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INSECTICIDAL ACTIVITY OF *JATROPHA* SEED OIL AGAINST *CALLOSOBRUCHUS MACULATUS* (FABRICIUS) INFESTING *PHASEOLUS ACONITIFOLIUS* JACQ

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ABSTRACT
The Pulse beetle, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) is a cosmopolitan insect pest of *Phaseolus aconitifolius* Jacq. (Mataki). The insect multiplies very rapidly in storage where it causes very high losses. In the present investigation insecticidal activity of *Jatropha* seed oil against *Callosobruchus maculatus* has been carried out. The eggs of *C.maculatus* were more susceptible to *Jatropha* oil and shows mortality to all selected dosage. *Jatropha* seed oil was highly toxic to the eggs of *C. maculatus* ($F = 76.17$; $p < 0.001$) at all dosage levels compared to other pre-adult stages (larvae: $F = 65.13$; $p < 0.001$, Pupae: $F = 17.43$; $p < 0.001$).

INTRODUCTION

The pulse beetle, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) is a cosmopolitan insect pest of *Phaseolus aconitifolius* Jacq. (Mataki) and cowpea, *Vigna unguiculata* Linnaeus (Walpers). The insect is a field-to-store pest as its infestation of cowpea often begins in the field as the mature pods dry (Haines, 1991). The insect multiplies very rapidly in storage where it causes very high losses (Ouedraogo *et al.,* 1996). Caswell (1980) reported that *C. maculatus* damage level to cowpea reached 50% after six months of storage. According to IITA (1989), *C. maculatus* consumes 50-90% of cowpea in storage annually throughout tropical Africa. The bruchid infestation also affects seed quality, market value and can reduce cowpea seed viability to 2% after months of storage (Caswell, 1980). Many plants have thus been explored for their insecticidal action. Kamakshi *et al.* (2000), Dwivedi and Kumari (2000), Sharma *et al.* (1999) have all studied the effect of various botanicals on the pest *Callosobruchus* sp. This notorious pest attacks the stored pulses and has dispersed throughout the tropics and subtropics through the medium of commerce and now has become a real menace. The female beetle lays eggs on the seed surface and the larva immediately after hatching bores into the seed. By the time it reaches the adult stage it consumes the seed cotyledons.

Injudicious use of chemicals as a pest management method has lead to the development of resistance in insects towards them and has a high degree of residual effect due to their no degradable nature. Hence biodegradable plant products have gained attention as a substitute to chemicals in the field of insect toxicology.

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 (Arthur and phillips, 2002). Attention has been focused on the use of indigenous plants as sources of cheap and locally available pesticides and over 200 plant species have been reported to have insecticidal properties capable of controlling insects (Golob and Webley 1980). Among the many plant species that have been used to control stored product pests is the physic nut, *Jatropha curcas* L. (Fam.: Euphorbiaceae) plant. The efficacy of *Jatropha* seed oil against insect has been reported by Huis (1991), Adabie- Gomez *et al.* (2006) and Henning (2007).

The objective of this study was therefore to evaluate the toxicity of *J. curcas* seed oil to *C. maculatus* infesting *Phaseolus aconitifolius* Jacq.

MATERIALS AND METHODS

Culturing of the experimental insect

The culture of pulse beetle *C. maculatus* was reared in laboratory. About 1 kg of grains were sterilised in an oven at 80ºC for 24 hr, cooled and divided in 200g lots into plastic kliner jars. About 100 adult *C. maculatus* from laboratory stock cultures were introduced onto the sterilized grains in each jars and allowed four days to lay eggs. The jars were covered with muslin cloth to facilitate ventilation and were placed on inverted Petri dishes kept in a large tray containing industrial oil to prevent any crawling insects and mites from invading and contaminating the cultures. The cultures were kept in the laboratory (32 ± 1°C, 60 ± 5% RH. and 12hr: 12hr light: dark regime)

Collection and extraction of *Jatropha* seed oil

The seeds of *Jatropha curcas* were harvested from trees From the Akurdi and Lonavala areas. They were dried in a shade for seven days, shelled and the batches ground into a fine powder, Five hundred and fifty gram of the powder and 2.5L of petroleum ether (40°C) were used in the extraction of the oil with a Soxhlet's extractor for 48 hr. This yielded 250mL of clean yellow oil and the ether was recovered through a rotary evaporator. The oil was kept in the dark at 4°C until it was needed.

