INTERNATIONAL JOURNAL OF PHYTOFUELS AND ALLIED SCIENCES
(A Journal of the Society for the Conservation of Phytofuels and Sciences)
(http://www.phytofuelsciences.com) (ISSN 2354 1784)

Studies on the Efficacy of *Jatropha curcas* L. Seed Oil on Adult Mortality and Emergence of Seed Beetle, *Callosobruchus maculatus* (F.) (Coleoptera:Chrysomelidae) in Cowpea

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Abstract

This study reports the efficacy of seed oil of *Jatropha curcas* L. (Euphorbiaceae) on adult mortality and emergence of seed beetle, *Callosobruchus maculatus* (Coleoptera:Chrysomelidae) of different age groups in the laboratory under ambient temperature of 25±2^0 C and relative humidity of 69±2%. It also examines the weight loss of the cowpea seeds infested with the insect. Four rates of the oil (0.5, 1.0, 1.5 and 2.0 ml/50 g cowpea seeds) were used against the test insect. Adults of both sexes were assigned into two age groups (1-3 days old) and (4-6 days old) before they were subjected to similar treatments to determine the response of their age differences. The oil-treated seeds showed significant ($p<0.05$) reduction in the population of adults and adult emergence of different age groups. The seed oil applied at the rate of 2.0 ml gave significantly ($p<0.05$) higher percentage mortality than the 0.5 ml/50 g cowpea seeds. Mean percentage mortality increased with increase in rate and exposure period under the two age groups. It was observed that early adults (1-3 days old) were more susceptible to the oil than late adults (4-6 days old). However, the seed weight loss was inversely proportional to increase in seed oil used, thus reducing with increase in seed oil rate. Also, the percentage seed weight loss was reduced by 19.01, 18.34 and 16.35% in 2.0 ml/50 g seeds at 30, 60 and 90 days after infestation, respectively. Jatropha seed oil could therefore, serve as a reliable approach to managing the insect pest under small scale storage, particularly with regard to its effectiveness, availability and ease of cultivation of the plant on marginal soil.

Key words: Fixed oil, bruchid, biopesticide, legume

Introduction

Cowpea, *Vigna unguiculata* (L.) Walp. is an important legume in the tropics. The crop is a principal source of protein for the rural and urban people (Lale, 1991) and used to combat malnutrition in young children in lieu of expensive animal protein sources such as meat, eggs and fish (Ileke *et al*., 2012). A substantial part of world cowpea production comes from Nigeria with about 4 million hectares and approximately 1.7 million tons of beans annually (Ofuya, 2003). Cowpea feeds millions of people in the developing world with annual world production estimated at 4.5 million metric tonnes on 12 to 14 million hectares (Diouf, 2011). The yield reduction of this crop and the post-harvest loss has been attributed to a number of field and storage insect pests.

In Nigeria, a field-to-store insect pest commonly found attacking the crop is the seed beetle, *Callosobruchus maculatus* (F.)(Coleoptera:Chrysomelidae). The beetle has drawn attention because it is very damaging and widely distributed throughout the tropical and subtropical regions (Lale, 2002). Ecologically, seed beetles are herbivores that have specialized on seed consumption (Boeke *et al*., 2001). Control of the insect pest has been through the use of conventional dusts, such as pirimiphos-methyl (Actellic* 25 dust), permethrin and fumigants such as aluminium phosphide. The effectiveness of synthetic chemicals has been accompanied by human hazards, effect on non-target organisms and insect resistance. Insect resistance is the most commonly encountered problem in control failures (Gbaye and Ogunlana, 2013). The pervasive use of these insecticides in granaries of small scale farmers has led to a number of problems, such as killing non-target organisms, user hazards, food residues, evolution of resistance to the chemicals, high cost of the chemicals and the destruction of the balance of the ecosystem (Boateng and Kusi, 2008). The fact that some of these poisonous chemicals have been banned in most developed and developing countries testifies to the need to finding sustainable alternative in pest control. The search for alternative insect pest control methods and materials which are relatively cheaper and less harmful to the user and the environment has therefore become essential (Athur and Phillips, 2002).

