

Full Length Research Paper

Insecticidal effect of *Jatropha curcas* L. seed oil on *Callosobruchus maculatus* Fab and *Bruchidius atrolineatus* Pic (Coleoptera: Bruchidae) on stored cowpea seeds (*Vigna unguiculata* L. Walp.) in Niger

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We report the insecticidal efficacy of *Jatropha curcas* seed oil against two bruchid beetle species, *Callosobruchus maculatus* Fab and *Bruchidius atrolineatus* Pic, devastating stored cowpea seeds (*Vigna unguiculata*). *J. curcas* oil concentrations ranging from 0.0, 0.25, 0.5, 1.5, 2.5, 5.0 and 7.5 ml were mixed with 200 g of cowpea seeds before introduction of 10 pairs (5 males and 5 females) of *C. maculatus* or *B. atrolineatus* as the case may be. Mortality, fecundity and rate of emergence were observed and compared with untreated control and a standard (Deltamethrin). *J. curcas* oil reduced adult survival in both species, *B. atrolineatus* being more sensitive than *C. maculatus*. Oviposition was also reduced by 85 to 90% in the females of both species after exposure to 2.5 ml of *J. curcas* oil solution. Only 9% of *C. maculatus* nymphs emerged as adults in seeds treated with 2.5 ml of oil. In *B. atrolineatus*, emergence was reduced to 12% in seeds treated with 1.5 ml of oil.

Key words: Natural insecticide, *Jatropha curcas* oil, pea beetle, cowpea seed.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is among the principal world food leguminous plants (Pasquet and Baudoin, 1997). In Africa, it is appreciated for its green leaves, pods and seeds, which can be consumed (ISRA, ITA, CIRAD, 2005). In Niger, it is one of the principal cultivated crops, and has an important place in the rural population's food (Ibro and Bokar, 2001).

Cowpea seeds are attacked severally during storage by a variety of insect pests (Douma et al., 2002). In a study comparing the susceptibility of twenty varieties of cowpea to insect infestations in Niger, Douma et al. (2002) concluded that all varieties were subject to infestations by two Bruchid beetles, *Bruchidius atrolineatus* and *Callosobruchus maculatus* (Coleoptera: Bruchidae).

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During the period of cowpea storage, losses associated with insect damages in stored cowpea could reach 30 to 100% (Singh and Allen, 1979).

The insecticidal effect of many locally harvested plants has been evaluated as protectants of stored cowpea seeds before the advent of synthetic insecticides. More than 2000 vegetable species have been prospected (Ngamo and Hance, 2007; Benayad, 2008). *Jatropha curcas*, locally named pourghère, has been the center of recent researches as its oil has been demonstrated to have insecticidal effect (Ratnadas et al., 1997; Abdoul Habou et al., 2011; Katoune et al., 2011). The seed oil of *J. curcas* has been shown to contain toxic substances called phorbol esters which exhibit insecticidal effects (Makkar and Becker, 1997; Solsoloy and Solsoloy 1997; Adebowale and Adedire, 2006). Boateng and Kusi (2008) and Abdoul Habou et al. (2011) have variously shown the effectiveness of *J. curcas* oil in controlling insects of cotton, rice and cowpea.

This study evaluates the insecticidal activity of *J. curcas* oil on two Bruchidae beetles *C. maculatus* Fabricius and *B. atrolineatus* Pic (Coleoptera: Bruchidae) on stored cowpea.

MATERIALS AND METHODS

Cowpea seeds

TN5-78 cowpea seeds, a variety susceptible to bruchid infestations, were conserved in the freezer for one week, in order to disinfect the seeds.

Breeding of the beetles

C. maculatus and *B. atrolineatus* were reared separately, but following the same procedure. 200 g of cowpea seeds were placed in large boxes (11.5 × 14 cm) and 20 beetle couples, previously collected from the field, introduced into them. After four days, the insects were removed and the seeds were left at 30°C for 21 days until adult emergence.

Evaluation of the insecticidal activity of *J. curcas* oil

An emulsifying concentrate (EC) was prepared using 50% of *J. curcas* oil, 30% of ethanol as a stabiliser and 20% arabic gum as an adjuvant in order to fix active molecules on the plant. Different dilutions of the EC were prepared in order to have seven solutions containing respectively 0.25, 0.5, 1.5, 2.5, 5.0 and 7.5 ml of *J. curcas* oil solution. 200 g of treated cowpea seeds were then introduced inside small boxes (4.6 cm high with 4 cm of superior diameter and 3 cm of inferior diameter) before introducing twenty insects aged 24 h. In each box, 13.5 g of one of the seven above-mentioned solutions of *J. curcas* oil were applied, corresponding to the following doses: 0.25, 0.5, 1.5, 2.5, 5.0 and 7.5 ml per 200 g of seeds. A control box was also set up with 13.5 g of EC prepared without *J. curcas* oil (50% distilled water, 20% arabic gum and 30% ethanol). For each treatment, 5 boxes were set up as replicates. All boxes were placed in an incubator (30°C and 70% relative humidity). As a reference, deltamethrin (Decis 25 EC) treated seeds were also evaluated.

