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Toxicity and persistence of *Boscia senegalensis* Lam. (Ex Poir.) (Capparaceae) leaves on *Callosobruchus maculatus* Fab. (Coleoptera:Bruchidae)

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ABSTRACT

In this study, we examined the toxicity and persistence of ground fresh leaves of *B. senegalensis* on the development of *Callosobruchus maculatus*, major insect pest of cowpea (*V. unguiculata*) in the sahelian area. The effect of the product has been evaluated over the three developmental stages (egg, larvae and adult) of *C. maculatus*. At high concentration (4 g/l of ground leaves), 100 % of adults were killed within 24 h of exposure to the leaf extract, and the development of newly laid eggs and neonate larvae was also inhibited. However, the ground leaves of *B. senegalensis* have a variable efficacy against the bruchid instars larva. The 2nd larvae stage (L2) of *C. maculatus* developing inside the seeds have proved to be highly susceptible while the 4th larval stage was relatively tolerant. The study of the toxicity persistence of the leaves of *B. senegalensis* showed that whatever its concentration, the efficacy of crushed leaves of *B. senegalensis* decreased with the storage time. At a concentration of 20 g/l, the effect of *B. seneglensis* can persist up to 7 days.

Keywords: *Biopesticides, Bruchids*, cowpea, pest control, Niger.

INTRODUCTION

The cowpea beetle, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) is a cosmopolitan insect pest of cowpea, *Vigna unguiculata* Linnaeus (Walpers). It is a field-to-store pest as the infestation of cowpea often begins in the field (Huignard et al., 1985) and then continues in storage where the damages can be important without any protect measure. Caswel (1960); Ouédraogo et al.,

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(1996); Sanon et al., (1998) reported that about 50% of the production was lossed after six months of storage.

According to IITA (1989) *C. maculatus* consumes annually 50-90% of cowpea in storage throughout tropical Africa. The bruchid infestation also affects seed quality, its market value and its viability up to 2 % after 3 months of storage (Caswel, 1980; Ofuya and Credland, 1995). Several control methods have been used over the years to protect cowpea grains in storage, but the use of synthetic insecticides has been very dominant (Mbata et al., 2005; Adabie-Gomez et al., 2006).

The problem of residues resulting after mixing these synthetic organic insecticides with pulses beyond the permissible tolerance levels for control of beetle's infestation, has forced researchers to look for alternate safe management of pulse beetles. The integration of natural insecticide products from locally available plants for use in storage by the farmers in developing countries appears to be quite safe and promising (Jilani et al., 1988; Dixit and Saxena, 1990; Varma and Dubey, 1999).

Leaf, bark, and seed powder or oil extracts of plants reduce oviposition rate and suppress adult emergence of bruchids and also reduced seed damage rate when mixed with stored grains (Keita et al., 2001; Tapondjou et al., 2002; Adedire and Akinneye, 2004; Rahman and Talukder, 2006; Shukla et al., 2007, Bamaryi et al., 2007). Powdered materials of orange, lemon, lime, lemon grass, cinnamon, derris, nutmeg, cactus and ginger were evaluated by Rajpakse and Vaneben (1997) against bruchids at high dose 300g Kg-1seeds.

However, low dose rate (25g kg-1) of dried powders of clove, red and black peppers have also been reported to prevent the infestation of bruchids (Aslam et al., 2002).

The possible mechanism of preventing insects to attack the grains have been described as the reduction of insect movement and death through occlusion of their spiracles, thereby, preventing respiration via trachea (Onayade, 2000; Raina et al., 2002; Semnani et al., 2006). The reduction in eclosion could either be due to egg mortality or larval mortality or even reduction in the hatching of the egg.

The present study was carried out to evaluate the effect of ground fresh leaves of *B. senegalensis* Lam. (Ex Poir.) on the developmental stages of *C. maculatus*.

MATERIALS AND METHODS

The study was conducted under natural conditions (October to December) in the Laboratory of Applied Entomology of the Faculty of Sciences of A.M. University of Niamey.

