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Insecticidal Activity of Three Plants Extracts on the Cowpea Pod Sucking Bug, *Clavigralla tomentosicollis*, STÄL (Hemiptera: Coreidae)

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Abstract: In Burkina Faso, farmers commonly use insecticidal plants for crop protection. To understand how insecticidal plant works (their mode of action), we carried out a bioassay on *Clavigralla tomentosicollis*, the cowpea pod sucking bugs with three insecticidal plants, *Cassia nigricans* V., *Cymbopogon schoenanthus* S. and *Cleome viscosa* L. Three modes of exposures (1) direct contact application, (2) stomach poisoning activity (3) and inhalation toxicity activity, were tested. The results showed a potent contact and stomach toxicity on 1st instars larvae regardless of the three crude extracts. But the plant extracts was less effective with older stages of the insects. A highest effectiveness was recorded with inhalation of vapours of crude extracts regardless of insect stages and type of plants. Implications of these findings are discussed regarding the use of plant extract for controlling pod sucking bugs in cowpea fields.

Key words: Cowpea, *Clavigralla tomentosicollis*, *Cassia nigricans*, *Cymbopogon schoenanthus*, *Cleome viscosa*

INTRODUCTION

Insect pests are an important constraint to cowpea (*Vigna unguiculata* Walp.) production in the tropics. In Burkina Faso, several species are of significant importance among these is the coreid pod sucking bug, *Clavigralla tomentosicollis* Stäl (Dabire, 2001; Dabire *et al*., 2005). So far the most effective control strategy relies on spraying of synthetic insecticides. However, these chemical are not affordable to small scale farmers and misuse can occurs with low literate farmers. Regaining interest on development of alternative control methods are ongoing among which is the using of bio-pesticides.

Traditionally farmers in Burkina Faso spray various plant crude extract for cowpea crop protection. To date a limited database is available on the efficiency of botanicals against cowpea field pests. Most of the data is about cowpea storage pest (Dabire, 1993; Dabire *et al*., 2008; Sanon *et al*., 2006). A few trials conducted so far using pod sucking bug resistant varieties in combination to neem seed extract spraying was promising (Ba *et al*., 2008). Additional investigations need to be done in order to develop other botanical insecticide for integrated pest management of cowpea field pests.

In this study, we focused investigations on, *Cleome viscosa* L. (Capparaceae), *Cassia nigricans* V. (Caesalpinaceae) and *Cymbopogon schoenanthus* S. (Poaceae) three annual subtropical sticky herb with strong penetrating odour (Berhaut, 1967). These plants are widely distributed in Burkina Faso. Their insecticidal activity by fumigation and repellent on the cowpea weevil, *Callosobruchus maculatus* under hermetic storage conditions is well documented (Belmain *et al*., 2001; Lambert *et al*., 1985; Dales, 1996; Ketoh *et al*., 2005; Dabire *et al*., 2008). Moreover several studies reported that botanicals used for storage pest are also efficient for field insect pest control on cowpea (Ofuya and Okuku, 1994; Ameh and Ogunwolu, 2000; Dabire, 2001; Oparaeke, 2005). However, few is known about their mode of toxicity on field insect pest. The mode of action of botanical is the basis of knowledge for the development of efficient bio-pesticide suitable for crop protection.

We carried out, under laboratory conditions, experiments to determine the mode of action of three plants on *C. tomentosicollis*. The present study is part of ongoing research in INERA for the development of botanical insecticide for small scale farmers.

MATERIELS AND METHODS

Study environment: The study was conducted in October 2003 under the prevailing ambient conditions of the

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INERA entomology laboratory at Kamboinse in central Burkina Faso. The average temperature and relative humidity during the study period were 29°C and 68%, respectively.

Source of insects: The larvae and adults of *C. tomentosicollis* were derived from a laboratory mass rearing facility. Insects were supplied with fresh pods of a continuous crop of the sensitive cowpea variety, KN1 and were reared in wood cages according to the technique describe by Dabire *et al.* (2005). Toxicity tests were carried out on 1st, 3rd and 5th instars larvae and adults of *C. tomentosicollis*.

Preparation of the plant extracts: Fresh *C. viscosa*, *C. nigricans* and *C. schoenanthus* plants were collected nearby the research station of Kamboinse. The whole plants, excluding roots were pounded in a mortar to produce a fine crushed plant material in accordance to Dabire *et al*. (2008). Inhalation toxicity test was carried out with fine crushed extract plant. Fine crushed is the formula that emits more vapours. For contact and ingestion toxicity we used aqueous plant extracts, the fine crushed material was pressed on clothing muslin to obtain pure extracts. A chemical insecticide deltamethrin (Decis EC, 25 g active ingredient/litre) purchased in the local market was used as positive control for comparison.