The number of dead insects was estimated, within one week of the experiment, in each box. The average mortality of the beetles (M_0) was expressed as a corrected mortality (Mc), taking into account the natural mortality observed on the control (M_t) according to Abbott's formula (Abbott, 1925):

$$Mc = \frac{M_0 - M_t}{100 - M_t} \times 100$$

To calculate LC 50, a binary logistic regression analysis was used:

$$\ln \left(\frac{p_i}{1 - p_i} \right) = \beta_0 + \beta_1 X_i$$

Where: p_i = probability; β_0 and β_1 = predictors, and X_i = doses (Duyme and Clautriaux, 2006).

Statistical analysis

The data collected were subjected to analysis of variance and the means separated using Fisher's least significant differences.

Adult bruchid emergence

In another set of experiments, we evaluated the potential effect of *J. curcas* oil on adult emergence. Twenty couples of *C. maculatus* or *B. atrolineatus* adults, aged 48 h, were introduced in small boxes containing 50 treated cowpea seeds. The same doses of *J. curcas* oil as presented above were applied. Each treatment was evaluated 5 times per bruchid specie. Five days after, corresponding to the maximum period of egg laying (Sanon, 1997) adult insects (alive or dead) were removed. Seeds with eggs were transferred into Petri dishes and placed in an incubator at 30°C. After 10 days, the rate of emergence was evaluated.

RESULTS

Effect of *J. curcas* oil on adult bruchids' survival

The percent mortality of *C. maculatus* and *B. atrolineatus* adults in cowpea grains with different concentrations of *J. curcas*' seed oil is presented in Table 1. The oil was highly toxic to both *C. maculatus* and *B. atrolineatus* after 7 days. For each concentration tested, a significant death rate was observed ($p \leq 0.05$), being higher in presence of high concentration of oil. Treatments with 10 and 15% (5.0 and 7.5 ml/200 g) *J. curcas* oil concentrations caused significant mortality on adult *C. maculatus* (42.0 and 48% respectively) after 72 h. This result was not different after 7 days of the introduction. Similarly, these treatments induced 65 and 84% mortality, respectively on *B. atrolineatus* after 3 days. The mortality rose to 95 and 98% respectively, after 7 days.

Effect of *J. curcas* oil on adult bruchid emergence

The oil of *J. curcas* seeds has an inhibitory effect on

Table 1. Effect of *J. curcas* oil concentrations on mean mortality of *C. maculatus* and *B. atrolineatus*.

