

# Biopesticidal Effect of Partitioned Extracts of *Zanthoxylum zanthoxyloides* (Lam.) Zepernick & Timler on *Callosobruchus maculatus* (Fab.)

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## Abstract

Botanicals have become the focus for discovery of novel bioinsecticides for protection of crops most especially because of their biodegradability, non-toxicity, target organism specificity and environmental friendliness. Partitioned extracts of *Zanthoxylum zanthoxyloides* were investigated for biopesticidal effect on *Callosobruchus maculatus* with aim of identifying the most active components and increase ease of handling when compared to bulk crude plant material used by farmers. Crude powder of rootbark of *Z. zanthoxyloides* was made as well as partitioned extracts using Kupchan partition extraction methods with methanol, acetone, ethyl acetate and n-hexane as solvents. Analysis of the rootbark of the plant showed 19.87% ash content, 24% crude protein, 24.85% crude fibre, 3.13% oil extract, 15.42mg/100g alkaloids, 45.90mg/100g tannins, 1039.14mg/100g saponins, 26.47mg/100g flavonoids, 150.0mg/100g iron, 244.70mg/100g calcium, 36.65mg/100g magnesium, 449.00mg/100g potassium and 128.30mg/100g of phosphorus. The crude powder and

extracts were bioassayed on *C. maculatus* in stored cowpea. Insecticidal activities of the partitioned extracts increased with increasing concentration; toxicity test revealed that n-hexane at 1%, 2% and 3%, acetone extract at 3%, and crude powder at 1.5g all have same effect on the insect as the positive control, achieving 100% mortality at 24 hours post treatment, number of adult emergence of 63.57 was recorded with 1% methanol extract against 28 in 3% of the extract. There was a significant difference in weight loss by the cowpea in the different fractional treatments while positive and negative controls also differed significantly ( $P>0.05$ ) with all the partitioned treatments. Partition extracts from *Z. zanthoxyloides* were more bioactive than the crude powder, and n-hexane fractions contain the most active compounds against *C. maculatus*. n-hexane extract contain most active biomolecules to be explored for bioinsecticide formulation with high potency for development of new biopesticide, eliminate toxic components and increase ease of handling when compared to bulk crude plant material used by farmers.

**Keywords:** *Zanthoxylum zanthoxyloides*, *Callosobruchus maculatus*, toxicity, partitioned extracts, insecticidal activity

## 1. Introduction

One of the greatest challenges facing the human race today is food insecurity. With the world population estimate of 8 billion today and projected to reach 10 billion persons in the year 2055 and 11 billion in the year 2088 (UN, 2017), the need to double stable food production for the growing populace becomes paramount. In Africa 257 million people (20%) are hungry and chronically undernourished and 28 African countries including Nigeria depends on food aid (Statista, 2020). One of the stable food crops and a major source of protein intake consumed world over and especially in Nigeria is cowpea (*Vigna unguiculata* (L) Walp.) (Leguminosae). Cowpea is the most important proteinous legume in West Africa responsible for between 60-80% protein intakes of its inhabitants; providing the only source of daily protein intake for a great number that cannot afford animal protein (Rajashekar *et al.*, 2016; Mobolade *et al.*, 2019). Annual production of cowpea (worldwide) is put at 7.4 million tonnes, with Nigeria accounting for 46%, valued at US\$ 633,956,000 (Xiong *et al.*, 2016; FAOSTAT, 2017; IITA, 2017). It is highly nutritive with 22% protein, 1.5% fat and 60% carbohydrate, and with protein digestibility higher than that of other legumes (Jayathilake *et al.*, 2018).

The major constraint to availability of this crop and stable food for Nigerians is the menace of insect pests (about 130 species associated with cowpea production); the most important pest of cowpea is *Callosobruchus maculatus* (Fab.) (Coleoptera: Chrysomelidae) and is responsible for up to 100% damage to cowpea in storage (Xiong *et al.*, 2016). Globally, insect damage to stored products range from 5-10% in temperate climes and 20-30% in tropical zones (Ashamo *et al.*, 2018).

