

*Full Length Research Paper*

# Chemical composition and insecticidal properties of the underutilized *Jatropha curcas* seed oil

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The chemical composition and insecticidal activity of *Jatropha curcas* L. seed were evaluated using standard techniques. The oil content of the seed is high (66.4%). Triacylglycerol was the dominant lipid species, while the major triacylglycerol was 1,2-Dioleoyl-3-linoleoyl-rac-glycerol. Linolenic acid was the dominant fatty acid in the oil. Physico-chemical properties of the oil indicated that the acid value, free fatty acids, peroxide value and iodine value were high. Ten sesterols and thirteen triterpene alcohol was identified in the unsaponifiable fraction of the oil. *Jatropha* seed oil at various serial dilution ranging from 0% to 2% (v/w) at 0.5% intervals were evaluated for anti-ovipositional activity and long-term protective ability of treated cowpeas against the seed beetle *Callosobruchus maculatus*. The oil significantly ( $P < 0.05$ ) reduced oviposition by *C. maculatus* in no-choice test in all the concentrations tested. The number of eggs laid by the seed beetle reduced from an average of  $54.33 \pm 3.53$  in the control to only  $4.00 \pm 1.53$  in 2% oil-treated seeds. There was no adult emergency in all the oil. However in choice-tested seed,  $6.67 \pm 1.33$  eggs were laid in cultures treated with 2% oil while  $21.67 \pm 1.45$  were laid in control cultures. In dual-choice tests, oviposition was significantly reduced at all the oil concentration evaluated. *J. Curcas* oil also offers a 12-week protection for treated seeds since there were neither seed damage nor adult emergency in treated cowpea seeds. The results of this study suggest *J. curcas* has antioviposition and ovicidal effects on *C. maculatus* therefore making it a viable candidate for incorporation into pest control program of grain legumes.

**Key words:** Natural insecticides, *Jatropha curcas* seed oil, unsaponifiable matter, *Callosobruchus maculatus*.

## INTRODUCTION

Cowpea, *Vigna unguiculata* L. (Walp) (family fabaceae) is a widely grown grain legume in the tropics and sub-tropics purposely for its edible seeds which are rich in protein (Jackai and Daoust, 1986). It is a major source of dietary plant protein for both humans and livestock (Okigbo, 1978; Adedire and Lajide, 1999). Profitable and economic production of cowpeas in developing countries,

particularly in Nigeria, is currently being hampered by its multitude of insect pests both on the field and at storage (Jackai and Adalla, 1997). In Nigeria, fresh cowpeas of high quality and relatively un-infested are often available around November-December while it becomes scarce and heavily infested by bruchids notably *Callosobruchus maculatus* during its off-season or barely 2-3 months after harvest (Caswell, 1976; Sowunmi, 1982). Bruchid infestation usually affects seed quality, market value and can reduce cowpea seed viability to 2% after 3 months of storage (Caswell, 1980, 1981; Ofuya and Credland, 1991).

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Over the years, several methods of bruchid control have been employed by farmers and researchers have identified some efficient control of the pests. Some of these methods range from store hygiene, physical and cultural control methods and use of invert materials. Chemical control appeared to be the most effective and efficient control method (Jackai and Daoust, 1986) but it has adverse effect on both man and environment (Makanjuola, 1989; Adedire and Lajide, 1999, 2000). Against this background, a search for natural-product based agrochemicals which are biodegradable, eco-friendly and safe to the environment has intensified (Jadhau and Jadhau, 1984).

*Jatropha curcas*, a potential anti-feedant candidate, belongs to the family, Euphorbiaceae. The seed which is black and oval in shape is rich in fixed oil (Shukla et al, 1996). The plant is a native of North America but now thrives well in Africa and Asia. It is easy to establish as it grows relatively quickly with high yields (Walls, 1967). Recently there was renewed interest on the utilisation of the seed oils in view of the relatively high oil content (Sujatha and Mukia, 1996).