In Nigeria, empirical investigations have reported the insecticidal efficacy of powders and oils from several plants such as neem, *Azadirachta indica* A. Juss, clove, *Eugenia ruchids* (L.) Baill; West African pepper, *Piper guineense* Schum and Thonn; pepper fruit, *Dennetia tripetala* Baker and toothache plant, *Zanthoxylum zanthoxyloides* (Lam); bush plant, *Hyptis suaveolens* Poit; (Ofuya and Salami, 2002; Ofuya *et al*., 2005; Ashamo, 2007; Musa, 2007). *Jatroppha curcas* L. is a widely available tropical plant that is often used for fencing by farmers (Jide-Ojo *et al*., 2013). Nash (2005) reported the use of the seed oil as biofuel and its potential as biopesticide. Various extracts of the plant have been reported to have insecticidal or molluscicidal/anthelminthic activities on vectors of medical or veterinary interest or on agricultural or non-agricultural pests (Ratnadass and Wink, 2012). This study therefore, examines the efficacy of *J. curcas* seed oil against adult mortality and emergence of

seed beetle, *C. maculatus*, in stored cowpea.

Materials and Methods

The study was carried out in the laboratory under temperature of 25±2^0 C and relative humidity of 69±2% in 2014.

Insect culture

The strain of *C. maculatus* used was obtained from already existing parent stock culture in the Crop Protection laboratory of the University of Ilorin, Nigeria. Susceptible cowpea seeds were infested with fifty adults of *C. maculatus* picked from the parent stock and left undisturbed for 10 days to oviposit before they were removed and discarded. The freshly emerged adults were keenly observed and separated into two age groups (i.e., 1-3 and 4-6 days old).

Source of seeds and their preparation

The cowpea seeds were purchased from the International Institute of Tropical Agriculture, Ibadan, Nigeria and placed in a polyethylene bag kept in a cool storage to inactivate developing insects and microbes. After 15 days, the seeds were equilibrated under laboratory conditions before commencement of the experiment.

Plant preparation

Mature *J. curcas* seeds collected from the University of Ilorin, Ilorin were de-pulped, washed, air-dried under shade and ground using a mortar and pestle. The fine powder was weighed into 200 g and extracted with 300 ml ethanol for 48 h at ambient temperature. The oil extract was stirred and filtered through Whatman No. 1 filter paper before it was concentrated in a steam bath at 50^{0}C. The choice of the plant part was based on the report of its pesticidal properties, which if found effective upon the test insect could be a potential source of biopesticide.

Experimental procedure

Four rates of *J. curcas* seed oil at 0.5, 1.0, 1.5 and 2.0 ml/50 g cowpea seeds were tested against two age groups of adults of *C. maculatus* (i.e., 1-3 and 4-6 days old). The oil and seed mixture was thoroughly agitated to obtain even spread of the oil and then allowed to stand for 1 h to permit the solvent to evaporate. The treated seeds were separately infested with five insects from each of the two age groups. The experimental set up was arranged in a completely randomized design with three replications on a laboratory desk. Water alone served as untreated control.

Data were taken on the adulticidal effect of the oil on the two age groups of *C. maculatus*. Adult mortality was determined at 30, 60 and 90 DAI. The insects were presumed dead if they remained immobile and did not respond to probing with a blunt dissecting probe (Asawalam and Emosairue, 2006). The number of dead insects was divided by the total number of insects expressed as percentage. Another experiment was set up to include the different rates irrespective of the age groups to determine the effect of the oil on adult emergence and seed weight loss at 30, 60 and days after infestation (DAI). The emerged progeny were removed to avoid overlap with subsequent generations. Progeny emergence was determined by counting the number of insects in each treatment and then expressed as percentage at 30, 60 and 90

DAI. Determination of seed weight loss involved reweighing of seeds after sieving and percentage weight loss was calculated by method of FAO (Ofuya and Lale, 2001) as:

$$\%\ Wt.loss = [UaN - (U + D)] \times 100/UaN$$

where

U = Wt. of undamaged fraction in the sample

N = total number of seeds in the sample

Ua = average weight of undamaged seeds

D = weight of damaged fraction in the sample

Data analysis

Data were subjected to analysis of variance and Duncan`s multiple range test was used to separate significantly different means at 5% level of probability.