<i>J. curcas</i> oil (ml)	<i>C. maculatus</i> (time of observation)							<i>B. atrolineatus</i> (time of observation)						
	1 days*	2 days	3 days*	4 days*	5 days*	6 days*	7 days*	1 day*	2 days*	3 days*	4 days*	5 days*	6 days*	7 days*
0	0±0.0 ^f	0±0.0 ^f	0±0.0 ^f	0±0.0 ^e	0±0.0 ^f	0±0.0 ^f	0±0.0 ^d	0±0.0 ^e	0±0.0 ^e	0±0.0 ^e	0±0.0 ^e	0±0.0 ^e	0±0.0 ^e	0±0.0 ^e
0.25	8±0 ^{de}	13±1.1 ^{ef}	18±0.5 ^{ef}	29±1.0 ^d	37±0.5 ^e	48±1.5 ^e	63±3.8 ^c	17±0.8 ^{de}	19±1.4 ^{de}	23±1.6 ^d	32±2.1 ^{cd}	45±1.4 ^d	66±0.4 ^d	75±3.0 ^d
0.5	14±0.8 ^{cd}	17±1.3 ^{ef}	25±1.2 ^e	33±1.3 ^d	46±1.7 ^{de}	62±1.8 ^d	72±2.5 ^{bc}	27±1.3 ^{cd}	37±2.1 ^{cd}	42±1.6 ^{cd}	50±2.4 ^{cd}	58±2.6 ^{cd}	74±1.7 ^c	81±0.8 ^c
1.5	19±1.3 ^{cd}	25±1.5 ^{de}	33±0.8 ^{de}	41±1.3 ^d	58±2.6 ^{cd}	72±1.8 ^{cd}	84±0.8 ^{ab}	26±1.0 ^{cd}	36±1.6 ^{cd}	47±3.6 ^{cd}	61±4.1 ^{bc}	74±2.9 ^{bc}	83±2.6 ^{bc}	86±1.7 ^{bc}
2.5	27±1.8 ^c	33±1.5 ^{cd}	41±2.3 ^{bc}	59±1.7 ^c	73±0.8 ^{bc}	82±1.1 ^{bc}	85±1.4 ^{ab}	51±2.1 ^c	53±3.7 ^{cd}	55±3.0 ^c	65±2.7 ^{bc}	79±2.5 ^{abc}	87±1.9 ^{abc}	95±1.4 ^{ab}
5.0	30±1.5 ^{bc}	35±1.8 ^c	48±2.3 ^{bc}	60±1.0 ^c	74±1.6 ^b	85±0.7 ^{bc}	87±0.8 ^{ab}	43±4.2 ^{cd}	56±4.4 ^{bc}	65±4.3 ^{bc}	80±2.4 ^{ab}	88±2.3 ^{ab}	94±1.6 ^{ab}	98±0.8 ^{ab}
7.5	32±1.3 ^{bc}	38±1.8 ^c	53±1.7 ^{cd}	61±3.1 ^c	75±2.0 ^b	84±1.4 ^{bc}	88±0.0 ^{ab}	67±3.2 ^b	74±2.0 ^{ab}	84±2.3 ^{ab}	91±2.6 ^a	92±2.6 ^{ab}	96±1.3 ^{ab}	100±0.0 ^a
Decis	92±0.8 ^a	93±1.1 ^a	99±0.4 ^a	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a	95±1.0 ^a	96±1.3 ^a	99±0.4 ^a	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a

The averages which have the same letter are appreciably not different. Turkey test, $\alpha=5\%$.

oviposition and subsequent emergence of adult *C. maculatus* and *B. atrolineatus*. The average number of eggs laid by *C. maculatus* in the control was 64.8±13.9 eggs. Under exposure to *J. curcas* oil, the oviposition rate reduced as indicated in Table 2. Oviposition was significantly inhibited in both insect species with concentrations of 5.0 and 7.5 ml of *J. curcas* oil solution, after four days infestation.

In the control, 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 as stated in Table 2.

The lethal concentration of *J. curcas* oil that effected 50% mortality of *C. maculatus* at the end of the 1st, 2nd and 3rd days were, respectively, 3.75, 3.10 and 2.75 ml. For *B. atrolineatus*, they were, 2.90, 2.45 and 2.25 ml, respectively (Table 3).

DISCUSSION

J. curcas oil contains phorbol esters with activate the protein kinase C (PKC) (Makkar et al., 1997). This phenomenon endues a cellular proliferation or apoptosis (Blumberg et al., 1987). The toxicity

of phorbol esters are demonstrated by many studies on insects pest species (Solsoly and Solsoly; Ratnadas et al. 1997; Abdoul Habou et al., 2011). This study compared the sensitivity of two beetles pea bruchid

In this study, we have demonstrated the toxic effect of *J. curcas* oil on *C. maculatus* and *B. atrolineatus* adult's survival and emergence. *J. curcas* oil reduced adult survival in both species, *B. atrolineatus* being more sensitive than *C. maculatus*. Oviposition is also reduced in the females of both species, reaching up to 85 and 90% fewer eggs deposited after exposure to a 2.5 ml of *J. curcas* oil solution. Only 9% of *C. maculatus* nymphs emerged to adults in the presence of a 5% solution of oil. In *B. atrolineatus*, emergence was reduced to 12% with the use of a 1.5 ml of oil solution. Similar result was found by Ravindra and Kshirsagar (2010) on the development of *C. maculatus*. In their work, these authors showed that the eggs of *C. maculatus* were the most susceptible at the developmental stage to *J. curcas* oil. Our results are comparable to those obtained by Udo (2011) and Singh et al. (1978) who used pure oil. Udo (2011) showed that palm oil has a significant effect on progeny development of *C. maculatus* as Singh et al.

(1979) demonstrated that groundnut oil at 5 ml/kg completely protected cowpea seeds in storage for the same insect for up to 180 days. The oil treatment prevented emergence of progeny rather than affecting oviposition or mortality of the adult weevils.