Once infestation of pests (including *C. maculatus*) is established in stored products, the only alternative available to the farmer is to apply synthetic insecticide otherwise loss of the entire stored product is inevitable. Synthetic insecticides have become very popular since their discovery in the 1940s because of their efficacy, availability and ease of application even on

large scale and they constitute man's first line of defence in pest outbreaks (Oni *et al.*, 2018). As the world population hits 8 billion mark, there is growing need to double food production and this would lead to threefold increase in fertilizer and pesticide usage (IITA, 2017). The world agrochemical market was worth US\$ 243.1 billion in 2019 and projected to hit US\$300 billion in 2024; insecticides account for 25% of this market (FAOSTAT, 2017; Statista, 2020). Unfortunately, the most deadly synthetic insecticides are used in Nigeria; these include Lindane, Malathion, Carbofuran, Dichlorvos, Chlorpyrifos, DDT, etc., (Ojo, 2016). Generally these insecticides have high mammalian toxicity, low biodegradability, pest resurgence and resistance, residue accumulation in farm products and soil, and the consequent environmental concerns. Another problem is the increasing cost of procurement in the wake of the dwindling farmers' income and value of local currency have rendered these insecticides too expensive with a call for switch to natural products (Ashamo *et al.*, 2018).

Peasant farmers all over the world use plant materials for storage of their excess harvest as protectants against pest infestation (Oni *et al.*, 2018). The past four decades have witnessed a new impetus in the development and evaluation of botanical insecticides in view of their relative safety to the environment (Soujanya *et al.*, 2016; Singh, 2017; Spochacz, *et al.*, 2018; Tazerouni *et al.*, 2019). Many plants and plant parts (root, stem, stembark, leaves, fruits, etc.) have been screened with positive results for development of biopesticides (Ojebode *et al.*, 2015; Gbaye *et al.*, 2016; Ileke *et al.*, 2017; Ashamo *et al.*, 2018).

Zhang *et al.*, (2017) reported that six essential oils from the genus *Zanthoxylum* have remarkable repellent activities against *Tribolium castaneum* and *Lasioderma serricorne* adults. Zhang *et al.*, (2018) reported that out of eleven lignans extracted from stem bark of *Zanthoxylum armatum*, four compounds have strong antifeedant activity against *T. castaneum*. Osabutey *et al.*, (2015, 2018) reported larvicidal potential of *Z. zanthoxyloides* in laboratory and field trials against *Plutella xylostella*. Buxton *et al.*, (2017) isolated pellitorine from *Z. zanthoxyloides* as the main bioactive compound responsible for insecticidal activities against *Sitophilus oryzae*.

The use of partitioned extraction methodology in this study is to allow for identification of components with high potency for development of new biopesticide, eliminate toxic components and increase ease of handling when compared to bulk crude plant material used by farmers.

## 2. Methods

Root bark of *Zanthoxylum zanthoxyloides* and *Vigna unguiculata* (seeds) were obtained from Bida, Nigeria (latitude 9.6° north and longitude 6.1° east) and *Z. zanthoxyloides* was dully identified at National Institute of Pharmaceutical Research and Development (NIPRD) Abuja with voucher number NIPRD/H/7101. The plant was dried under laboratory conditions (ambient temperature of 34±6°C and relative humidity of 41±5%). A stock specimen of *C. maculatus* was obtained from Biological Science Department of Federal Polytechnic, Bida. Analytical grade of solvents were obtained for the work.

### 2.1 Preparation of Extracts

The root bark of *Z. zanthoxyloides* was grounded into fine powder (using electric blender; CM/L-7480681 and sieved using 1mm<sup>2</sup> mesh) and 1000g (weighed with Ohaus analytical balance PA214 and Labtech BL7501; China) was used for partitioned extraction using modified Kupchan partition methodology (Emran, *et al.*, 2015). The following solvents were used for partition extraction; methanol, acetone, ethyl acetate and hexane in order of decreasing polarity. Soxhlet extraction apparatus, water bath DK600 and rotary evaporator RE-6000 (China) were used for extraction and evaporation.