In our earlier studies, we considered the composition and insecticidal properties of *Monodora tenuifolia* seed oil (Adedire et al., 2003). Therefore in continuation of our studies on the sourcing of natural product agrochemicals, this article considers the physicochemical properties, lipid classes, fatty acids and triacylglycerols of the *Jatropha* seed oil. Unsaponifiable content of the oil will also be analysed for the triterpene alcohols and sterols. The work also involved studies on the insecticidal activity of the oil on the cowpea storage bruchid, *C. maculatus*.

## MATERIALS AND METHODS

### Collection and preparation of samples

The samples were obtained from markets in Ibadan, Akure, Ilorin, Warri and Kaduna. They were identified at the Genetic Resources unit of the International Institute of Tropical Agriculture, IITA, Ibadan, Nigeria. They were subsequently ground in a Christy laboratory mill and stored in a cellophane bag at 4°C prior analysis.

### Triacylglycerol standards

All the standards used for the studies were purchased from SIGMA Chemical Company. They include: Tripalmitin PPP; 1, 2-Dipalmitoyl – 3-oleoyl- rac- glycerol PPO; Dipalmitoyl – 2-oleoyl – rac- glycerol POP; 1, 2- Dipalmitoyl – 3-oleoyl –rac-glycerol PPO; 1, 2- Distearoyl – 3 – palmitoyl – rac – glycerol SSP; 1- Stearoyl – 2- Oleoyl –3-palmitoyl – rac-glycerol SOP; 1,3- Dioleoyl – 3- palmitoyl – rac- glycerol OPO; 1- Palmitoyl – 2- 3 dioleoyl – rac- glycerol POO; 1, 3 – Dioleoyl –1,3 stearoyl – rac- glycerol OSO; 1, 3 – Distearoyl – 2- oleoyl – rac –glycerol SOS; 1,2- Distearoyl – oleoyl – rac – glycerol SSO; Triolein OOO; 1,3 Dipalmitoyl – 2 – linoleoyl – rac- glycerol PLP; Trilinolein LLL; 1,2 - Dilinoleoyl – 3-oleoyl – rac- glycerol LLO; 1- Palmitoyl – 2- oleoyl-3 linoleoyl-rac-glycerol POL; 1,2 Dioleoyl- 3- linoleoyl – rac-glycerol OOL; 1- Behenoyl-2,2-dioleoyl-rac-glycerol BOO.

## Analytical methods

The oil extracted with petroleum ether (40 – 60°C) using the soxhlet procedure for 6 h. Iodine value, saponification value, peroxide value, free fatty acid and refractive index were determined using the method already described in the AOAC (1980).

The fatty acids in the oil were determined by gas chromatography. To 0.1 g of the oil, 5 ml methanol and 1 ml  $\text{CH}_2\text{Cl}_2$  were added. The mixture was cooled in ice and  $\text{CH}_3\text{COCl}$  (0.6 ml) was added. 1 ml of the solution was withdrawn into the hydrolysis tube and heated for 1 h at 110°C. The mixture was cooled and discharged into a 10 ml NaCl solution (1%) in a separating funnel. The organics were extracted with 3 x 4 ml hexane and 4 ml  $\text{CH}_2\text{Cl}_2$ . The  $\text{CH}_2\text{Cl}_2$  phase was separated on a DB5 30 m x 0.25 mm, capillary column (J and W Scientific, Köln, Germany) installed in a GC Chrompack CP 9001 equipped with a flame ionization detector and Mosaic integrator. The temperature was programmed as follows: 35°C for 3 min; temperature increased at 20°C per min up to 120°C; 5°C per min up to 230°C and retained at 230°C for 5 min. Heptadecanoic acid was used as the internal standard.

Lipids were separated on 0.75 mm plates (20 X 20 cm) coated silica gel (MERCK). Plates were developed vertically in a 80/20/1 volume mixture of petroleum ether: diethylether: acetic acid. Details of the procedure have been described earlier (Esuoso et al., 1998). The triacylglycerol fraction was identified, scrapped off and eluted with  $\text{CH}_2\text{Cl}_2$ . The samples and standards was injected into Gas Chromatography (CHROMPACK CP 9000) using Chrompack TAP capillary column 25m x 0.25mm (J and W Scientific, Köln, Germany). The carrier gas was hydrogen maintained between 95-96 kpa. The temperature was programmed as follows: 80°C for 2 min; temperature increased to 280°C at 30°C per min; temperature increased to 355°C at 3°C per min.