Bruchids rearing

Callosobruchus maculatus adults were collected from cowpea seeds bought on the local market of Niamey. They were brought back to the laboratory and then mass reared under specific laboratory conditions.

Fifty pairs of 2- or 3-days -old *C. maculatus* adults were placed for 48 h in rearing boxes containing seeds of *V. unguiculata* of the variety, TN 5-78. The females laid their eggs on these seeds and their offsprings complete their development within the cotyledons. The adults were isolated after emergence and used either for the production of a new generation or for direct experiments.

Experimental design in the laboratory studies

Toxicity of the ground leaves on C. maculatus adults

The various concentrations (1.25 g/l, 2 g/l, 4 g/l) of *B. senegalensis* ground fresh leaves were applied in one liter glass bottle

containing 50 g of sterilized cowpea grains. Each bottle was infested with 20 unsexed *C. maculatus* adults and the number of dead insect(s) was assessed after 24 and 48 and 72 hours. Insects that did not respond to pin probe were considered dead. Untreated grains were used as control. Each treatment was repeated three times

Persistence bioassay

For each of the following concentrations (0; 2; 4; 6; 8; 10 and 20 g/l), eight (8) batches of three (3) one liter glass bottles containing 50 g of cowpea seeds were prepared. After 24 h, 20 unsexed of *C. maculatus* were daily introduced in a batch until all the eight (8) batches of each treatment were used.

In each experimental situation, the insects were removed after 24 hours. They were then kept in the laboratory for 24 h before counting the dead adults.

The experiment was terminated when the mortality rate in the treated batches became the same as in the control.

Effects of ground leaves of B. senegalensis on reproductive activity and the development of C. maculatus

Twenty pairs of 48 h old *C. maculatus* were introduced into a bottle of one liter, containing 50 g of healthy seeds of cowpea and ground fresh leaves of *B. senegalensis*. The concentrations of 0 g/l (control), 1.25, 2 and 4 g/l were applied. Each treatment was replicated three times. Five days later, the dead insects were removed from bottles and the seeds bearing the insects eggs were transferred into Petri dishes and then maintained in the laboratory. At the 10th day after the infestation, the number of hatched eggs was counted, and then the seeds were incubated until adults' emergence. The number of eggs laid, the percentage of egg mortality, the larval mortality and rate the of adult emergence were determined. The treatment was repeated three times.

Effect of *B. senegalensis* leaves on the eggs

100 seeds, each bearing a fresh egg of *C. maculatus* were introduced into a 1 liter bottle treated with a single concentration of ground leaves of *B. senegalensis*. Concentrations of 0 g/l (control), 1.25 g/l, 2.5 g/l and 4 g/l were applied. For each concentration, the test was carried out in 24 hours. The seeds were then transferred to Petri dishes. 10 days later, the egg mortality was estimated by counting the eggs hatched and sterile in each bottle. The treatment was repeated three times.

Effect of *B. senegalensis* leaves on immature stages of *C. maculates*

The experience consisted to study the effect of green ground leaves of *B. senegalensis* on 2^{nd} instar larvae and 4^{th} instar larvae of *C. maculatus*. Concentrations of 0 g/l (control), 1.25 g/l, 2.5 g/l and 4 g/l were applied. For each concentration and each experimental situation, the test was conducted with 100 individuals of each pre-imaginal stage. These individuals were spread due to one larva per seed. 24 hours, the seeds were transferred into Petri dishes and monitored until the end of the emergence in the control. The treatment was repeated three times.

Statistical analysis

All experiments were in triplicate (except the last one). The percentage of mortality at each developmental stage was subjected to an analysis of variance and differences found were assessed using Fisher tests at the 5% level of significance using the software package XLSTAT.

RESULTS

Effects of leaves on the bruchid adults

The presence of ground leaves of *B. senegalensis* had significantly affected survival of adult beetles. Mortality increased with increasing concentration of groud leaves.

With the lowest concentration (1.25g/l), 22% mortality rate of *C. maculatus* was observed after 24h of exposure and with the higher concentration (4 g/l) 100% of adults exposed were killed after 24h (Figure 1). The analysis of the figure 1 let appear that with the concentrations 1.25g/l and 2 g/l, the mortality rates did not vary in function of the exposure time. Whatever the concentration, the mortality rate remained the same regardless of the duration of exposure.