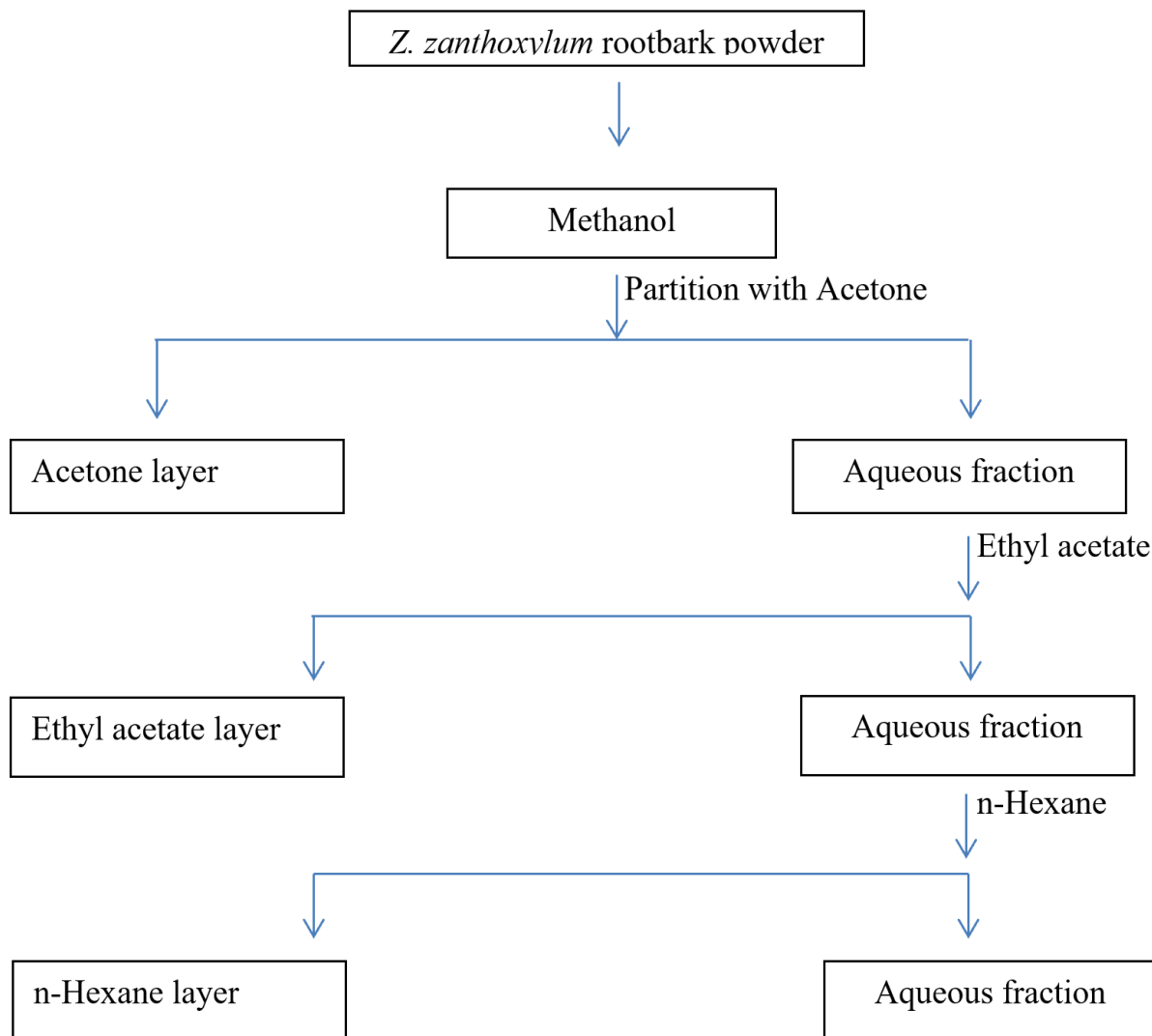


Figure 1. Schematic representation of modified Kupchan partition methodology

## 2.2 Proximate, Phytochemical and Mineral Composition Analysis

Determination of percentage moisture, ash, crude protein, crude fibre, oil extract and nitrogen free extract (NFE) of root bark of *Z. zanthoxyloides* was carried out by AOAC (1995) standard methods. Phytochemical screening was done in accordance with Harbone (1973) methods. Mineral analysis was done using Buck Scientific Atomic Absorption/Emission Spectrophotometry (AAS) and molybdenum blue method for phosphorus. UV-visible

spectrophotometer Shimadzu UV1800 (Japan) was utilized.