Adebowale and Adedire (2006) conducted a similar experiment in the laboratory on *C. maculatus* Fabr devastating insects of cowpea in Nigeria. They observed a significant reduction in laying for all tested concentrations and a total inhibition of eggs and larvae. The number of eggs laid by *C. maculatus* females was also reduced. They also observed that the emergence rate of the adults is null for all the concentrations applied. These authors observed that the seeds are thus protected during 12 weeks and advance that the insecticidal effect could originate in sterols and terpenes alcohol contained in *J. curcas* seeds' oil. Boateng and Kusi, 2008 highlighted the insecticidal effect of *J. curcas* oil on *C. maculatus* (Coleoptera: Bruchidae) and its parasitoid *Dinarmus basalis* (Hymenoptera: Pteromalidae). The adults of the two species have the same sensibility for the toxic effect of *J. curcas* oil. *C. maculatus*' eggs are more sensible than those of *D. basalis* because they are protected by the

Table 2. Effect of *J. curcas* oil concentrations on oviposition and rate of emergence of *C. maculatus* and *B. atrolineatus*.

<i>J. curcas</i> oil (ml)	<i>C. maculatus</i>			<i>B. atrolineatus</i>		
	number of eggs*	number of adults emergent's*	rate of emergent*	number of eggs*	Number of adults emergent's*	rate of emergent*
0	64.8±13.8 ^a	48.8±8.7 ^a	76.2±0.1 ^a	75.4±11.5 ^a	57±1.9 ^a	76.1±1 ^a
0.25	40.8±10.2 ^b	25.8±10.5 ^b	63.4±0.1 ^b	59.6±8.5 ^b	16.8±6.3 ^a	27±01 ^b
0.5	38.6±6.4 ^b	11.6±8.9 ^b	29.1±0.1 ^b	47.2±4.3 ^b	6.6±8.0 ^b	13.2±0.1 ^b
1.5	36.6±12.0 ^b	7.8±5.1 ^b	19.1±0.1 ^b	18.8±7.0 ^c	2.6±7.6 ^c	11.3±0.1 ^b
2.5	9.6±3.0 ^c	1.0±1.2 ^c	11.6±0.0 ^c	8.2±4.3 ^c	0±0.0 ^c	0±0.0 ^c
5.0	0±0.0 ^c	0±0.0 ^c	0±0.0 ^c	0±0.0 ^c	0±0.0 ^c	0±0.0 ^c
7.5	0±0.0 ^c	0±0.0 ^c	0±0.0 ^c	0±0.0 ^c	0±0.0 ^c	0±0.0 ^c
Delthametrin	0±0.0 ^c	0±0.0 ^c	0±0.0 ^c	0±0.0 ^c	0±0.0 ^c	0±0.0 ^c

Averages which have the same letter are not different, Turkey test $\alpha=5\%$.

Table 3. Regression coefficients estimated by logistic binary analysis.

Parameter	observation time					
	<i>C. maculatus</i>			<i>B. atrolineatus</i>		
	1 day	2 days	3 days	1 day	2 days	3 days
Probability	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Cstant (β_0)	-2.51	-2.19	-1.65	-2.13	-1.86	1.62
Coef β_1	0.033	0.033	0.029	0.036	0.047	0.049
DL 50 (ml)	3.75	3.10	2.75	2.90	2.45	2.25

seeds. Solsoloy and Solsoloy (1997) also showed the effectiveness of *J. curcas* seeds oil on *Sitophilus zeamais* Motschulsky and *Callosobruchus chinensis* L. insects devastating seeds of maize and *Phaseolus vulgaris* L.

Ogunleye et al. (2010) tested the properties of seed oil of three botanicals namely *J. curcas*, *Heliathus*

annus (sunflower) and *Cocos nucifera* (coconut) on maize weevil, *Sitophilus zeamais* Mots. The results of insects treated with all dosage rates of *C. nucifera* showed a significantly higher mortality when compared with the control. The least rate of application of *J. curcas* produced 70% mortality after 24 h, while the dosage of 0.3 ml and 0.4 ml produced 80% mortality after 24 h. The control experiment remained at 0% level throughout the period of the experiments.

J. curcas seeds' oil has a toxic effect on the adults of *C. maculatus* and *B. atrolineatus*. This toxicity increases with the concentration. A reduction of laying of the females of these two species is observed for weak concentrations. The emergence rate of adults is more significant to *B. atrolineatus* than *C. maculatus*. In Niger, the peasants' cans used 7.5 ml/200g to detrued 100% of beetle's pea contained in the grains which will be preserving after 24 h. Germination test performed on treated seeds showed germination rate above 80%. *J. curcas* oil does not affect the germination of seeds.

Conflict of Interest

The authors have not declared any conflict of interest.

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