Persistence bioassay of ground fresh leaves of *B. senegalensis*

Whatever the concentration of product, the crushed leaves of *B. senegalensis* efficiency decreases according to the conservation time (Figure 2). Indeed, after 24h of exposure, it appeared that *B. senegalensis* fresh leaves had no effect on adult weevils at the concentration of 2 g/l. But for the higher concentrations, the product continues to have a lethal effect on *C. maculatus* adults. With the concentration of 20 g/l, the results showed a mortality rate more than 50% 3 days after the introduction of the product.

Effects of ground material on laying and egg development of C. maculatus

The fresh leaves of *B. senegalensis* affected considerably the laying and egg development of *C. maculates*. This effect results from significant reduction ($\alpha \leq 5$ %) in the number of egg laid and the hatchability of eggs. Thus the number of eggs laid varied from 51 in the control to 9 at 4 g/l with respectively 7,84 and 100% not hatched eggs.

Analysis of results pointed out that the emergence of adults was significantly affected by the treatment. A rate of emergence of 67.89 was observed in the control and 0% when a concentration of 2 g/l was applied.

Effect of B. senegalensis ground material on immature stages of bruchid

Eggs developing outside the seed were very susceptible to *B. senegalensis* fresh leaves extract. At the lower concentrations (1.25g/l and 2 g/l) percentage of egg mortality of 85% and 99% were respectively observed within 24h. With 4 g/l, no larvae were hatched from the egg during the same exposure time (Figure 3).

The effect of the ground leaves extract on stages developing inside the seed was influenced by larval stage (Figure 4). In our experimental conditions, the 2nd larval stage (L2) was more susceptible than the 4th larval stage (L4).

Table 1 : *B. senegalensis* concentrations effect on *C. maculatus* fecundity and egg mortality.

Concentrations (g/l)	Egg number	Unhatched eggs	Rate of egg mortality (%)
Control	511,13 ±21,12 a	41 ± 6,31	8,02 ± 0,15% a
1.25	317,33 ± 12,05 b	72 ± 11,23	22.71 ± 3,31 %a
2.5	161,45 ± 17,77 c	147 ± 2,34	91,30 ± 19,07%b
4	76,15 ± 19,02d	76 ± 19,02d	100% ab

In each column, values followed by different letters were significantly different.

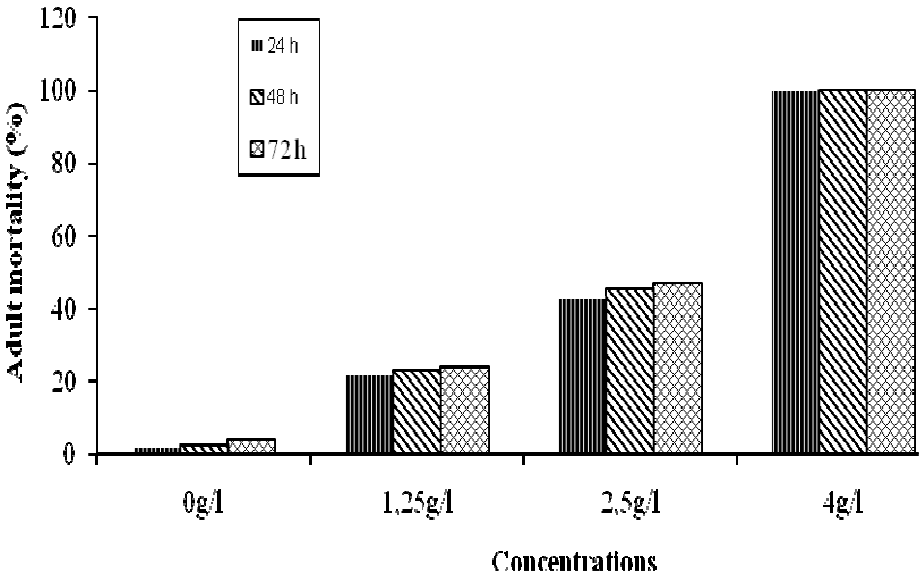

Figure 1: Mean rate of *C. maculatus* adult mortality in function of exposure time.