### 2.3 Bioassay

20g of cowpea was weighed into each of the 51 plastic containers. Each partitioned extract of *Z. zanthoxyloides* (methanol, acetone, ethyl acetate and n-hexane) was applied at 1%, 2% and 3% to the cowpea in the plates and thoroughly mixed, and every concentration for each extract was replicated three times. Crude powder of the root bark of *Z. zanthoxyloides* was applied at 0.5g, 1.0g and 1.5g to 20g of cowpea and each concentration replicated three times. Positive control was set up using Dichlorovinyl dimethyl phosphate (Sniper) and a negative control (without extract or powder) was also set up and replicated three times. Five (5) pairs of *C. maculatus* was added to each plate (i.e. 5 males and 5 females) and allowed to oviposit for seven days following inoculation. The set up was left at ambient laboratory conditions for observation and monitored for oviposition.

Toxicity test was carried out by dipping method (Paramasivam and Selvi, 2017), the insects were returned to food source and mortality was measured over a period of five days. Mortality was calculated using Abbott's formula:

$$CM = \left[ \frac{MT - MC}{100 - MC} \right] 100$$

CM = Corrected mortality

MT = Mortality in test

MC = Mortality in control

Four parameters were measured; toxicity, number of adults emerged (number of adult insect emerging in first filial generation), weight loss and viability test of seeds (percentage germination of cowpea). Weight loss and Percentage germination were calculated as follows:

$$WL = \left[ \frac{OW - FW}{OW} \right] 100$$

WL = Weight loss

OW = Original weight

FW = Final weight

$$PG = \left[ \frac{NSG}{TSP} \right] 100$$

PG = Percentage germination

NSG = Number of seeds that germinated

TSP = Total number of seeds planted

### 3. Data Analysis

Data obtained for toxicity was transformed using Abbott's formula. Other data were subjected to one way Analysis of Variance (ANOVA) and Tukey's Multiple Comparison Test (SPSS version 21 and Graphpad Prism version 8.0).

### 4. Results

#### 4.1 Proximate, Phytochemical and Mineral Composition Analysis

Proximate analysis of root bark of *Z. zanthoxyloides* revealed 7.46% moisture content, 19.87% ash content, 7.00% crude protein, 24.85% crude fibre, 3.13% oil extract and 37.69% NFE (Table 1)

Table 1. Proximate analysis of root bark of *Z. zanthoxyloides*

Sample	% Moisture	% Ash content	% Crude protein	% Crude fibre	% Oil extract	% NFE
<i>Z. zanthoxyloides</i>	7.46	19.87	7.00	24.85	3.13	37.69

NFE: Nitrogen free extract (carbohydrate)

Phytochemical analysis (Table 2) showed that root bark of *Z. zanthoxyloides* contained 15.42mg/100g alkaloids, 45.90mg/100g of Tannins, 1039.14mg/100g of Saponins, 26.47mg/100g of Flavonoids and 7630.04mg/100g of reducing sugar.

Table 2. Phytochemicals analysis of root bark of *Z. zanthoxyloides*

Sample	Alkaloids	Tannins	Saponins	Flavonoids	Reducing sugar
<i>Z. zanthoxyloides</i> (mg/100g)	15.42	45.90	1039.14	26.47	7630.04

Mineral analysis of root bark of *Z. zanthoxyloides* indicated it contained 150.00mg/100g of Iron, 244.70mg/100g of Calcium, 36.65mg/100g of Magnesium, 24.20mg/100g of Sodium, 449.00mg/100g of Potassium, and 128.30mg/100g of Phosphorus (Table 3).

Table 3. Mineral analysis of root bark of *Z. zanthoxyloides*

Sample	Iron	Calcium	Magnesium	Sodium	Potassium	Phosphorous
<i>Z. zanthoxyloides</i> (mg/100g)	150.00	244.70	36.65	24.20	449.00	128.30

#### 4.2 Bioassay

The result of toxicity test showed that n-hexane extract at 1%, 2% and 3%, acetone extract at 3%, and crude powder at 1.5g all had same effect on the insect as the positive control, achieving 100% mortality at 24 hours post treatment (Table 4). All treatments of methanol extract was only able to achieved 90% mortality after 120 hours, all treatment of acetone extract achieved 100% mortality at 120 hours while ethyl acetate did the same under 96 hours.