Experimental design of laboratory studies

Four oil treatments; 0.5, 1.0, 1.5 and 2.0 mL of *Jatropha* seed oil on 100g of grains and untreated grains (control) were used in the experiments. Each treatment was replicated four times and kept for 15 / 30 days.

Toxicity of the oil on *C. maculatus* adults in grains

The various rates of the *Jatropha* seed oil were applied to 100g of sterilized cowpea grains in one litre kilner jars and covered with muslin cloth. Each jar was infested with 20 unsexed *C. maculatus* adults. The number of dead insect(s) in each jar was assessed after 72 hr. Insects not responding to pin probe were considered dead. Untreated grains were used as control.

Data analysis

Data collected on insect counts were transformed using square root $(x+1)$ whereas percentage data were transformed using Arcsine (x /100)1/2 transformations. Abbot's formula was also used to adjust the data where there were deaths in control treatment.

RESULTS AND DISCUSSION

Effect of oil on eggs and immature stages inside cowpea seeds

Toxicity is one of the various effects of terrenes plant fixed oils to insects. Adebowale and Adedire (2006) found that *Jatropha* seed oil contained a high proportion of sterols and terpene alcohols responsible for insecticidal action (Duke, 1992). The current study showed *Jatropha* seed oil was highly toxic to the eggs of *C. maculatus* ($F = 76.17$; $p < 0.001$) at all dosage levels compared to other pre-adult stages (larvae: $F = 65.13$; $p < 0.001$, pupae: $F = 17.43$; $p < 0.001$). This resulted in a significantly reduced number of adults emerging from the seeds (Table 4). A limited toxicity was observed with 0.5mL/100g grain treatment especially on egg and larval stages. However, the oil treatments produced significantly lower adult numbers ($p < 0.05$) compared to the untreated control. In insect development, the eggs tend to be more tolerant to chemical treatments (Giga and Smith, 1987). However, in this experiment the *Jatropha* seed oil significantly inhibited egg development as observed by Adebowale and Adedire (2006)

Table 1: Percent adult mortality of *C. maculatus* after 72hrs exposure to *Jatropha* seed oil treated grains

Treatment mL/100g	% Mortality
Control	0.0 ± 0a
0.5 mL	76.6 ± 2.2a
1.0 mL	94.2 ± 2b
1.5 mL	98 ±0.2c
2.0 mL	99±0.2c

Table 2: Mean (±S.E) *C. maculatus* adults repelled by *Jatropha* seed oil

Treatment mL/100g	% Repellency
Control	0.0±0.0a
0.5 mL	47.5 ± 4.8b
1.0 mL	78.0± 3.9c
1.5 mL	96 ±2.8d
2.0 mL	98±2.8d

Table 3: Mean (±S.E) percent adult mortality on *Jatropha* seed oil treated grains store for up to 30 days

Treatment mL/100g	15 days	30 days
Control	0.0 ± 0.0a	0.0 ± 0.0a
0.5 mL	9.75 ± 2.4b	7.50±1.4b
1.0 mL	20.0 ± 2.0c	16 ± 3.4c
1.5 mL	28.25 ±1.4d	17.75 ±1.2c
2.0 mL	28.75 ± 2.0d	8.75 ± 2c

Table 4: Mean (±S.E) number of *C. maculatus* adults emerged from treated eggs and immature stages inside cowpea grains

Treatment mL/100g	egg	larva	pupa
Control	140.8 ± 20.6b	140.8 ± 20.6c	140.8 ± 20.6b
0.5 mL	4.5 ± 1.3a	22.3± 2.2b	84±11.4 a
1.0 mL	3.5 ± 0 a	15.3± 1.6 a	78±13.4 a
1.5 mL	1.2 ± 0.5 a	10.8± 1.9 a	67±10.3 a
2.0 mL	1.2 ± 0.5 a	10 ± 0.8 a	60±3.1 a

