

# Anacardium Occidentale and Jatropha Curcas Seeds' Alkaloids: Their Feeding Inhibitory and Growth Retardatory Potentials on Rhyzopertha Dominica

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**Abstract**— The damaging effect of *Rhyzopertha dominica* larvae and adult insects on the nutritive content and economic value of *Zea mais* seeds, and the cytotoxic nature of most phyto-alkaloids prompted this research work to be carried out. *A. occidentale* and *J. curcas* seeds' alkaloids were extracted and experimented for feeding inhibition and growth retardation on callow and mature adult *R. dominica* (Lesser grain borer) using maize seeds. Feeding inhibition for *A. occidentale* and *J. curcas* seeds' alkaloids on adult insects were 39.40%, 49.88%, 81.37%, 100%, 47.48%, 55.12%, 93.31%, 100% respectively at 50 µg/mL, 150 µg/mL, 300 µg/mL and 400 µg/mL concentrations. Alkaloids from *Jatropha curcas* seeds demonstrated higher feeding inhibitory potentials than alkaloids from *Anacardium occidentale* seeds. Relative Growth Rates (RGR) of callow and mature *R. dominica* were retarded by *A. occidentale* and *J. curcas* seeds' alkaloid treatments. The feeding inhibitory potentials of *A. occidentale* and *J. curcas* seeds' alkaloids at 400 µg/mL concentration on both the callow and mature adult insects were translated to growth retardation of the insects when compared to maize seeds without alkaloid treatment (controls) fed by the insects. Relative Consumption Rates (RCR) of maize by callow and mature *R. dominica* were also reduced for maize seeds treated with alkaloids, indicating growth is a function of consumption. Thus alkaloids from *Anacardium occidentale* and *Jatropha curcas* seeds demonstrated feeding inhibitory and growth retardatory potentials on callow and mature adult *R. dominica*.

**Keywords**— Alkaloids, *Anacardium occidentale*, Consumption, Extraction, Feeding Inhibition, Growth, *Jatropha curcas*, *Rhyzopertha dominica*.

## I. INTRODUCTION

### 1.1 *Rhyzopertha dominica* and its feeding behavior

*Rhyzopertha dominica*, also known as lesser or stored grain borer is in a monotypic genus of beetle, *Rhyzopertha* in the Bostrichidae family. It is a black-brown, slim, cylindrical, small insect of three millimeters length with shape similar to a bullet. Its head is attached to its thorax by a rounded neck shield covered with pits that get smaller toward the posterior end. A ten-segmented antenna is clubbed with last three segments forming a loose club. Each female lays up to five-

hundred eggs either on kernels of grain or loosely in frass produced by the insects. Egg development takes five days at 36 °C and hatches into a larva which is white in color and c-shaped. Larval development often takes twenty seven to thirty one days at 28 °C and forty six days at 25 °C respectively. Pupation takes approximately five to six days at 28 °C and eight days at 25 °C respectively in an enlarged cell where the larval feeding tube ends, and the adult insect can live up to two hundred and forty days (Toews *et al.*, 2006; Koehler *et al.*, 2011; Mason, 2003).

Infestation of grains by *Rhyzopertha dominica* larvae and adults originate from either residual population inside storage structures or immigration from outside sources, and insects have been shown to enter grain silos and other storages through vents and poorly sealed silo bases (Jia *et al.*, 2008). Both the larvae and mature adult insects are voracious feeders that cause severe damage to chick peas, dried potatoes, herbs, wood, wheat, maize, rice, millet, peanuts, cocoa beans as well as processed products such as macaroni, tobacco and dried spices (Toews *et al.*, 2006; Doug, 2009). They feed on the endosperm of grains by chewing their way through the bran layer, reducing grains to shells of bran, leaving behind large amounts of flour, tunnels, irregular shaped holes, and a sweet odor in grains as signs of infestation. Interestingly, the sweet odor produced by male *Rhyzopertha dominica* contains pheromones which have shown effectiveness as lures for use in insect traps. On a contrary, the larvae and adult insects do not infest standing grain and can survive outside the grain environment on seeds and acorns of other plants (Koehler *et al.*, 2011; Doug, 2009).

### 1.2 *Anacardium occidentale*

*Anacardium occidentale* (Cashew) plant, a member of the Anacardiaceae family is a small tree having coriaceous leaves, polygamous flowers, with five petals and nine stamens. Fruits are fleshy, dark yellow or orange coloured (Ghodgate *et al.*, 2013). Cashew nut shell, a honeycomb structure contains a natural resin, cashew nut shell liquid (CNSL) which is used as a raw material to develop drugs, antioxidants, fungicides etc., and for treatment of wood against termites. Medicinal properties of phytochemicals present in CNSL have been reported, and include cytotoxic activity against several tumor lines, anti-diabetic, anti-inflammatory and analgesic effects (Kanan *et al.*, 2009). Cashew nut seeds are ground into paste to treat snake bites. Fruits, stem-bark and leaves have anti-fungal, antipyretic and antidiarrheal applications. They are used to treat sores and rashes. Leaf extracts have demonstrated growth inhibition against several species of bacteria and fungi (Azam-Ali and Judge, 2004; Akash *et al.*, 2009).