Table 4. Percentage mortality of *Callosobruchus maculatus* post treatment

TREATMENT		0HR	24HR	48HR	72HR	96HR	120HR
METHANOL EXTRACT	1%	0.00	10.00g	10.00f	40.00f	70.00c	80.00c
	2%	0.00	20.00f	20.00e	50.00e	80.00b	90.00b
	3%	0.00	40.00e	40.00d	60.00d	70.00c	90.00b
ACETONE EXTRACT	1%	0.00	50.00d	70.00b	70.00c	80.00b	100.0a
	2%	0.00	70.00c	100.00a	100.00a	100.00a	100.0a
	3%	0.00	100.00a	100.00a	100.00a	100.00a	100.0a
ETHYL ACETATE	1%	0.00	20.00f	40.00d	80.00b	100.00a	100.0a
	2%	0.00	80.00b	100.00a	100.00a	100.00a	100.0a
	3%	0.00	80.00b	100.00 a	100.00a	100.00a	100.0a
N-HEXANE	1%	0.00	98.33a	100.00a	100.00a	100.00a	100.00a
	2%	0.00	100.00a	100.00a	100.00a	100.00a	100.00a
	3%	0.00	100.00a	100.00a	100.00a	100.00a	100.00a
CRUDE POWDER	0.5g	0.00	40.00e	40.00d	60.00d	70.00c	90.00b
	1.0g	0.00	50.00d	50.00c	69.33c	100.00a	100.00a
POSITIVE CONTROL	1.5g	0.00	100.00a	100.00a	100.00a	100.00a	100.00a
NEGATIVE CONTROL		0.00	100.00a	100.00a	100.00a	100.00a	100.00a
		0.00	0.00h	0.00g	0.00g	0.00d	0.00d

Values with different small letters within the columns are significantly different ( $F_{pr} < 0.05$ ). based on Multiple Comparison Test following ANOVA. HR = Hour

Figure 2 shows adult emergence in F1 generation. The extracts reduced number of adult emergence with increasing concentration: methanol extracts from 63.57 in 1% treatment to 28 in 3% treatment, acetone extract from 52 in 1% treatment to 17 in 3% treatment, ethyl acetate from 46 in 1% treatment to 14 in 3% treatment, n- Hexane extract and positive control reduced number of adult emergence to zero while in negative control 95 adults emerged (these result were significantly different at  $p < 0.05$ ).

Weight loss by cowpea showed similar variation: in methanol extract it varied from 0.73g in 1% treatment to 0.43g in 3% treatment, in acetone extract from 0.83g in 1% treatment to 0.57g in 3% treatment, in ethyl acetate extract from 0.87g in 1% treatment to 0.56g in 3% treatment, n-hexane did better than the positive control with values of 0.23g in 1% treatment, 0.13g in 2% treatment and 0.1g in 3% treatment while positive control had 0.33g (Figure 3).

Figure 4 shows the viability of the cowpea seeds after F1 emergence, expressed as percentage germination. Percentage germination of cowpea after adult emergence showed that methanol extract varied from 73% in 1% treatment to 86% in 3% treatment, acetone extract from 63% in 1% treatment to 76% in 3% treatment ethyl acetate extract from 63% in 1% treatment to 76% in 3% treatment, n-hexane extract from 96% in 1% treatment to 100% in 2% and 3% treatments, similar values were obtained from positive control. The negative control gave 23% germination.



The effect of crude powder of *Z. zanthoxyloides* is shown in Figure 5. Crude powder applied at 0.5g, 1.0g and 1.5g gave 29, 22 and 14 for number of adult emergence, 0.47g, 0.5g and 0.43g for weight loss, 76%, 80% and 83% for germination respectively.

