

Full Length Research Paper

Insecticidal activity of *Boscia senegalensis* (Pers.) Lam ex Poir. on *Caryedon serratus* (Ol.) pest of stored groundnuts

Momar Talla GUEYE^{1*}, Dogo SECK², Seynabou BA¹, Kerstin HELL³, Mbacké SEMBENE⁴, J-P. WATHELET⁵ and Georges LOGNAY⁶

¹Institut de Technologie Alimentaire, Hann-Dakar, BP 2765, Sénégal.

²Centre Régional de Recherche en Ecotoxicologie et Sécurité Environnementale (CERES/Locustox), km 15 Route de Rufisque, BP 3300 Dakar, Sénégal.

³CIP (International Potato Center) – BP 08 0932- Cotonou, Bénin.

⁴Département de Biologie Animale, Faculté des Sciences de Dakar, UCAD, BP 5005, Dakar, Sénégal.

⁵Université de Liège, Gembloux Agro-Bio Tech, Unité de Chimie Générale et Organique, 2 Passage des Déportés -5030 Gembloux, Belgique.

⁶Université de Liège, Gembloux Agro-Bio Tech, Unité de Chimie Analytique, 2, Passage des Déportés -5030 Gembloux, Belgique.

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This publication describes the efficacy of *Boscia senegalensis* for the control of the groundnut bruchid, *Caryedon serratus* (Ol.). Crushed *B. senegalensis* fresh leaves and fruits were mixed with groundnuts and stored in hermetic containers. A high sensitivity of *C. serratus* adults at relatively low concentrations of the plant was observed. After 24 h of exposure, fresh crushed leaves mixed with peanuts seeds at 6% (w/w) induced 100% mortality of adults. Same results were obtained with fresh crushed fruits at only 3% (w/w). Only 6% leaves or fruits of *B. Senegalensis* ensured a complete protection of groundnuts pods and shelled nuts against the weevil *C. serratus*. The results show that this natural product, which liberate enzymatically Methyl isothiocyanate could potentially protect stored groundnuts at farm level in Africa.

Key words: *Boscia senegalensis*, *Caryedon serratus*, fumigant effect, stored groundnuts, insecticidal activity.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the major sources of protein that can significantly improve the livelihood of the rural poor and increase foreign exchange earnings for many West African countries. It generates 60, 42 and 21% of the rural cash earnings among groundnut producers in Senegal, Niger and Nigeria respectively, and accounts for about 70% of rural employment in Senegal (Ndjeunga et al., 2006). Groundnut is the major agricultural product of Senegal after millet/sorghum. The two crops occupy about 80% of

the cultivated land in Senegal. Groundnut contributes more than 28% of daily calories intake of Senegalese (Evers, 1997). Apart from being a staple food, groundnut production, traditional processing and marketing are a main source of income for many rural households (Gaye, 2008).

Insect damage to basic food, cash crops or seeds appear to be one of the most serious constraints to increasing agricultural production and rendering systems more sustainable (Guèye et al., 2011). Groundnut (*Arachis hypogaea* L.) was the primary cash crop for three West African countries (Senegal, Nigeria, Gambia), but is prone to post-harvest insect infestation and losses. Due to severe losses caused by the groundnut bruchid,

*Corresponding author. E-mail : gueyemt@gmail.com.

Caryedon serratus (Ol.), which may reach 40% in six months (Gillier and Bockelée-Morvan, 1979), chemical pesticides are often applied under farm level conditions (CORAF, 2007). Unfortunately, their use is not without inconvenience and risk, as reported by many authors (Wilson and Tisdell, 2001; Maumbe and Swinton, 2003, World Health Organization, 2008; Sow et al., 2008). Repeated applications of these insecticides without respecting the recommended dose can lead to resistance that appears increasingly in target insects (Delobel and Tran, 1993; Fields and Muir, 1995; Haubruge and Amichot, 1998, Nauen et al., 2003). Bell (2000) reported that the global withdrawal of methyl bromide that was formerly used for the protection of groundnuts will be widely used in Senegal, by 2015. Until recently, methyl bromide and phosphine were the most common fumigants used in post-harvest protection due to their wide spectrum of action, but there is need to look for alternatives including potential natural pesticides. Beyond ecological and environmental problems, the main constraint to the use of synthetic insecticides by African farmers is insufficient financial means to procure pesticides and lack of basic training on the use of pesticides.