Results

Adulticidal effect of *Jatropha curcas* seed oil on *Callosobruchus maculatus* of different age groups

Analysis of variance shows significant ($p<0.05$) differences in percentage mortality of adult *C. maculatus* in the two age groups of the insect with the various rates (Table 1). Percentage mortality of *C. maculatus* adults (4-6 days old) in the rates of 1.5 and 2.0 ml/50 g was significantly ($p<0.05$) higher than the mortality in 1.0 ml/50 g cowpea seeds at 1 DAI. During the same period, the rate of 2.0 ml/50 g cowpea seeds had significantly higher mortality of *C. maculatus* adults (1-3 days old) than lower rates. The trend was similar for adults (1-3 days old) mortality at 2 and 3 DAI. It was observed that the highest rate (2.0 ml/50 g) had significantly higher mortality of *C. maculatus* (4-6 days old) than lower rates at 2, 3 4 and 5 DAI.

It was observed that *J. curcas* seed oil applied at the rate of 0.5 ml/50 g cowpea seeds caused 13.3 and 26.7% adult (1-3 days old) mortality at 1 and 2 DAI, respectively. These early adults may have been more susceptible to the same rate of the oil because there was no mortality of the late adult beetle during the same exposure period. Cowpea seeds treated with 2.0 ml/50 g had significantly higher adult mortality for 4-6 days group (86.7%) than 1.0 and 1.5 ml/50 g with 26.7 and 46.7% adult mortality at 2 DAI, respectively. However, significantly higher mortality was recorded in 1.5 ml when compared with 1.0 ml in 2 DAI. The results also show that 2.0 ml/50 g seeds had remarkable improvement in its insecticidal potential over 1.5 ml/50 g from 2 to 5 DAI. Cowpea seeds treated with 2.0 ml of the oil had significantly higher adult (4-6 days old) mortality (80.0%) than the mortality (13.4 to 20.0%) recorded in lower rate at 1 DAI.

At 3 DAI, the seeds with 2.0 ml had significantly higher ($p<0.05$) adult (4-6 days old) mortality (93.3%) than the mortality (6.7 to 46.7%) in lower rates. Cowpea seeds treated with 2.0 ml of the oil had significantly higher ($p<0.05$) adult (1-3 days old) mortality (93.3%) than the mortality (33.3 – 60.0%) in the lower rates. Seeds treated with 1.0, 1.5 and 2.0 ml rates of jatropha seed oil had significantly

higher adult (4-6 days old) mortality of *C. maculatus* (53.3, 60.0 and 93.3% respectively), than 0.5 ml with 6.7% mortality at 4 DAI. Cowpea seeds treated with 1.5 and 2.0 ml of the oil had adult mortality (1-3 days old) of 66.0 and 93.4%, respectively which were significantly higher ($p<0.05$) than mortality (40.0%) in the lowest rate and the control 4 DAI. At 5 DAI, cowpea seeds treated with 2.0 ml of the oil had significantly higher ($p<0.05$) adult (1-3 days old) mortality (93.4%) than the mortality in lower rate at 5 DAI. The adult mortality of 1-3 days old insects in 0.5 and 1.0 ml was not significantly different from the mortality in 1.5 ml/50 g seeds. In both age groups of *C. maculatus*, the percentage mortality caused by the highest rate was statistically comparable ($p=0.05$).

Comparison of adult (4-6 days old) and (1-3 days old) in seeds treated with *J. curcas* seed oil

The mortality of *C. maculatus* adults (4-6 days old) and adults of age group 1-3 days old were compared based on the insecticidal effect of *J. curcas* seed oil (Table 2); Eighty percent (80.0%) adult (1-3 days old) mortality was obtained in 2.0 ml/50 g seeds in 1 DAI. At 2 DAI, 86.6% adult (1-3 days old) mortality was obtained in 2.0 ml rate. This study shows the excellent performance of the oil against early adults (1-3 days old), indicating that the early adults were significantly more susceptible to the treatments than late adults (4-6 days old). The ability of the seed oil to cause mortality was rate dependent; it increased with increase in the rate of the seed oil.

Effect of *J. curcas* seed oil on adult emergence of *C. maculatus* and seed weight loss

