6, 0.9 and 1.2 mL dosages of leaf extracts and covered with Petri plates. Each treatment and the control were replicated four times. A Complete Randomized experimental Design was adopted for the experiment. The experiment was left for 7 days after which both dead and live insects were removed and the number of eggs laid were counted and recorded. Thereafter, the experiment was left in the laboratory until adult weevils started emerging. The number of emerged weevils were also counted and recorded.

2.6 Effect of *A. muracata* leaf extract on the germination of cowpea seeds

Clean and wholesome cowpea seeds were sorted out by removing the shaft and disinfested by putting them in a deep freezer for 72 h. Afterward the seeds were removed and air-dried for 1 h in the laboratory. Twenty grams (20 g) of the seeds were weighed into Petri-dishes and 0.3, 0.6, 0.9 and 1.2 mL of *A. muricata* leaf extract were added and thoroughly mixed with the aid of a glass rod in order to enhance uniform coating. They were left for 1 h to air dry and then covered with Petri plates to prevent weevil infestation and allow for ventilation. Four replicates were prepared. The control experiment consisted of samples that were not treated with any of the extracts. Both the treated and control experimental set ups were left in a wooden cage in the laboratory for 90 days. Afterward, the germination experiment was performed by picking 20 seeds at random and germinating it on a moistened filter paper in Petri dishes. This was done for all the dosages (0.3, 0.6, 0.9 and 1.2 mL). After seven days of germination, the number of germinated seeds were counted and converted to percentage.

2.7 Effect of *A. muricata* leaf extract on seed damage

Fifty grams (50 g) of clean wholesome cowpea seeds were measured into transparent plastic cups and mixed with 0.3, 0.6, 0.9 and 1.2 mL of *A. muricata* leaf extract. They were air-dried for 1 hour. Afterward, 10 pairs of adult *C. maculatus* were introduced into each plastic cup. A control treatment without any extract was set-up. Each treatment was replicated four times. The plastic containers were covered with muslin cloth held tightly in place by rubber bands and kept in a wooden cage in the laboratory. After 90 days, each replicate was assessed for seed damage and weight loss. Percentage seed damage was determined thus:

$$\% \text{ damage} = \frac{\text{No of seeds damaged x 100}}{\text{Total no of seeds}}$$

Seed damage was also assessed after 90 days using the weevil perforation index (WPI) as described by Fatope *et al*. (1995) [13]. WPI value exceeding 50 was regarded as enhancement of infestation by the weevil or negative protectant ability of the extract tested.

2.8 Qualitative phytochemical screening of *A. muricata* leaf

The phytochemical analysis was carried out using the method of Trease and Evans (1989) [38].

2.8.1 Test for alkaloids

Mayer's test: One milliliter (1 mL) of the extract was taken and placed into a test tube. Then, One milliliter (1 mL) of potassium mercuric iodide solution (Mayer's reagent) was added and shaken. The emergence of whitish or cream precipitate implies the presence of alkaloids.

2.8.2 Test for glycosides

Legals test: One milliliter (1 mL) of an extract was taken, and then an equal volume of Sodium nitroprusside was added followed by a few quantities of sodium hydroxide solution and shaken. The formation of pink-to-blood red precipitate signifies the existence of cardiac glycoside.

2.8.3 Test for steroids

Liebermann Burchard's test: The extract was dried out first through evaporation and extracted again with chloroform. Few drops of acetic anhydrides were added, followed by H_2SO_4 (sulphuric acid) from the side of the test tube. The formation of violet to blue-colored ring at the junction of the two liquids indicated the presence of steroids.

2.8.4 Test for Tannins

Gold Beater's skin test: A Gold Beater's Skin was obtained from Ox skin. The Gold Beater's Skin was soaked in 2% hydrochloric acid and washed with distilled water. Then, it was placed in a solution of an extract for 5minutes and washed with distilled water. Finally, it was placed in 1% ferrous sulfate solution. If the Gold Beater's Skin changes to brown or black tannins are present.

2.8.5 Test for flavonoids

Alkaline reagent test: One milliliter (1 mL) of the leaf extract was taken and placed into a test tube. Then few drops of sodium hydroxide solution were added and shaken. The emergence of intense yellow color that turns to colorless after adding dilute acid implies the existence of flavonoids.

2.8.6 Test for phenols

Ferric chloride test: One milliliter (1 mL) solution of the leaf extract was taken and placed into a test tube. Then, 1% gelatin
solution containing sodium chloride was added and shaken. The formation of bluish-black color indicated the presence of phenols.

2.8.7 Test for Saponins: The presence of saponin was determined using the methods stated below.

Libermann Test (Foam Test): When stable, characteristic honeycomb-like froth was obtained. This showed the

presence of saponins.