### Isolation of unsaponifiables

Oil (10 g) dissolved in 200 ml of ethanol potassium hydroxide (2 M) was refluxed for 1 h. The reaction mixture was diluted to 400 ml with distilled water and transferred to a separating funnel. The unsaponifiables were extracted three times with 100 ml of diethylether. The ether extracts was first washed with 100 ml of aqueous solution of potassium hydroxide (0.5 M) to remove any residual free fatty acids. Further washing and cleaning was carried out five times with 100 ml distilled water, and the ether layer removed in a rotary evaporator. The value was expressed in weight percent.

### Separation of the unsaponifiables

A chloroform solution (50%) of unsaponifiable material (30 cm/plate) was when applied uniformly along a line from the edge of a 20 x 20 cm plate coated with 0.55 mm layer of silica gel and developed three times with hexane/ethyl acetate (6:1, v/v) as mobile phase. After development, the plate were sprayed with a solution of Rhodamine-6G in ethanol (0.5%) and observed under UV light. Three different zones were marked:  $R_f$  0.9-0, hydrocarbon (n-alkane);  $R_f$  0.02-0.04, sterols. Each zone were carefully scraped from the plates and extracted thoroughly with diethylether. Sterols and triterpene alcohols were silanated with 10  $\mu\text{l}$  of bis (trimethylsilane) – trifluoroacetamide (BSTFA) at 60°C for 1 h. The residue obtained was with diethylether and the solvent was removed at reduced pressure in a rotary film evaporator.

### Analysis of unsaponifiables

For the determination of hydrocarbons, the fraction was injected

**Table 1.** Percentage oil composition and lipid classes of *Jatropha curcas*.

Composition	Percentage
Oil	66.4
Unsaponifiable	3.8
Hydrocarbons/sytereo esters	4.8
Triacycerols	88.2
Free fatty acid	3.4
Diacylglycerols	2.5
Sterols	2.2
Monoacylglycerols	1.7
Polar lipids	2.0

into the Gas Chromatography without derivatization using a capillary column (SE-54, 20 m x 0.27 mm, J and W. Scientific, Köln, Germany). The programming was as follows: 35°C for 3 min, temperature increased at 5°C/min to 280°C for 5 min. Further determination was carried out on a GC – MS. Varian MAT 112S using an ionization voltage of 60 eV. For sterols and triterpene alcohols, the determination was carried out on GC with an OV-17 glass capillary column (30 m x 0.3 mm i.d). Relative retention times (RRT) were expressed on silanated cholesterol ester (1.00). The RRT values of authentic samples of sterols and triterpene alcohols given in Table 5.

#### Insect culture and maintenance

*Callosobruchus maculatus* used in this study were derived from field infested cowpeas bought from Oba Market in Akure, Nigeria and reared in the laboratory on clean uninfected kilner jars while cultivars of cowpeas was carried out in kilner jars at fluctuating ambient temperature and relative humidity. The kilner jars were covered with muslin cloth held tightly in place for adequate aeration of the culture and precluded entry or exit of insects. New generations of bruchids were derived from this stock culture by infesting clean un-infested beans with 10 pairs of teneral adult bruchids.

#### Oviposition and adult emergency

Disinfested lfe brown variety cowpea seeds weighing 20 g were serially treated with *Jatropha* oil corresponding to 0, 0.5, 1.0, 1.5 and 2% (v/w). The cowpeas were thoroughly coated with the oil using a glass rod and then allowed to air-dry for about 1 h prior to the extraction solvent. Each experimental plate was infested with a copulating pairs of male and female *C. maculatus* of about 0-24 h old.

Each treatment was prepared in triplicates. After 14 days when the insects have died, the number of eggs laid on the seeds were counted and recoded while the number of  $F_1$  progeny was determined at 42 days post commencement of the experiment.