The use of plants against stored grain pests is a well-known practice prior to the advent of synthetic pesticides in rural areas. Several studies conducted in Senegal and other African countries (Seck et al., 1993; Sanon et al., 2002, 2005; Thiaw, 2007) reported on the biological activities of local plants for crop protection and pest management. Seck et al. (1993; 1994) studied the biological activities of *Senna occidentalis* L., *Calotropis procera* Ait. and *Boscia senegalensis* (Pers.) Lam. ex Poir. on five insect species different from *C. serratus* and identified the active ingredients. These encouraging results were the motivation to evaluate the efficacy of *B. senegalensis* for the control of *C. serratus*.

MATERIALS AND METHODS

Insect cultures

The mother strains of *C. serratus* were collected from pods of *Piliostigma reticulatum* at Keur Baka (15°57'W, 13°56'N), in the region of Kaolack, Senegal. Insects were maintained using groundnut seeds in glass jars of 1 L in the laboratory. After 2 to 3 days of oviposition, infested seeds were removed and put in Petri dishes and then placed in a controlled incubator at 33 ± 2°C and 70 ± 5% relative humidity until cocoon formation. Cocoons were transferred into other Petri dishes at ambient temperature (22 ± 4°C) and relative humidity 80 ± 5% till the emergence of adults.

Plant materials

Peanut pods used in this experiment were collected at the same locality. They were stored in the freezer for at least one week to eliminate any hidden infestation. Leaves and fruits of *B. senegalensis* were collected on a daily basis according to experimental needs, at Thies city (16°56'W, 14°48'N).

Fumigation tests without groundnuts

One day old adults of *C. serratus* were submitted to fumigation tests over a period of 10 days with fresh leaves and fruits of *B. senegalensis*, which were finely crushed with a laboratory mill (Blender type). Experiments were conducted in four replications with 25 unsexed insects using 1 L glass jars. Increasing concentrations of 1, 2, 3, 4, and 6 g/L of either fresh crushed leaves or crushed fruits were tested in 4 replicates. In parallel, experiments with whole fresh leaves and fruits were tested under the same conditions, but only at concentrations of 6 and 10 g/L. 25 unsexed untreated insects were followed as the control. In each jar, the plant substrate was placed at the bottom, inside a Petri dish with a perforated top. Insects were placed in the jar, outside the Petri dish to avoid their contact with the product, while exposing them to the volatiles emanating from the plant material. Jars were hermetically sealed and stored at 22 ± 4°C. Jars were open every 24 h for counting and removal of dead insects. Corrected mortality rates were calculated according to Abbott formula (Abbott, 1925):

$$\text{Observed mortality rate (\%)} = 100 \times \frac{\text{Number of dead beetles}}{\text{Total number of beetles}}$$

$$\text{Corrected mortality rate (\%)} = 100 \times \frac{(\text{Observed mortality} - \text{Control mortality})}{100 - \text{Control mortality}}$$

Fumigation tests in presence of groundnuts

Fumigation tests were repeated on 100 g of groundnut kernels or groundnuts within pods, mixed with the same doses of fresh leaves or fruits of *B. senegalensis*, finely crushed as previously described prior to introducing the perforated Petri dishes containing 25 adults of *C. serratus*.

Statistical analysis

Observed mortalities were subjected to Analysis of Variance with SPSS 15.0 software followed by Newman-Keuls' test in order to evaluate the differences between treatments. Moreover, LD50 were calculated by LD50 RIZA software.

RESULTS

Evaluation of the insecticidal activity of *B. senegalensis* fresh leaves and fruits on *C. serratus* adults

Preliminary biological tests of *B. senegalensis* fresh leaves and fruits (Table 1) showed that intact organs have no effect on *C. serratus* adults, even after 10 days of exposure at 10 g/L. Maximum percentage mortalities recorded for whole leaves and fruits were only 2 and 3%, respectively. Under the same conditions, crushed fresh fruits and leaves showed 100% mortality of the insects within 24 h at low dosages of 2 and 3 g/L, respectively. Lower doses are not effective. Behavioral observation of the insects exposed to the crushed *B. senegalensis* leaves and fruits showed a significant agitation sometime after the exposure, followed by increased tibia volume

Table 1. Mortality of *Caryedon serratus* adult after 10 days of fumigation with crushed fresh leaves (CFL) or whole fresh leaves (WFL) and crushed fresh fruits (CFF) or whole fresh fruits (WFF) of *Boscia senegalensis*.