2.9 Data Analysis

The data obtained in this study were subjected to analysis of variance (ANOVA) and where significant differences existed, treated means were separated, using the New Duncan's Multiple Range Test.

3. Results

1. Effect of *A. muricata* leaf extracts on mortality of *C. maculatus*

Mortality of *C. maculatus* increased with increased in dosage levels of the leaf extracts of *A. muricaa* (Table 1). Mortality in extract-treated seeds is significantly higher (P≤ 0.05) than that of the control experiment. Complete mortality (100%) of adult *C. maculatus* was recorded when exposed to 0.9 mL of the extracts within 96 h post treatment. Also, 100% mortality was recorded when exposed the highest dosage of 1.2 mL extract within 72 h.

Table 1: Mortality of *C maculatus* exposed to leaf extracts of *A. muricata* for 96 hours

	% at hours	Post	Treatment	
Dosage (mL)	24 h	48 h	72 h	96 h
0.3	42.10±2.15^{d}	49.10±2.33^{d}	58.50±2.73^{d}	68.21±2.84^{c}
0.6	53.25±1.91^{c}	61.15±2.16^{c}	74.25±3.43c	85.30±4.14^{b}
0.9	62.50±2.26^{b}	71.48±3.19^{b}	83.25±3.72^{b}	100.00±0.00^{a}
1.2	76.25±2.42^{a}	89.15±3.26^{a}	100.00±2.87^{a}	100.00±0.05^{a}
Control	0.000±0.00^{e}	0.000±0.00^{e}	0.000±0.00^{e}	0.000±0.00^{d}

Means within the same column followed by the same letter(s) are not significantly different (P ≤ 0.05) using New Duncan`s Multiple Range Test.

4.2 Effect of *A. muricata* leaf extracts on oviposition and adult emergence of C. *maculatus*

All the leaf extracts of *A. muricata* significantly (p≤ 0.05) reduced the number of eggs laid by the cowpea weevil compared to the untreated seeds (Table 2). Oviposition and adult emergence decreased with increased in dosage levels of the leaf extracts. Oviposition was totally suppressed when exposed to 1.2 mL of the extract, there was no adult emergence.

Table 2: Effect of *A. muricata* leaf extract on oviposition and adult emergence by cowpea weevil

Dosage (mL)	Number of eggs laid	% of eggs hatched
0.3	32.26±1.93^{b}	32.25±1.28^{b}
0.6	22.15±1.66^{c}	18.10±1.11^{c}
0.9	9.20±0.18^{d}	0.00±0.00^{d}
1.2	0.00±0.00^{e}	0.00±0.00^{e}
Control	67.15±2.13^{a}	88.25±4.23^{a}

Means within the same column followed by the same letter(s) are not significantly different (P ≤ 0.05) using New Duncan`s Multiple Range Test

4.3 Effect of *A. muricata* leaf extracts on grain viability

Percentage germination of all treated seeds after 7th day was generally high (Table 3). Almost all the treated seeds germinated regardless of the leaf extract dosages. The untreated cowpea seeds had the highest germination of 100%, followed by seeds treated with 0.3, 0.6 and 0.9 mL which are not significantly different (p ≤ 0.05). Seeds treated with 1.2 mL dosage level had the lowest percentage germination of 92.15%.

Table 3: Percentage germination cowpea seeds that were previously protected for 90 days

Dosage (mL)	Percentage germination
0.3	96.50±3.32^{b}
0.6	94. 25±2.22^{b}
0.9	94.56±2.18^{b}
1.2	92.15±3.07^{c}
Untreated	100.00±0. 00^{a}

Means within the same column followed by the same letter(s) are not significantly different (p≤ 0.05) using New Duncan`s Multiple Range Test

4.4 Protectant ability of *A. muricata* leaf extract on cowpea seeds after 90 days of storage

A. muricata leaf extract of dosages level 0.6, 0.9, 1.2 mL, completely prevented infestation and damage of the treated cowpea seeds (Table 4). There was neither seed damage nor weight loss recorded in the treated cowpea seeds and WPI was zero excepts in seeds treated with 0.3 mL which had WPI of 12.38. However, the WPI of the treated seeds were significantly different from WPI of the control. In the untreated cowpea seeds, 68.50% damage occurred as revealed by emergent holes of the weevils. The weight of the untreated seeds was significantly higher than the treated seeds.