#### Oviposition deterrent activity

Two approaches were used to accomplish the oviposition-deterrent effect of *Jatropha* oil on female bruchids. The first procedure involved in a dual-choice system while the second approach was accomplished in a multi-choice chamber. The dual-choice bioassay

procedure involves 5 treated cowpea seeds of the same concentration and 5 solvent treated cowpeas arranged alternatively in ring-like manner in a Petri-dish coated with paraffin wax (Adedire and Lajide, 1999). The multi-choice system is similar except that all the treated cowpea seeds of all the concentrations and control were arranged in the same manner in Petri-dish. Then two copulating pairs of *C. maculatus* (0-24 h old) were introduced into each Petri-dish and covered. Three replication of each experiment was prepared. The number of eggs laid on each heated and untreated (solvent control) cowpea seed was enumerated after the demise of the female bruchids.

#### Growth performance at 12 weeks post-treatment

Serial dilution (0, 0.5, 1 1.5 and 2%, v/w) of *Jatropha* oil were used to surface-treat 50 g grams of susceptible IFE brown variety of cowpea in a 100 ml volumetric flask. The uniform mixing of the oil and the seeds was accomplished by manual agitation with the aid of a glass rod. The seeds were then air-dired for 1 h before introducing five couples of teneral adult *C. maculatus* into each conical flask. Each treatment was carried out in triplicates while the oil was replaced with petroleum ether (the control flask). Each flask was covered with muslin cloth held firmly in place with a rubber band to prevent escape of the bruchids or entry of some other insects and at the same time ensure adequate aeration. The flasks were kept in a completely randomized manner and left for 12 weeks in an open laboratory at ambient temperature (30±2°C) and relative humidity. After 12 weeks, the number of damaged and unaffected seeds were counted and recorded. Also the numbers of dead and live bruchids were enumerated.

#### Statistical analysis

All the data obtained were subjected to analysis of variance (ANOVA) and where there are significant differences, means were separated the range test (DMRT). Data on oviposition- deterrent activity of the oil in dual-choice chamber was analysed by students t- test.

## RESULTS AND DISCUSSIONS

Percentage oil composition and lipid classes are presented in Table 1. The oil content is high (66.4%). This percentage is much higher than those recorded for most oil-rich seeds (Esuoso and Bayer, 1998; Esuoso et al., 1998). This would therefore be an advantage in terms of the exploitation of the oil. Triacylglycerol was the dominant lipid specie (88.2%). The high unsaponifiable matter (3.8%) is an advantage for use as natural insecticide. This is because unsaponifiable matter contains sterols and triterpene alcohols which is responsible for the insecticidal properties of fixed oils (Jalad et al., 1977; Haftmann, 1970). The composition of the unsaponifiable matter is presented in Tables 2 and 3 Ten sterols and thirteen terpene alcohol were identified in the oil. The dominant sterols were 24-ethylcholesterol and  $\beta$ -sitosterol, while 24-methylene-24-dihydroparkeol and taraxasterol were the major triterpene alcohol in the oil. Shankaranarayana et al. (1980) have shown the insect growth-inhibiting properties and chemosterilant activity of a pentacyclic triterpenoid(urs-12-ene-3 $\beta$ -

**Table 2.** Fatty acid composition of *Jatropha curcas*.

Composition	Percentage
Palmitic acid (C16:0)	11.3
Stearic acid (C18:0)	17.0
Oleic acid (C18:1)	12.8
Linoleic acid (C18:2)	47.3
Arachidic acid (C20:0)	4.7
Arachidoleic acid (C20:1)	1.8
Behenic acid (C22:0)	0.6
C24:0	44

**Table 3.** Triacylglycerols of *Jatropha curcas*

Composition	Percentage
PPO/POP	2.7
PLP	1.5
SOP	2.0
OPO/POO	12.5
POL	10.0
SOO/OSO	3.3
OOO	0.4
OOL	31.2
LLO	19.8
LLL	10.7
BOO	3.5
Unidentified	2.4