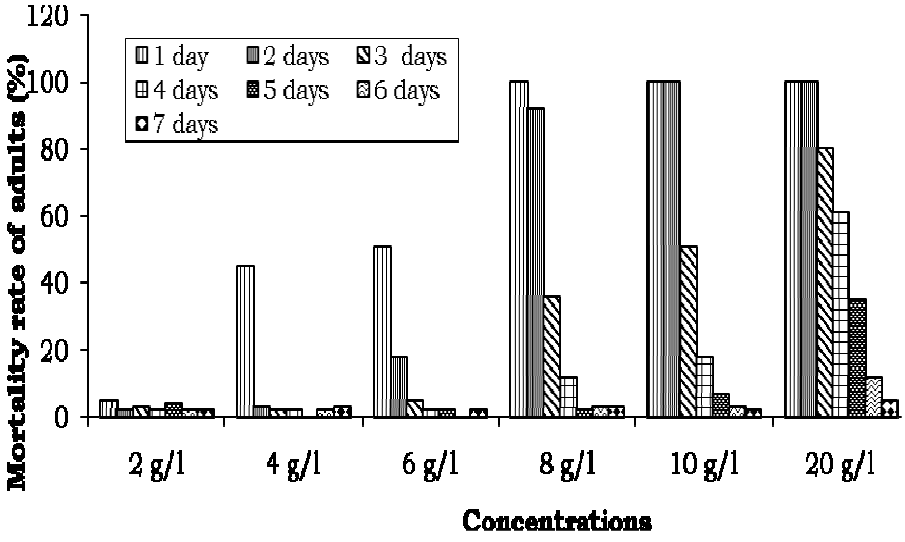

Figure 2: Persistence of *B. senegalensis* leaves effect in function of the concentration.

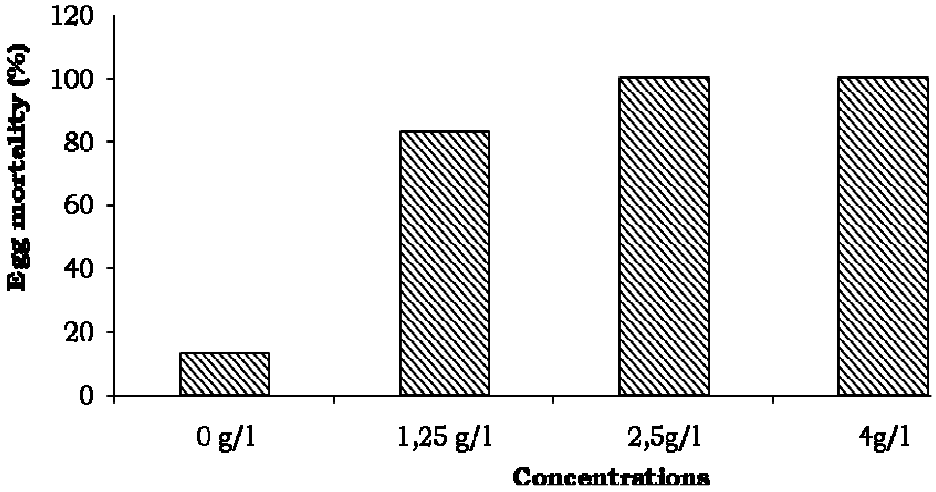

Figure 3: *C. maculatus* egg mortality after 24h exposure to increasing concentrations of *B. senegalensis.*