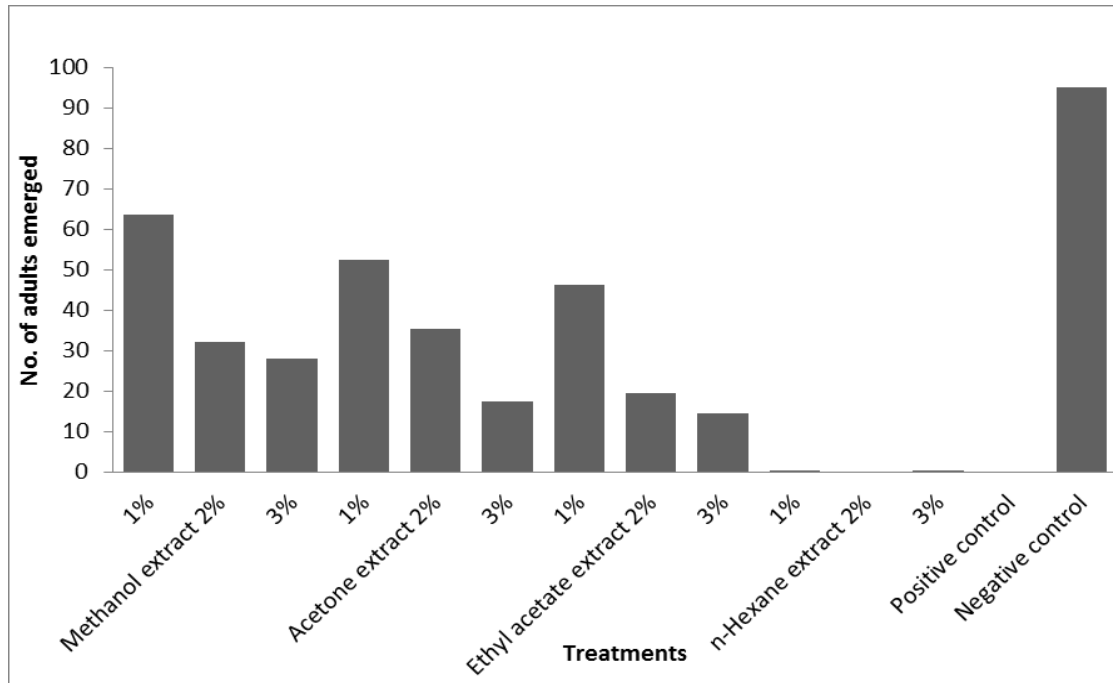


Figure 2. Effect of partitioned extracts on adult emergence

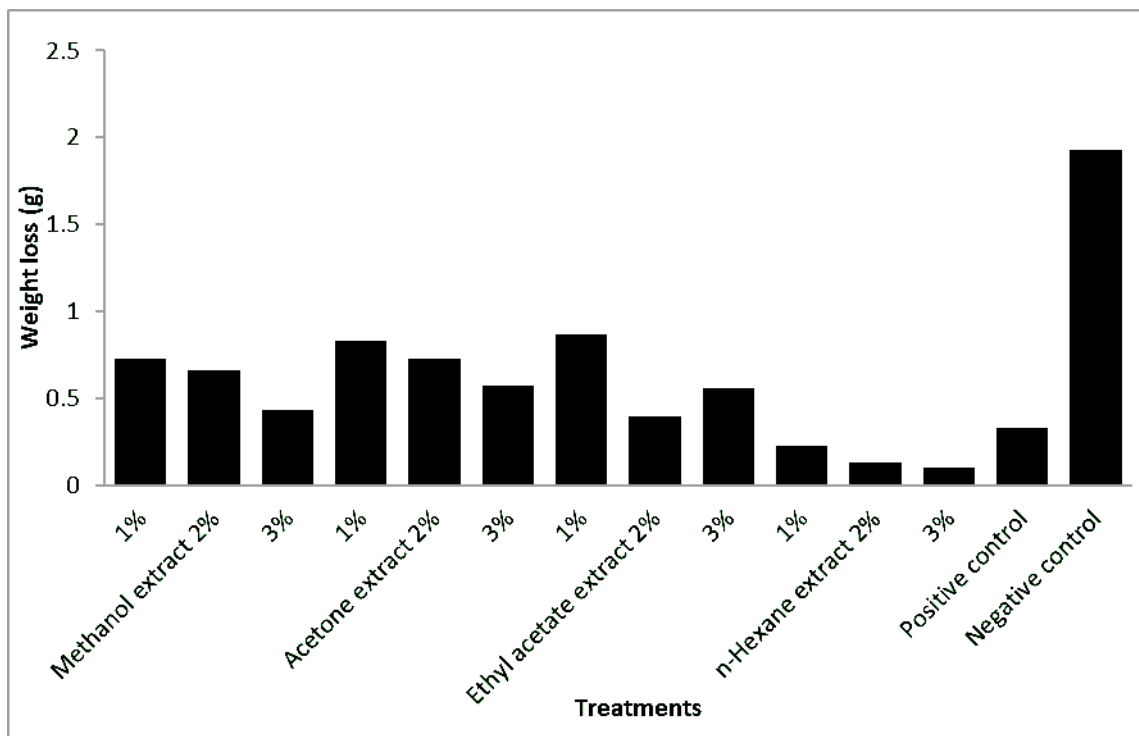


Figure 3. Effect of partition extracts on weight loss



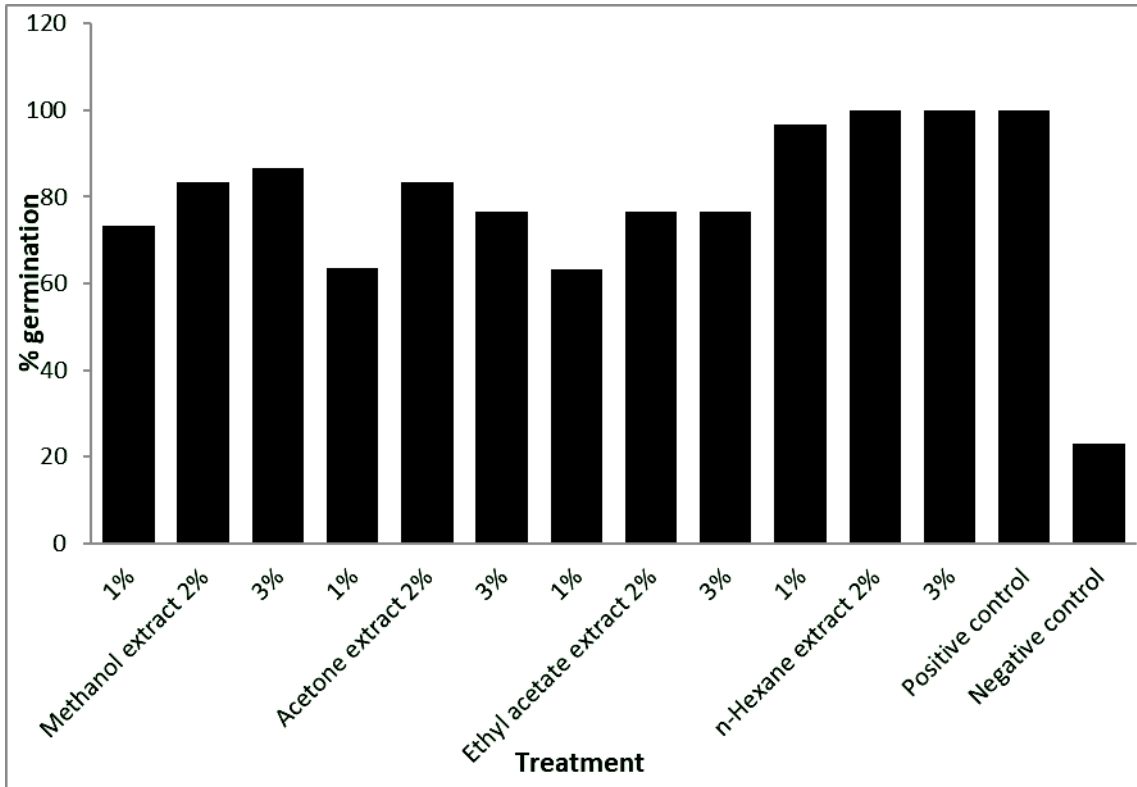


Figure 4. Effect of partition extracts on germination

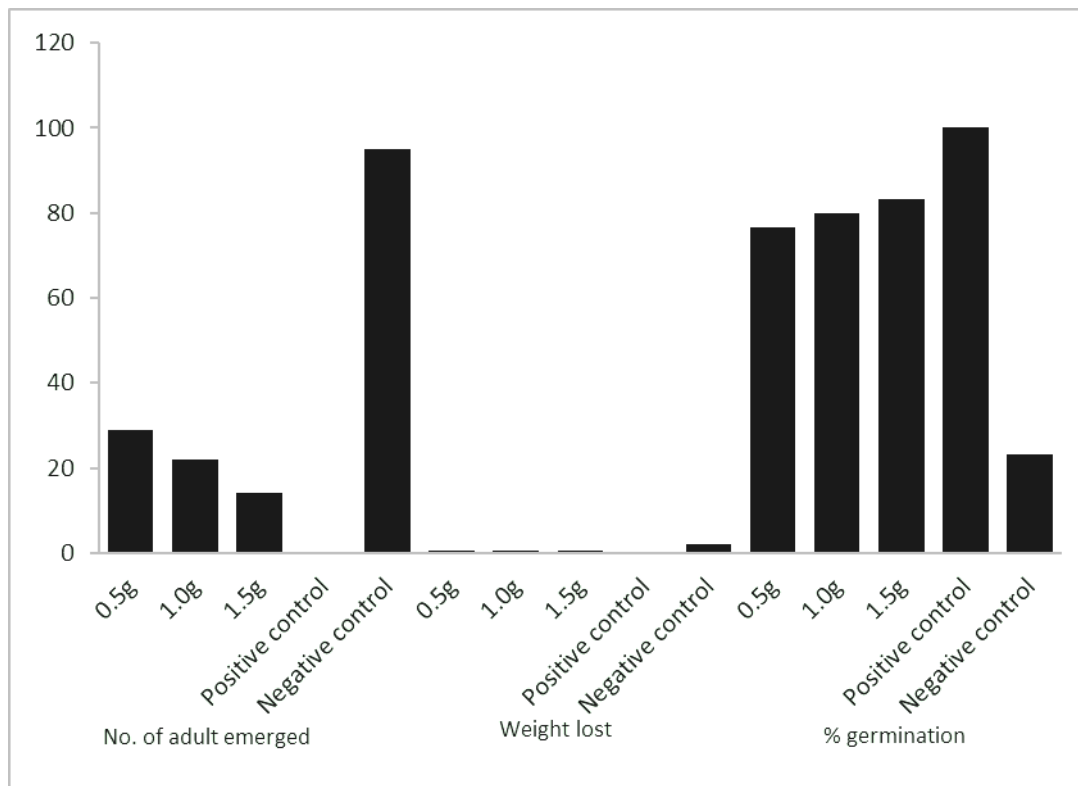


Figure 5. Effect of crude powder on *C. maculatus*

## 5. Discussion

The result for proximate and elemental analysis corresponds to those obtained by Ilodibia *et al.* (2017) working on stem of *Z. zanthoxyloides*. Results obtained for the phytochemical analysis were quite different from those of Aworinde *et al.* (2016); their values were all higher except for saponins where 1039.14mg/100g was obtained compared to 378.33mg/100g. This can be attributed to the differences in the part analysed (root and stem, respectively).

n-hexane extract achieved 100% mortality within 24 hours, this is consistent 100% mortality obtained by Buxton *et al.* (2017) using crude powder and methanolic extract of *Z. zanthoxyloides* as contact insecticide against *C. maculatus*, *Sitophilus oryzae*, *Oryzaephilus mercator* and *Rhyzopertha dominica* respectively; Ojebode *et al.* (2016) using essential oil of *Cymbopogon citratus* and *Citrus sinensis* and Ileke *et al.* (2017) using leaves and seed extract of *Aframomum melegueta*.