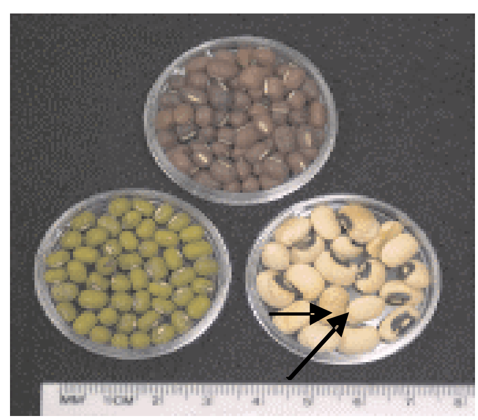

Figure:1 Culturing beans *Phaseolus aconitifolius Jacq*

Toxicity, repellency and persistence of *Jatropha* seed oil to *C. maculatus*

The percent mortality of *C. maculatus* adults in cowpea grains with different dosages of *Jatropha* seed oil is shown in Table 1. The oil was highly toxic ($F = 4214.61$; $p<4, 15\ 0.001$)

Figure 2: Damaged eggs

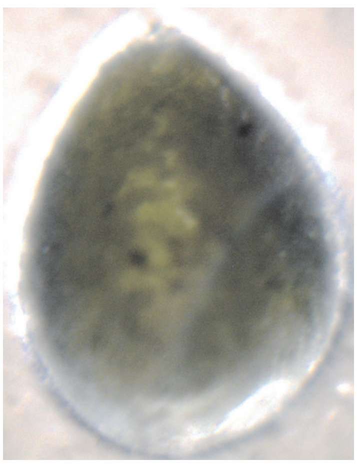

Figure 3: Single egg (400x)

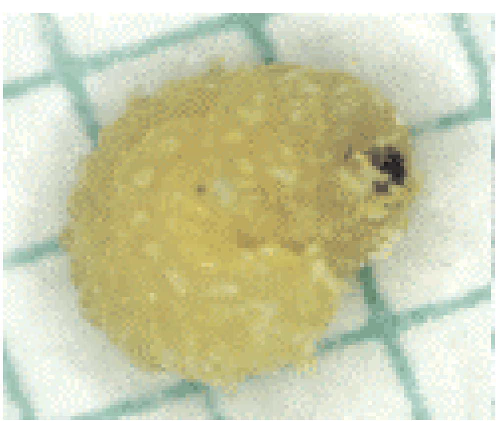

Figure 4: Larva

Figure 5: Early pupa

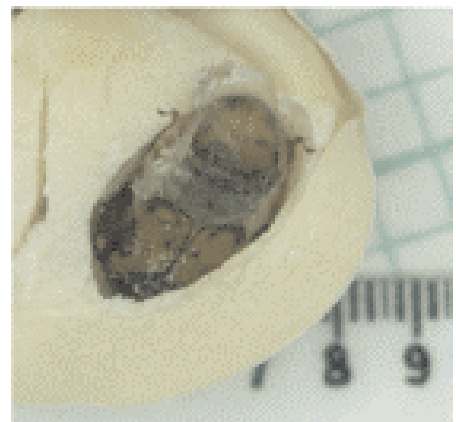

Figure 6: late pupa

Figure 7: Male *C. maculatus*

after 72-hour exposure at all dosage levels. The lowest dosage (0.5mL) recorded a significantly lower toxicity against C. maculatus. Paired t-test of the toxicity of the oil to insects showed the ability of the oil to repel *C. maculatus* was dosage dependent (($F = 145.44$; $p<0.001$); it increased with increase in the dosage of the oil (Table 2). The lowest dose (0.5mL) repelled 47.5% where highest dose (2.0mL) repelled 98.0%. There was no significant difference ($p>0.05$) between 1.5mL and 2.0mL. Persistency of the oil on the cowpea grains to cause mortality to *C. maculatus* at all. Treatment rates generally declined in storage from 15 to 30 days (Table 3). Within the treatment levels, the 2.0mL and 1.5mL doses caused significantly higher mortality ($F = 56.58$; $p< 0.001$) than the 0.5mL level after 15day of storage. The control of *C. maculatus* larvae and pupae inside the seeds will require higher oil concentrations. Accumulation of higher concentrations of the *Jatropha* seed oil in the grains could affect their quality and use as human food. *Jatropha* seed oil has shown promise as a

Figure 8: Female *C. maculatus*

stored product protectant against insect pests. However, with the closeness of stored grains to the table, the behavior of curcin, a toxic protein found in its extracts or oils should be monitored over time and establish how much of it ends up in various foods after processing the grains.

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