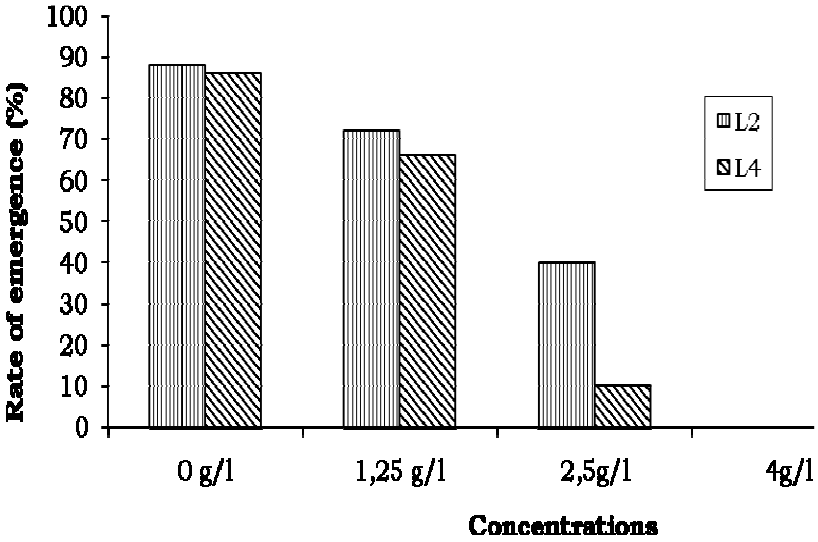

Figure 4: Rate of *C. maculatus* adults emergence from treated larvae inside cowpea seed.

DISCUSSION

The results showed that ground fresh leaves of *B. senegalensis* affected significantly the development of *C. maculatus*.

In our experimental conditions, we observed that all adults of *C. maculatus* died after 24 h of exposure to ground leaves at 4 g/l. There was no oviposition as mentioned by Glitho et al., (1997). The concentration of 4 g/l was also very toxic to eggs and larvae of *C. maculatus*. However the persistence of the action of that ground material would depend on the concentration applied. Indeed, at a

concentration of 2 g/l, it appears that the mortality rate remained relatively constant after 24 h of exposure. With 20 g/l, the effect persisted seven days after the introduction of the product. This weak efficiency of leaves observed with concentration of 2 g/l was related to a little concentration of these components. Also, the leaves of *B. senegalensis* caused a significant reduction in the number of eggs laid and the inhibition of hatching.

The reduction of laying could be the fact of the early death of *C. maculatus* adults due to the ground leaves, as showed by Mazibur and Gerhard (1999) and Ketoh et al. (2002) by studying the effect of the essential oil of *Acorus calamus* on *Callosobruchus phaseoli* and that of *Cymbopogon goganteus* on *C. maculatus*.

The product had also a quite larvicide effect as showed by Toufique et al. (2009).

The findings of the present investigation are in accordance with those of other workers who have previously reported that plant material affected significantly the development of bruchids (Adedire and Akinneye, 2004; Rahman and Talukder, 2006; Shukla et al., 2007; Bamaryi et al., 2007; Seck et al., 1993; Doumma et al., 2006; Toufique et al., 2009).

Studies carried out by Toufique (2001) reported that the toxicity of Niger's *B. senegalensis leaves*, reduce *Bruchidius atrolineatus* Pic. and *C. maculatus* oviposition. Kjaer et al. (1973) have reported that *B. senegalensis* leafless twigs contain methyl and isopropyl glucosinolates.

Seck (1994) showed that fresh crushed leaves mixed with seeds of cowpea at a concentration of 4% (P/P) killed *C. maculatus* adults within 24 h and inhibited the production of the F1 progeny.

According to the same author, at a concentration of 2% (w/w), Bruchid emergence was reduced at 95% and the reduction of its damage was estimated to 93% compared to the control.

The findings of the present investigations indicate that botanical sources might be useful as insect control agents for commercial use. Ground leaves of *B. senegalensis* were effective in reducing the oviposition, emergence and survival of *C. maculates.*

Application of plant material to grain seeds for storage is an inexpensive and effective technique as these substances are of low cost for household storages of chickpea especially during months of infestation

Conclusion

This study showed that *B. senegalensis* had a lethal effect on all the development stages of *C. maculatus.*

The results indicated that ground fresh leaves of this plant affected significantly the longevity, egg laying and the larval development of this insect.

However the persistence of the insecticidal activity of the ground fresh leaves seemed to depend on the concentration applied. Our data showed that more the concentration was important, more the insecticidal activity persisted.

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