Suppression of adult emergence and weight loss, and percentage germination of cowpea seeds were all highest in n-hexane extract, the values here compared well with those obtained for positive control and collaborates results obtained by Buxton *et al.* (2017) and Ashamo *et al.* (2018). All the partitioned extracts did better in terms of weight loss, germination capacity and adult emergence than results reported in Obembe and Ogundipe (2017) which neither achieved 100% mortality after 96 hours nor reduced weight loss by same magnitude. The performance of extracts increased with increasing concentration in conformity with earlier works (Ileke *et al.*, 2017 and Ashamo *et al.*, 2018). This is indicative of the fact that all the partitioned extracts and particularly n-hexane component of the partitioned extracts contained secondary metabolites which are responsible for the effect exhibited in this study. Such secondary metabolites associated with *Z. zanthoxyloides* may be responsible for insecticide activity; repellence, mortality, anti-feeding, anti-oviposition, anti-morphogenesis, etc. Some of these metabolites already identified include: zanthoxylol (Elujoba and Nagels, 1985), benzophenanthridine, furoquinoline, aporphine alkaloids and several aliphatic amides (Matu, 2011), pellitorine, (Aruda *et al.*, 1994; Buxton *et al.*, 2017), several chemical compounds from oils of six species of *Zanthoxylum* (Zhang *et al.*, 2017). The current study will add to the number of insecticidal compounds for *Zanthoxylum* as we explore the compounds in the most active extract (n-hexane extract) of *Z. zanthoxyloides*.

## 6. Conclusion

This result shows that n-hexane extract contains the most active ingredients as insecticide against *C. maculatus* (comparable to positive control) and had no negative impact on the germination of cowpea seeds. The work provides valuable evidence for the potential of n-hexane extract in development and commercialization of novel pesticides in the future. The focus of future work would be on characterisation, purification and formulation of the active ingredients for use by our industries and farmers.

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