**Table 4.** Physicochemical parameter *Jatropha curcas* oil.

Composition	Percentage
Colour	Golden Yellow
Specific gravity	0.8601
Refractive index	1.4735
Free fatty acids (%)	4.54
Acid value (mg. KOH. g <sup>-1</sup> )	4.24
Saponification value (mg.KOH.g <sup>-1</sup> )	169.9
Iodine value (mg. I <sub>2</sub> . g <sup>-1</sup> )	111.6
Peroxide value (mg reac.O <sub>2</sub> g <sup>-1</sup> )	3.5

palmitate) extracted from the bark of *Santalum album*. In addition the anti-feedant activities of pentacyclic triterpene acids have been demonstrated (Shukla et al., 1996; Jagdeesh et al., 1998; Uppuluri et al., 2003; Makonjuola, 1989). Some oils might be responsible for their anti-oviposition activity.

Fatty acid composition is presented in Table 2 Linoleic acid constitutes the dominant fatty acid (47.3%). High molecular weight fatty acid (C24:0) was identified on the

**Table 5.** Retention times of Sterols and Triterpene Alcohols used as References in Gas Chromatography

Code	RRT	Compound
<b>Sterol</b>		
1.	1.00	Cholesterol (Cholest-5-enol)
2.	1.17	27. methylcholesta- 5, 22E – dienol
3.	1.34	Campesterol (24-methylcholesterol)
4.	1.46	Stigmasterol (24-ethylcholesta – 5, 22E-dienol)
5.	1.47	24 –ethylcholesterol (24-cholesterol)
6.	1.66	β- sitosterol (24- cholesterol)
7.	1.68	24- ethylcholesta- 7, 22E 25- trienol
8.	1.84	Isofucosterol (24Z-ethylidne cholesterol)
9.	1.95	24 –ethylcholesta – 7, 25-dienol
10.	2.17	Avenasterol (24Z-ethylcholesta-7,24-d, enol)
11.	2.19	Peposterol (24-ethylcholesta – 7, 24-d, enol)
<b>Terpene Alcohols</b>		
12.	1.30	Euphol (eupha – 8, 28-dienol)
13.	1.33	24- dihydrolanosterol (24 – lanost- 8-enol)
14.	1.50	Tirucallol (tirucalla – 8, dienol)
15.	1.58	Teraxerol (D- fiedoolean – 14 –enol)
16.	1.66	(3-amyrin (olean – 12- enol)
17.	1.72	Butrospermol (eupha – 7, 24 – dienol)
18.	1.77	Isomultiflorenol (D:c – fiedoolean – 8-enol)
19.	1.79	24 – methyleneost – 8- enol
20.	1.87	∞ -amyrin (urs – 12 –enol)
21.	1.89	24 – methylene – 24- dihydro parkerol
22.	1.90	Cycloartenol (9β-; 19 – cycloartenol)
23.	1.96	Lupeol
24.	2. 10	24 – methylenecycloartenol
25.	2.40	Taraxasterol
26.	2.52	γ taraxasterol

oil. In their studies Khan et al. (1983) reported that the insect repellent activity of fixed oil of *Annona Squamosa* and *Polyalthes longifolia* were due mainly to the presence of high molecular weight fatty acids among other active agents.

The composition of triacylglycerols is presented in Table 3 OOL (1,2,-dioleoyl-3- linoleoyl-rac - glycerol) and LLO (1,2-dilinoleoyl-3-oleoyl rac-glycerol) were the dominant triacylglycerol species.

The physico-chemical parameters of *J. curcas* oil are presented in Table 4. The high iodine value (116 mg.I<sub>2</sub>.g<sup>-1</sup>) indicates a preponderance of unsaturated fatty acid. The fatty acids, acid value and peroxide values are relatively high compared with most seed oils.