Table 3 shows the percentage emergence of adult *C. maculatus* in cowpea seeds treated with *J. curcas* seed oil. The analysis of variance reveals that there was significant difference ($p<0.05$) in the percentage adult emergence of *C. maculatus* in the cowpea seeds at 30, 60 and 90 DAI. The seeds treated with 1.0 ml of *J. curcas* seed oil had lower adult emergence (30.0%) than those seeds treated with 0.5 ml (45.0%), though the difference between the treatments was not significantly different ($p>0.05$). Similarly, the seeds treated with 1.0 ml of *J. curcas* oil had significantly lower ($p<0.05$) percentage emergence than seeds treated with higher rate. Adults emerged from seeds treated with varying rates, but the seeds with the lowest rate and the untreated seeds (control) had the highest adult emergence 60 and 90 DAI. Results show that adult emergence in 1.5 ml/50 g was not significantly different when compared to emergence in the highest rate of treatment. Results obtained from the analyzed data reveal that all the treated seeds performed better than the seeds in the untreated control. Table 3 also shows the percentage seed weight loss as a result of *C. maculatus* infestation. All rates did not differ significantly ($p>0.05$) in mean percentage weight loss at 30, 60 and 90 DAI. However, the seed weight loss was inversely proportional to increase in seed oil used, thus reducing with increase in seed oil rate. Also, the percentage seed weight loss was reduced by 19.01, 18.34 and 16.35% in 2.0 ml/50 g seeds at 30, 60 and 90 days after infestation, respectively.

Table 1: Efficacy of *Jatropha curcas* seed oil on different age groups of adults of *Callosobruchus maculatus*

Rate	% adult mortality (1-3 days old)DAI					% adult mortality (4-6 days old) DAI				
(ml/g)	1	2	3	4	5	1	2	3	4	5
0.0	0.0^{d}	0.0^{c}	6.67^{e}	13.3^{d}	20.0^{d}	0.0^{c}	0.0^{d}	0.0^{e}	0.0^{e}	6.7^{e}
0.5	13.3^{c}	26.7^{b}	33.3^{d}	40.0^{c}	53.3^{c}	0.0^{c}	0.0^{d}	6.7^{d}	6.7^{d}	20.0^{d}
1.0	20.0^{b}	26.7^{b}	40.0^{c}	40.0^{c}	53.3^{c}	20.0^{b}	26.7^{c}	40.0^{c}	53.3^{c}	53.3^{c}
1.5	20.0^{b}	26.7^{b}	60.0^{b}	66.7^{b}	73.3^{b}	40.0^{a}	46.7^{b}	46.7^{b}	60.0^{b}	66.7^{b}
2.0	80.0^{a}	86.7^{a}	93.3^{a}	93.3^{a}	93.3^{a}	40.0^{a}	86.7^{a}	93.3^{a}	93.3^{a}	100.0^{a}
F-Value	15.34	28.41	19.58	10.04	8.31	12.30	32.30	15.66	15.32	8.57

Values with the same superscript are not significantly different at p=0.05 using Duncan` s multiple range test

Table 2: Comparison of percentage mortality of *Callosobruchus maculatus* adults (1-3 days old) and (4-6 days old) in cowpea seeds treated with *Jatropha curcas* seed oil

Age group of adults of *C. maculatus* (days)	Rates				
	DAY ONE				
	0.0	0.5	1.0	1.5	2.0
1-3	0.0	13.4	20.0	20.0	80.0
4-6	0.0	0.0	15.0	30.0	30.0
	DAY TWO				
1-3	0.0	26.6	26.6	26.6	86.6
4-6	0.0	0.0	20.0	35.0	65.0
	DAY THREE				
1-3	6.6	33.4	40.0	66.0	93.4
4-6	0.0	5.0	30.0	35.0	70.0
	DAY FOUR				
1-3	13.4	40.0	46.0	66.0	93.4
4-6	0.0	5.0	30.0	45.0	70.0
	DAY FIVE				
1-3	20.0	53.4	53.4	73.4	93.4
4-6	5.0	15.0	40.0	50.0	75.0

Table 3: Percentage emergence of adult *Callosobruchus maculatus* and seed weight loss as influenced by *Jatropha curcas* seed oil

Rate (ml/50 g)	Mean percentage adult emergence (DAI)			Mean percentage seed weight loss (DAI)		
0.0	45.0[a]	75.0[a]	75.0[a]	14.78[a]	15.16[a]	17.06[a]
0.5	45.0[a]	75.0[a]	75.0[a]	13.52[b]	14.73[b]	15.82[a]
1.0	30.0[b]	45.0[b]	45.0[b]	12.93[b]	13.64[b]	15.56[a]
1.5	5.0[c]	40.0[c]	45.0[b]	12.15[b]	12.44[b]	14.31[a]
2.0	5.0[c]	20.0[d]	25.0[c]	11.97[b]	12.38[b]	14.27[a]
F-Value	4.24	5.26	3.98	2.68	2.72	3.01