Treatments	Doses in g/L	Percentage corrected mortality (cumulative)				
		1 day	2 days	3 days	5 days	10 days
CFL	2	17	25	25	27	31
	3	100	100	100	100	100
	4	100	100	100	100	100
	6	100	100	100	100	100
WFL	6	0	0	0	0	1
	10	0	0	0	0	2
	2	100	100	100	100	100
CFF	3	100	100	100	100	100
	4	100	100	100	100	100
	6	100	100	100	100	100
WFF	6	0	0	0	0	0
	10	0	0	1	2	3

Table 2. Toxicity of crushed fresh leaves (CFL) and crushed fresh fruits (CFF) of *Boscia senegalensis* on *Caryedon serratus* adults in presence of groundnut pods (GP) and groundnut kernels (GK).

Treatments	Doses in g/L	Corrected mortality after 24 h	Treatments	Doses (g/L)	Corrected mortality after 24 h
CFL + GP	3	0	CFF + GP	1	0
	4	2		2	0
	6	100		3	100
	8	100		4	100
CFL + GK	2	0	CFF + GK	1	0
	3	0		2	0
	4	0		3	0
	6	100		4	100

and a permanent immobilization, leading to death within one to two hours.

Efficiency of *B. senegalensis* fresh leaves and fruits on *C. serratus* adults in the presence of groundnut

Results presented in Table 2 show 100% mortality of insects within 24 h of fumigation with crushed fresh leaves at 6 g/L mixed with groundnuts either as kernels or in pods. Lower doses were ineffective. For fresh crushed fruits, mixed with groundnuts pods, a concentration of 3 and 4 g/L killed all adults of *C. serratus*. Lower doses did not show any insecticidal activity.

Calculated LC_{50} (Table 3) showed that LC_{50} of 2.7 and 0.5 g/L were obtained respectively for leaves and fruits of *B. senegalensis* without substrate. The LC_{50} increased in the presence of groundnuts whether in pods or as kernels. On groundnut pods, the LC_{50} was 3.3 g crushed fresh leaves/100 g of groundnuts against 2.4 g crushed fresh fruits/100 g of groundnuts. On groundnut kernels, the LC_{50} of leaves and fruits was 3.3 and 3.5 g/100 g, respectively.

DISCUSSION

Results demonstrate a significant fumigant effect of *B. senegalensis* crushed fresh leaves and fruits on *C. serratus*

Table 3. CL50 after 24 h of crushed fresh leaves (CFL) and crushed fresh fruits (CFF) of *Boscia senegalensis* on *Caryedon serratus* adults in presence (pods, kernel) or without groundnut.

Treatments	Substrate	CL50 (g/L)	95% confidence
CLF	Without	2.7	2.5 - 2.9
CFF	Without	0.5	0.2 - 0.9
CLF	Pods	3.3	3.1 - 3.5
CFF	Pods	2.4	-
CLF	Kernels	3.3	3.1 - 3.5
CFF	Kernels	3.5	-