Table 4: Effect of cashew kernel oil on long term storage of cowpea seed

Dosage (mL)	Mean total number of seeds	Percentage seed damage	Mean weight loss (g)	Weevil Perforation Index (WPI)
0.3	190.50	8.25±0.42^{b}	5.50±0.21^{b}	12.38±0.21^{b}
0.6	192. 25	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}
0.9	189. 00	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}
1.2	190.00	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}
Untreated	191. 25	68.50±2.34^{a}	26.15±1.13^{a}	50.25±1.33^{a}

Means within the same column followed by the same letter(s) are not significantly different (P ≤ 0.05) using New Duncan`s Multiple Range Test

4.5 Qualitative Phytochemical composition of Ethanol leaf extracts of *A. muricata*

The qualitative phytochemical screening of A. muricata conducted revealed the presence of Glycoside, Flavonoids, Saponins and Steroids, while Alkaloid, Phenols and Tannins were not detected.

Table 5: Qualitative Phytochemical composition of Ethanol leaf extracts of *A. muricate*

Phytochemical	Composition
Alkaloids	-
Glycoside	+
Flavonoids	+
Phenols	-
Saponins	+
Tannins	-
Steroids	+

+ = Present, - = Not detected

Discussion

The insecticidal activity of *A. muricata* leaf extract on cowpea weevil, *C. maculatus* was investigated. The results of this study have revealed the efficacy of *A. muricata* plant leaf extracts on *C. maculatus. A. muricata* had high efficacy in the management of the weevils due to it insecticidal effects on weevil mortality, ovipostion and adults emergence. This finding corroborates the finding of Lala *et al*., (2014) [20] WHO reported that the aqueous and oil extracts of *Annona squamosa* and *Annona muricata* were effective against *Aedes albopictus* and *Culex quinquefasciatus* at varying levels of application. The ability of the extract to cause high mortality of the insects, low oviposition rate and low adult emergence varied with the dosages of the extract used. This corroborates the study conducted by Ajayi *et al.* (2018) [5] which investigated the combined toxicity of the extracts of *M. oleifera* and *Z. officinale* on cowpea seeds pest, *C. chinensis.* It was also established by Riser (1996) [32] that *Annona muricata* plant extracts were effective in the management of field insect pests of cowpea. Similarly Ishuwa *et al*. (2016) [17] also reported that *A. muricata* was very effective against *Callosobruchus maculatus* on stored cowpea. This result also supports the findings of Padma *et al*. (1998) [27] who stated that *Annona muricata*-based products were more effective than synthetic insecticides in the control of different orders of insect pests. According to Lala *et al.* (2014) [20], extracts of *A. muricata* and *A. squamsa* contain alkaloids and flavonoid compounds that perhaps confer their biological insecticidal proprieties. The insecticidal effect of the plants extracts on *C. maculatus* in the treated cowpea seeds might be as a result of contact toxicity. Most insects carry out gaseous exchange by means of trachea which usually open at the surface of the body through spiracles. There is a likelihood that the extracts that were mixed with the seeds blocked the spiracles thereby leading to suffocation and death of the insects (Rahman and Talukder, 2006; Adedire *et al*., 2011) [30, 3]. Indeed, the extracts act by contact toxicity (Nuto, 1995) [25] and the bioactivity of the leaves extracts on the mortality of the insects seems to be due to the presence of chemical compounds having insecticidal, oviposition inhibiting, fecundity, and fertility effects on the insects. The fact that the plant extracts induces reduction of oviposition by female *C. maculatus* and mortality of the developmental stages had been reported by a number of authors and has been well documented (Boukar *et al.*, 2018) [9]. The effect of the extracts on oviposition in the present study could be linked with respiratory impairment, which probably affects the process of metabolism and consequently other systems of the body of the bruchids (Adedire *et al*., 2011) [3]. The plants extracts probably inhibited locomotion, hence impeding free locomotion of the weevils, thereby affecting mating activities and fecundity. The inability of the eggs to stick to the treated cowpea seeds due to the presence of the extracts may also reduce survival after adult emergence.

Some plant oils and extracts have been tested for long-term protectant ability on seeds and grains with positive results. It was reported by Pereira (1983) [28] and Shaaya *et al.* (1997) [34] that oils extracted from crude palm kernel and rice bran at the rate of 1.5 g and 3 g kg–1 cowpea seeds offered full protection from *C. maculatus* for a period of 4 to 5 months. The insecticidal activity of *A. muricata* extract could be linked to the presence of secondary plant compounds (Rehm & Espig 1991) [31] which had been implicated in their immu-nomodulatory, haemolytic, allelopathic and insecticidal activities (Echendu 1991, Golob *et al.* 1999) [12. 14].

Conclusion

The extract of *A. muricata* used in this work has proven insecticidal properties against cowpea beetle, *C. maculatus*. Nevertheless, the insecticidal potential of this plant extract is depended on the dosages and the period of application. The result showed that the extract had contact toxicity effects on mortality, oviposition and adult emergence of the weevil, hence, could serve as alternative to the chemical insecticides used in controlling the insect pest.

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