Values with the same superscript are not significantly different at p=0.05 using Duncan`s multiple range test

Discussion

In this study, the topical application of the fixed plant oil shows insecticidal properties against *C. maculatus* adults. It was found that *J. curcas* seed oil had faster and greater insecticidal activity against younger adults of 1-3 days old than older adults of 4-6 days old. The adult mortality in the treated seeds at varying rates of treatment performed better than the untreated control. This study also shows that there was an improvement in the potency of the oil against the test insect with increase in exposure period. The excellent performance of the oil in killing the early and late adults in 1.5 ml/50 g and 2.0 ml/50 g rates reveals that the seed oil has potential for short-term storage of cowpea seeds against *C. maculatus*. Abdoul Habou *et al*., (2014) reported that the oil was highly toxic to both *C. maculatus* and *Bruchidus atrolineatus* after 7 days. They also reported that the rates of emergence of *C. maculatus* and *B. atrolineatus* were 76.2 and 76.1% respectively, indicating that exposure to *J. curcas* oil drastically reduced adult emergence in the two insect species. In a similar experiment, Boateng and Kusi (2008) reported that *J. curcas* seed oil applied at 0.5 ml/150 g recorded a significantly lower toxicity against *C. maculatus* when compared to 2.0 ml/150 g grains. Plant secondary metabolites have been subjected to empirical investigation to unravel those constituents that have insecticidal properties (Akhtar and Isman, 2004). The mode of action of fixed plant oil could be asphyxiation (physical action) whereby oil droplets act as physical barrier to respiration due to their clogging air passages (spiracles) on the insect body (Credland, 1992; Umar, 2013). However, there could be secondary action of the chemical constituents (Don-Pedro, 1990). Extracts from different plants have been known to possess insecticidal properties against a wide range of insect pests (Abdullah and Muhammad, 2004). Other

authors opined that plants offer a cheaper sustainable alternative to synthetic insecticides, store design, fumigation and thermal distribution methods (Mukanga *et al*., 2010). Adebowale and Adedire (2006) and Harborne (2001) found that Jatropha seed oil contained a high proportion of sterols and terpene alcohols responsible for insecticidal action.

The progeny reduction in this study might be presumed to be caused by inhibitory action of the seed oil against egg hatchability and larval emergence. Boeke *et al.*, 2004; Koul *et al*., 2008 reported that the effect of plant extracts caused reduced larval, pupal and adult weight, prolonged larval and pupal period, reduced pupal recovery and impaired rate of adult emergence. The efficacy of fixed plant oil as insecticide have been attributed to ovicidal effects of the oil (Khalequzzman *et al*., 2007) leading to reduction in adult emergence. It could be suggested that the oil probably had effect on reproductive ability of the insect thus leading to reduction in adult emergence.

Seed weight loss could be associated with the larvae of *C. maculatus* in the seeds. Results from this study shows that *J. curcas* seed oil had high mortality effect against the adults irrespective of the age group. The reasons for seed weight loss have been explained in literature. Kshirsagar (2010) reported that the female beetle lays eggs on the seed surface and larva immediately after hatching bores into the seed and by the time it reaches the adult stage it consumes the seed cotyledons. The oil had effect on the insect since there was reduction in seed weight loss of treated cowpea seeds caused by the insect.

Research has been undertaken in order to evaluate the potential of many local plant species to control insect pests during grain storage in Africa (Bekele and Hassanali, 2001; Keita *et al*., 2000; Tapondjou *et al.*, 2005). For example, among the many plant species that have been used to control stored product pests is the physic nut, *J. curcas* plant. The efficacy of the plant seed oil against cowpea beetles has been reported by van Huis, 1991; Adabie-Gomez *et al*., 2006; Henning, 2007). The seed oil of *J. curcas* has been shown to contain toxic substances called phorboesters which exhibit insecticidal effects (Solsoby and Solsoby, 1997; Adebowale and Adedire, 2006). Human toxicity is ascribed to the curcin which occurs in the seed meal and not in the oil (Ratnadass and Wink, 2012).

Conclusion

This study elucidates the insecticidal potential of *J. curcas* seed oil against *C. maculatus.* Further studies should examine the isolation and characterization of the oil components with a view to formulating a biopesticide that will fit into pest management programme in storage. This is particularly possible in Nigeria because of the availability of the plant and ease of cultivation on marginal soil.

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