Direct contact effects of pure plant extracts on *C. tomentosicollis* larvae and adults: The tests were carried out in 374 cm^3 capacity plastic flasks. For each insect stage (1st, 3rd and 5th instars larvae and adults) three treatments equivalent to the three plants were setup. Far each insect stage, 200 individuals were split in 20 flasks (10/flask). Each batch of 5 boxes representing one replicate and 4 replicates were done. Three flasks correspond to the 3 plants treatments and the remaining, the two control treatments (deltamethrin and untreated control). Insects were dipped into the plant extracts for 5 sec then allowed to dry for 5 min and put in the flask. Insect were fed on fresh pods (5 pods/flask) of the KN1 cowpea variety (sensitive to *C. tomentosicollis*). Instead of plant extract, the untreated control insects were dipped in tap water and the insecticide treatment insects in a solution of 2 mL deltamethrin/l water. Insects were kept in flasks for 24 h and the number of dead insects was recorded for each treatment.

Effects of ingesting pure plant extracts on *C. tomentosicollis* larvae and adults: The same number of flask was used as above. Fresh cowpea pods were soaked in the different plant extracts for 24 h and dried prior to be supplied to insect feeding. Instead of plant extract, in the untreated control, pods were dipped in tap water and for the deltamethrin treatment; pods were dipped in a solution of 2 mL deltamethrin/l water. Insects were kept in flasks for 24 h and the number of dead insects was recorded for each treatment.

Effects of inhalation of plant extract vapours on *C. tomentosicollis* larvae and adults: This test was done with increasing quantity of each crushed fresh plants to record the highest mortality. We used 0.5, 1, 1.5 and 2 g plant material per flask for *C. viscosa*; 1, 3 and 5 g crushed per flask for *C. nigricans* and 5, 10 and 15 g per flask for *C. schoenanthus*. For each doses 4 replications were done. The crushed fresh plant were packed in mosquito netting and suspended in the flasks with a thread tied to the flask lid. The insect do not have any direct contact with the plant material. The control flasks contained no plant material; but a moist cotton wool was placed in the flask to ensure humidity comparable to other treatments. Ten insects were kept in each flask for 24 h and the number of dead insects was recorded for each treatment.

Data analysis: Data on adult mortality were submitted to a two-way Analysis of Variance (ANOVA), with doses and insect stages as factors, using SAS software version 8 (2001). When the analysis probability indicated significant differences, a separation of means was made by the Student Newman Keuls Test at the 5% level.

RESULTS

The direct contact of the extracts killed 100% of 1st instars larva regardless of the plant extract (Table 1). But this mortality decreased to less than 40% when 1st instars larvae where provided pods soaked with plant extracts (Table 2). Both direct contact toxicity and stomach poisoning were significantly low with 3rd, 5th instars larvae and adult insect regardless of the plant extracts (Table 1 and 2). This mortality decreased to 0-5% while

Table 1: Percentage contact mortality (±SE) of larva and adult of *C. tomentosicollis* treated with three different plants aqueous extracts and deltamethrin

	Larva			
Treatments	1st instars	3rd instars	5th instars	Adult
C. viscosa	100Aa	5±0.6Cb	2.5±0.5Cb	0Bb
C. nigricans	100Aa	17.5±0.5Bb	2.5±0.5Cc	2.5±0.5Bc
C. schoenanthus	100Aa	15±0.6Bb	10±0.1Bb	5±0.6Bb
Deltamethrin	100A	100A	100A	100A
Untreated control	0B	0C	0C	0B

Means within a column followed by the same uppercase letter are not significantly different; and means within a line followed by the same lowercase letter are not significantly different (p<0.05; SNK-test)

Table 2: Percentage mortality (±SE) of larva and adult of *C. tomentosicollis* fed with pods of cowpea soaked in three different plants aqueous extracts and deltamethrin

	Larva			
Treatments	1st instars	3rd instars	5th instars	Adult
C. viscosa	37.5±1.0Ba	2.5±0.5Cb	0Bb	5±0.6Bb
C. nigricans	30±0.8Ba	17.5±1.3Bb	10±1.1Bb	2.5±0.5Bb
C. schoenanthus	37.5±1.5Ba	10±0.8Bb	10±0.8Bb	2.5±0.5Bb
Deltamethrin	82.5±0.5Aa	65±1.7Ab	65±1.7Ab	55±0.6Ab
Untreated control	0C	0C	0C	0C

Means within a column followed by the same uppercase letter are not significantly different; and means within a line followed by the same lowercase letter are not significantly different ($p<0.05$; SNK-test)

Table 3: Percentage mortality (±SE) of larva and adult of *C. tomentosicollis* kept with increasing vapours