### 1.3 *Jatropha curcas*

*Jatropha curcas* plant, a member of the Euphorbiaceae family is a small drought resistant tropical tree or shrub which exudes whitish coloured, watery latex when cut. Normally, it grows between three and five meters high, but can attain a height of up to eight or ten meters under favorable conditions. It has large green to pale-green leaves which are alternate with a spiral phyllotaxis. Petiole length ranges between 6-23 mm,

and flowers are formed terminally and individually, with female flowers usually slightly larger and occur in the hot seasons. The plant is often used for fences or as hedges by locals especially farmers because it is not fed on by most animals and insects. Its latex contains an alkaloid known as "Jatrophine" which is believed to have anti-cancerous properties. It is applied externally as treatment for skin diseases, rheumatism, and sores on domestic livestock. Tender twigs of the plant are used for cleaning teeth, while juice of the leaves is externally applied against piles (Gupta *et al.*, 2011). Its roots are used as an antidote for snake-bites. Seeds of this plant have been used traditionally to treat ailments like burns, convulsions, fever and inflammation. Seed oil is used externally for the treatment of sciatica, dropsy and paralysis. It has a strong purgative action, and has been reported to be a remedy for syphilis. Also, seed oil is known to be useful as an insecticide, for soap production and as a fuel substitute to produce biodiesel via transesterification. Explored chemical constituents of *Jatropha curcas* includes diterpenes, triterpenes, lignanes, coumarines, flavonoids, phytosterols (Demissie *et al.*, 2013; Prasad *et al.*, 2012; Ikbāl, 2009).

## II. MATERIALS AND METHODS

### 2.1 Sample collection and authentication

Fruits of *Anacardium occidentale* and *Jatropha curcas* were randomly harvested from trees in Kintampo and Navrongo respectively in the Brong Ahafo and Upper East Regions of Ghana. Plants were authenticated at the Department of Applied Biology, University for Development Studies, Ghana.

### 2.2 Preparation of plants' seed samples

Fruits of the plants were dried under shade for about a week at 25 °C. Shells were removed and seed contents were dried under shade for 72 hours, and then ground into fine powder using a blender. The powders were stored in containers and later subjected to alkaloid extraction process.

### 2.3 Alkaloid extraction

Powdered seeds (50 g) of *Anacardium occidentale* and *Jatropha curcas* were separately soaked in 200 mL 95% ethanol solvent for three days and then filtered. Residues obtained were mixed with 1M HCl and allowed to stand for 1 hour and then filtered. Ethanol solvent was evaporated from crude extracts in a water bath at 45°C leaving the concentrates. Concentrates were combined with HCl filtrates and mixed

with excess dilute 1M HCl. Acid mixtures were basified by adding equal amounts of dilute 5M Na<sub>2</sub>CO<sub>3</sub> until swirls of white precipitates which were alkaloids clouded the solution. Next, an equal volume of n-hexane solvent was added to the mixture, poured in to a separating funnel and shaken. It was then allowed to stand overnight for the separation to take place. This step was repeated twice to get as much alkaloids as possible into the n-hexane solvent. When the separation was over, the n-hexane solvent was evaporated in a water bath leaving the concentrated alkaloids.

## 2.4 Test for crude alkaloids

Few drops of Wagner's reagent were added to 1mL solution of alkaloids, and brownish red precipitates indicated the presence of alkaloids.

## 2.5 Insect rearing

*Ryzopertha. dominica* insects (Callow adults of 21 days old and mature adults of 26 days old) reared in aerated containers at an average temperature of 32°C and relative humidity of 75%, in light and dark conditions of 12:12 hours daily, and fed with maize grains at Savannah Agricultural Research Institute (SARI), Nyankpala in the Northern Region of Ghana were used for the experiment.

## 2.6 Feeding inhibition test

*R. dominica* insects were kept in aerated containers with each container having 6 insects. Containers with distilled water treated maize grains served as controls (three each for callow and mature adult insects), while those with alkaloids treated maize grains in distilled water solvent served as treatments. Alkaloids were tested in four different concentrations (50, 150, 300 and 400 µg/mL) on callow and mature adult insects, with each concentration in triplicate. Maize grains were soaked in these concentrations for 20 seconds and dried under shade for 24 hours. Insects in treatment containers fed on alkaloids treated maize while those in controls fed on distilled water treated maize. Weights of food consumed within 24 hours were recorded for six days, and the formula below was used to estimate feeding inhibition index of alkaloid extracts:

- Feeding Inhibition Index (FII) =  $\frac{C-T}{C+T} \times 100$ , where;

C = amount of distilled water treated maize grains eaten

T = amount of crude alkaloids treated maize grains eaten

Relative consumption rate was calculated as weight of food eaten divided by initial weight of insect.