The insecticidal properties of the *J. curcas* oil are presented in Tables 7-10. *J. curcas* has long been implicated in traditional medicine and also used as an insect repellent, a molluscicide and a rodenticide (Duke, 1985). The oil significantly reduced the number of eggs laid by *C. maculatus* in no-choice experiments. In choice-

**Table 6.** Composition Sterols and Trieterpene Alcohols in *Jatropha* Seed Oil.

Sterols		Terpene alcohols	
1.17(2) <sup>a</sup>	4.5 <sup>b</sup>	1.30(12) <sup>a</sup>	3.5 <sup>b</sup>
1.34(3)	3.7	1.33(13)	6.1
1.46(4)	4.8	1.50(14)	5.2
1.47(5)	41.5	1.58(15)	4.3
1.66(6)	18.5	1.66(16)	-
1.84(8)	5.9	1.72(17)	3.1
1.95(9)	2.2	1.79(18)	1.5
2.17(10)	3.8	1.78(20)	2.0
2.19(11)	3.5	1.89(21)	37.8
Unidentified peaks	5.7	1.90(22)	-
		1.96(23)	8.8
		2.10(24)	7.9
		2.40(25)	10.8
		2.52(26)	5.6
		Unidentified peaks	3.4

<sup>a</sup>relative retention times ; <sup>b</sup>Components add to 100% in each, - Not detectable (below) detection limit.

**Table 7.** Effect of *Jatropha curcas* oil oviposition and adult emergence of *Callosobruchus maculatus*.

Oil conc. (%)	Mean number of eggs laid	Adult emergency (%)
0.00	54.33a ± 33.53	100
0.50	30.67b ± 1.86	0.00
1.00	21.33b ± 1.76	0.00
1.50	10.00c ± 1.53	0.00
2.00	4.00c ± 1.53	0.00

Mean followed by the same letters are not significantly different (P>0.05) by Duncan's multiple Range test.

**Table 8.** Oviposition – deterrent effect of *Jatropha curcas* oil on female *Callosobruchus maculatus* in a dual choice chamber.

Oil conc. (%)	Mean number of eggs laid	Adult emergency (%)
0.00	54.33a ± 33.53	100
0.50	30.67b ± 1.86	0.00
1.00	21.33b ± 1.76	0.00
1.50	10.00c ± 1.53	0.00
2.00	4.00c ± 1.53	0.00

tests, ovipositing adult female *C. maculatus* discriminated between oil treated cowpeas and the untreated ones.

All the *Jatropha* oil concentrations tested totally prevented adult emergence, indicating that the oil exhibited ovicidal activity. Egg and larval mortalities were

**Table 9.** Ovisposition–deterent effect of graded concentration of *Jatropha curcas* oil on adult *C. maculatus* in a multi – choice chamber.

Oil concentration (%)	Mean number of eggs laid
0.00	21.67 ± 1.45a
0.50	16.67 ± 67b
1.00	14.33 ± 0.33b
1.50	10.67 ± 0.67c
1.51	6.67 ± 1.33c

**Table 10.** Effect of *Jatropha curcas* oil on growth performance of *C. maculatus* at 12 weeks post-treatment.

Oil concentration (%)	Oil seed damages	Mean number of F <sub>3</sub> Progeny
0.00	1.00	1177z
0.50	0	0
1.00	0	0
1.50	0	0
2.00	0	0

probably due to suffocation and or lethal chemical poisoning of the immature forms by the oil. This observation is in agreement with that of Jadher and Jadher (1984) who reported that 0.2% (v/w) of *J. curcas* oil reduced oviposition by *C. maculatus* and totally prevented egg hatch even after 33 days of treatment. Egg stage is one of the stages that is usually very tolerant to chemical treatment (Giga and Smith, 1987). Thus this present study shows that *Jatropha* oil was able to inhibit oviposition and egg development even at a lower concentration (0.5% v/w) since no adult bruchid emerged. This oil was also effective as a grain protectant against bruchid attack during short-term storage since there was no bruchid development in oil treated grain legumes stored for 12 weeks. The insecticidal activity of this seed oil could be due to the presence of several sterols and terpene alcohols which have been known to exhibit insecticidal properties (Heftmann, 1970; Adolf et al., 1985; Duke, 1985).

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