	Larvae			
Treatments	1st instars	3rd instars	5th instars	Adult
Cleome viscosa				
0.5 g	0Ba	2±0.45Ca	10±1.0Ca	12±1.3Da
1 g	92±1.3Aa	64±1.3Bb	24±0.5Cc	34±1.3Cc
1.5 g	100Aa	94±0.9Aa	66±2.2Bb	54±0.55Bb
2 g	100A	100A	100A	100A
Untreated control	0B	0D	0D	0D
Cassia nigricans				
1 g	74±1.8Ba	22±1.3Cb	18±1.5Cb	8±0.8Cb
3 g	86±1.7BAa	68±1.3Bb	54±1.1Bb	20±0.7Bc
5 g	100A	100A	100A	100A
Untreated control	0C	0D	0D	0D
***Cymbopogon schoenanthus* S.**				
5 g	68±0.8Ba	56±0.5Cb	22±0.8Cc	8±0.8Cd
10 g	94±0.9Aa	80±0.7Ba	54±1.5Bb	36±1.1Bc
15 g	100A	100A	100A	100A
Untreated control	0C	0D	0D	0C

Means within a column followed by the same uppercase letter are not significantly different; and means within a line followed by the same lowercase letter are not significantly different ($p<0.05$; SNK-test)

insect developed to adulthood. When insects were confined with crushed plant extracts, a mortality of up to 100% was reached for each of the development stage with increased quantities of the crushed plants (Table 3). The lowest quantity of crushed plant necessary to obtain a total mortality of the insects was recorded with *C. viscosa* (2 g) followed by *C. nigricans* (5 g) and *C. Schoenanthus* (15 g).

DISCUSSION

All the three plants exhibited insecticidal activity against *C. tomentosicollis*. Their insecticidal properties have been reviewed by Dales (1996). Most of reports assumed insecticidal/repellent activities with stored products insects (Belmain *et al*., 2001; Lambert *et al*., 1985; Lale, 1991; Ketoh *et al*., 2005; Ndungu *et al*., 1995, 1999; Dabire *et al*., 2008). A repellent activity of relative species of *Cleome* is also reported on spider mites (Nyalala and Grout, 2007). These studies focused on the insecticidal properties with storage insects without mentioning the mode of action of plant extracts.

In this study we observed a potent toxicity due to inhalation of plant vapours and in that case *C. viscosa* was the more efficient. This mode of toxicity was reported for several insect pests of stored commodities (Seck *et al*., 1996; Bouda *et al*., 2001; Dabire, 2001; Dabire *et al*., 2008). Since, this mode of toxicity is due to volatile chemical compound of essential oils (Kini, 1993; Seck *et al*., 1993; Tapondjou *et al*., 2002), it is difficult to expect it working in field application.

In this study we also confirmed a potent contact and stomach poisoning activity of the plant extracts on the 1st instars larvae. However, this insecticidal activity decreased sharply while insect developed to adulthood. The development of the exoskeleton might provide protection to botanical penetration in the insect body as the larvae develop to adulthood. But Williams *et al*. (2003) demonstrated a pyrethiroid knock down type of contact insecticidal toxicity of *C. viscosa* on adult of *Cylas formicarius elegantulus* S. (Coleoptera: Curculionidae). An antifeedant activity of chemical extracts of *C. nigricans* was also reported on larvae of *Heliothis zea* and *H. virescens* (Georges *et al*., 2008). In this study we used aqueous extract, while both previous studies used organic solvent extract. Chemical extractions might have necessarily enhanced the active ingredient concentration and the efficiency of insecticidal plants (Williams *et al*., 2003; Georges *et al*., 2008). The main active ingredient of *C. nigricans* is emodin and when using it, a potent contact insecticidal activity was achieved on adult of *Bemisia tabacci* (Georges *et al*., 2008). Insecticidal contact activity of emodin extracted from *Rhamnus dispermus* was also reported against adults of the peach trunk aphid (Ateyyat and Abu-Darwish, 2009).

Thus, we can expect that organic solvent extractions of active ingredient might enhance the contact insecticidal activity of *C. viscosa* and *C. nigricans* on all the development stages of *C. tomentosicollis*. Otherwise targeting the 1st instars larvae might also be the best way to keep *C. tomentosicollis* population under control in cowpea field. The first generation of *C. tomentosicollis* invading cowpea field used to come from wild alternate host plants (Dabire *et al*., 2005). The insect population build-up quickly and cause severe damage to cowpea (Dabire, 2001). By focusing interventions on the first generation of progeny, reduced damage would occur as a result of the control of the neonates on the plant which might stop the population of the insect to build up on cowpea. The mixture of plant extracts could also be tried to enhance the contact toxicity activity. Improvement of formulation of the extracts for field applications might also involved using stabilizer like bar soap and starch solution.

This kind of formulation was successful in Nigeria with plant extracts for controlling cowpea insect pest (Oparaeke *et al*., 2005).

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