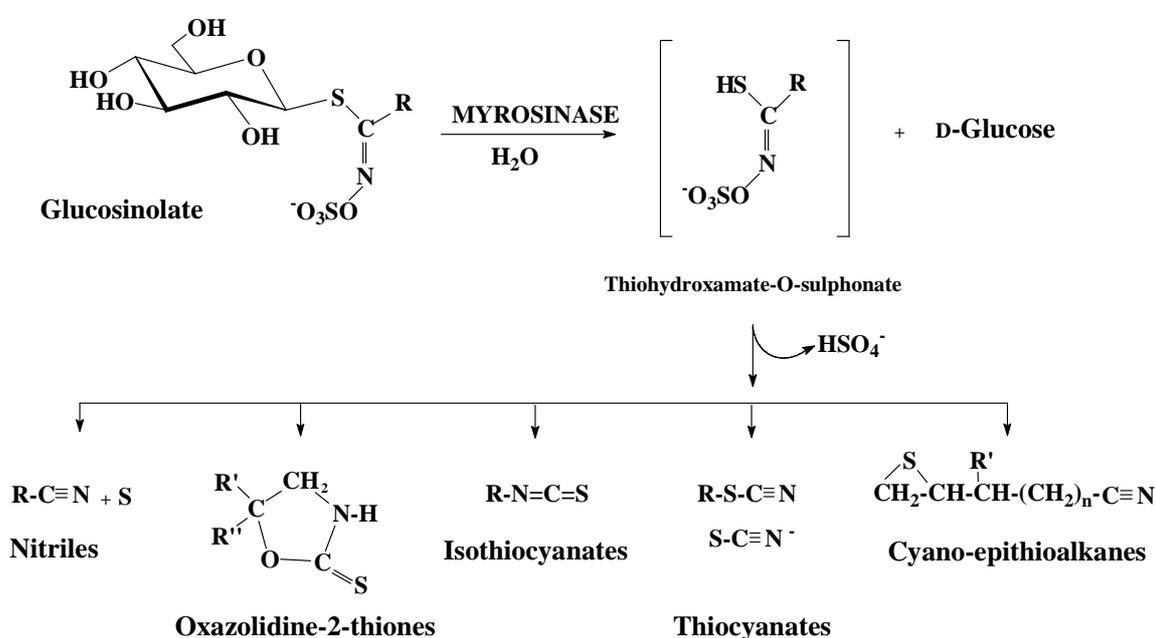


Figure 1. Biological degradation pattern of glucocapparin.

on like previous studies reported on *C. maculatus* (Seck et al., 1993; Seck, 1994; Sanon et al., 2005). Observations made on the behavior of insect from exposure to death showed a high knock-down effect similar to the mode of action of a pyrethroid, with resultant 100% mortality from the first day of treatment. Nevertheless, the release of the molecule responsible for the toxicity requires crushing of the organs, so that compounds can become volatile. It is during crushing that the contact between the activating enzymes myrosinase (EC 3.1.6.1 Thioglucosidase) and glucocapparin is established (Vig et al., 2009; Morra and Borek, 2010). Figure 1 describes the mode of degradation of Glucosinolates (GLs) leading to isothiocyanates (ITCs). GLs are sugar anionic thioesters containing a β -thioglucosidase-type bond, which can be quickly

hydrolyzed by Myrosinase-catalyzed reaction to give stoichiometric amount of D-Glucose, hydrogen sulfate ion, generating a series of diverse aglucons such as ITCs, nitriles (Nis), thiocyanates, thiones (OZTs), etc. (Bernardi et al., 2000). Glucocapparin is the main glucosylated molecule responsible for the release of natural Methylisothiocyanate (MITC), which have been described as a powerful insecticide (Brown and Mora, 2005; Zhenga and al., 2006). The higher observed biological activity of fruits as compared to leaves is consistent with the results obtained by Seck (1994) who tested the efficacy of this plant against *Callosobruchus maculatus*. Observed differences between Seck (1994) and the results of this study, could be due to differences in the concentration of active material. Concentrations of active material in plants are known to differ with location,

phenological stage and the ripeness of the organs (Fahey et al., 2001; Seck, 1994). MITC has ovicidal and larvicidal effects on bruchids (Auger et al., 1994) and also exhibit insecticidal, nematicidal, fungicidal and herbicidal properties (Jewess et al., 1999; Tomlin, 2003), so that the observed effects could be due to release of MITC.

LC₅₀ values obtained in our experiment are very similar to those measured by Seck et al. (1993), which ranged from 1 to 4.2 g/L for fresh crushed leaves and 0.4 to 1.7 g/L for fresh crushed fruits for the control of *C. maculatus*, *Sitophilus zeamais* and *Tribolium castaneum*. Our present results seem to indicate a mass effect when comparing the differences noted between the LC₅₀ without substrate and those obtained in the presence of groundnuts. Sanon et al. (2002) made similar observations on cowpea and suggested a partial absorption of the active product by the seeds (without determining the residual MITC). This hypothesis has to be investigated, and residual MITC levels need to be analyzed.

From a practical point of view, the results suggest that *B. senegalensis* can be used as an alternative to synthetic insecticides for the protection of groundnut seed at village level under Sahelian conditions, regions where this plant is native (Baumer, 1995). Efficacy of leaves against *C. serratus* should be further tested, in view of their greater availability during the year for the protection of shelled, as well as groundnuts in pods. However, it should be pointed out that the presented results were obtained under hermetic conditions with no loss of fumigant substance. It would be interesting to conduct the study in accordance with farmers' practices, because airtightness would be difficult to guarantee in the case of granaries.

On-going studies are oriented towards the establishment of a validated method for the determination of MITC traces (GC-MS and GC with Flame Photometric Detector). It is, indeed necessary, to assess the persistence of this fumigant in stored grains because it seems to have significant toxicity. The analysis of residues will also indicate whether the treated peanuts would be safe for human consumption; although further toxicity studies are needed to effectively exclude human health risk and investigate if the taste of groundnuts is altered by these volatiles.

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