- Relative Consumption Rate =  $\frac{Wc}{W}$ , where;

Wc = weight of maize grains eaten.

W = weight of insect at the beginning of the experiment.

Relative growth rate was calculated as the difference in weight of the insects between the last day and initial day divided by the initial weight of the insect *R. dominica*.

- Relative Growth Rate (RGR) =  $\frac{A-B}{B}$ , where;

A = weight of live insects on 6<sup>th</sup> day

B = initial weight of insects.

## III. RESULTS

Table 1: Results for presence and % yield of alkaloids

Seed Alkaloids	Inferences	% yield
<i>A. occidentale</i>	+	10.34
<i>J. curcas</i>	+	13.23

Key: (+) = present: (−) = absent

Table 2: Feeding inhibitory indices of *A. occidentale* seed alkaloids on mature and callow adult *R. dominica*

Concentration (µg/mL)	% FII		Average % FII
	Mature adults	Callow adults	
50	7.83	70.97	39.40
150	16.99	82.76	49.88
300	62.73	100.00	81.37
400	100.00	100.00	100.00

Key: FII= Feeding Inhibitory Indices (Expresses feeding inhibition potential)

Table 3: Feeding inhibitory indices of *J. curcas* seed alkaloids on mature and callow adult *R. dominica*

Concentration (µg/mL)	% FII		Average % FII
	Mature adults	Callow adults	
50	37.88	56.98	47.43
150	47.60	62.63	55.12
300	86.62	100.00	93.31
400	100.00	100.00	100.00

Key: FII= Feeding Inhibitory Index (Expresses feeding inhibition potential)

Table 4: Effects of *A. occidentale* and *J. curcas* seed alkaloids on Relative Growth and Consumption Rates of *R. dominica* at 400 µg/mL

Parameter	<i>J. curcas</i>		<i>A. occidentale</i>	
	Mature adults	Callow adults	Mature adults	Callow adults
Control (RGR)	0.46	0.21	0.44	0.26
RGR	-0.28	-0.13	-0.18	-0.13
Control (RCR)	48.26	88.71	24.43	72.14
RCR	24.95	70.70	14.29	44.20

Key: RGR= Relative Growth Rate, RCG= Relative Consumption Rate

#### IV. DISCUSSION

Alkaloids were found present in the seeds of *Anacardium occidentale* and *Jatropha curcas* respectively (table 1). Feeding inhibitory potentials (% FII) for *A. occidentale* and *J. curcas* seeds' alkaloids on callow and mature adult *R. dominica* were concentration dependent, and increased as alkaloids' concentrations were increased. The alkaloids exhibited 100 % feeding inhibition on the callow and mature adult insects at 300µg/mL and 400 µg/mL respectively, implying the alkaloids were more effective feeding inhibitors on the callow adult insects than mature adults (tables 2 and 3). This is in line with documented reports that the mature adults are more voracious feeders and resistant to pesticides than the callow adults (Koehler et al., 2011; Doug, 2009). % FII of *A. occidentale* seed' alkaloids on the callow adult *R. dominica* (P-value = 0.0140914) was higher than that on the mature adult *R. dominica* (P-value = 0.024513). % FII of *J. curcas* seed' alkaloids on callow adult *R. dominica* (P-value = 0.002212 ) was also higher than that on the mature adult *R. dominica* (P-value = 0.007682). Thus the *J. curcas* seed' alkaloids had slightly higher feeding inhibitory indices compared to *A. occidentale* seed' alkaloids (tables 2 and 3). Growth rates of callow and mature adult insects that fed on maize treated with alkaloids were retarded, and consumption rates of maize reduced when compared with those of the controls (table 4). Hence the growth of callow and mature adult *R. dominica* is a function of consumption of maize. Prasad et al., 2012 reported that alkaloids in the seed, leaf and stem-bark extracts of *J. curcas* plant possessed antifeedant

properties. Praveena et al., 2012 have also reported the antifeedant activity of *A. occidentale* seed oil to be concentration dependent.

#### V. CONCLUSION

Alkaloids from *Jatropha curcas* seeds exhibited slightly higher feeding inhibitory potentials than alkaloids from *Anacardium occidentale* seeds, which were later translated to slightly lower growth rates of both callow and mature adult insects at 400 µg/mL concentration. Therefore the *Anacardium occidentale* and *Jatropha curcas* seeds' alkaloids possess feeding inhibitory and growth retardatory potentials.

#### RECOMMENDATION

Further research works should be conducted to isolate specific alkaloidal compounds responsible for the feeding inhibitory and growth retardatory potentials of *Anacardium occidentale* and *Jatropha curcas* seeds' alkaloids on callow and mature adult *R. domiica*. Investigating these alkaloids to prove their non-toxic nature to mammalian cells will create an avenue for usage as protectants against *Rhyzopertha dominica* infestation of maize.

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