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TESTING OF INSECTICIDAL EXTRACT FROM *MITRACARPUS SCABER* ZUCC. (RUBIALE: RUBIACEAE) AGAINST FOUR STORED FOOD PRODUCTS INSECT PESTS IN IVORY COAST

[a]Mamadou Doumbia, [a]Kouassi Yoboue, [a]Dagobert K. Kra, [b]Jan Klimaszewski*, [a]Koffi E. Kwadjo, [a]Bleu G. Douan, [a]Mamadou Dagnogo

[a]UFR SN, University Nangui Abrogoua, Abidjan, Ivory Coast.

[b]Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, Quebec, QC, Canada.

A B S T R A C T

The comparative insecticide efficiency tests of saturated ethanol and acetonic extracts from leaves of *Mitracarpus scaber* Zuccarini (Rubiale: Rubiaceae) and *Phillanthus amarus* Schum and Thonn (Euphorbiale: Euphorbiaceae) against four (4) stored food insect pests showed for the first time that the extracts from *Mitracarpus scaber* are significantly toxic for *Cryptolestes* sp, (Coleoptera: Cucujidae), *Palorus subdepressus* Wollaston (Coleoptera: Tenebrionidae), and *Rhyzopertha dominica* Fabricius (Coleoptera : Bostrichidae). However, no toxic impact from the two plant extracts has been observed in regard to *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae).

Keywords: Insecticide, Efficiency, Insects pests, Plant extracts, Stored food products, *Mitracarpus scaber*.

INTRODUCTION

In Ivory Coast, the main crops such as coffee, cocoa, rice, maize and imported wheat are attacked by insects (Lavabre, 1970; Agbaka, 1991). To mitigate damage caused by insects, synthetic chemical insecticides are applied by spraying or fumigation (Monro, 1970; Appert, 1985). However, it is recognized that the extensive use of synthetic chemicals creates pesticide residue problems and risks of developing insect resistant strains (Mills, 1983; Haubruge & Amichot, 1999). For this reason, it is recommended to explore alternative crop protection methods by plant derived insecticides (Afifi *et al*., 1989; Ahamad & Ahmed, 1991; Foua-Bi, 1993; Seck *et al*., 1993; Gakuru & Buledi, 1995; Owusu, 2001; Ogendo *et al*., 2008; Rajendran and Sriranjini, 2008). The insecticidal activity of other natural insecticides such as diatomaceous earth against stored maize pests has been also studied in Ivory Coast (Doumbia *et al*., 2014). In northern rural Ivory Coast, for protecting stored food against insect damage, people use dried leaves of lemongrass *Cymbopogon nardus* (L.) Rendle (Glumales: Poacea) for maize, bean and millet (Doumbia M., unpublished data). The lemongrass plant is also used by native people for making tea and a tonic. It was noticed in the past that plants used in traditional medicine against various diseases also show insecticidal properties (Aziza, 1988; Dunkel *et al*., 1991). *Mitracarpus scaber* Zucc. (Rubiale: Rubiaceae) and *Phillanthus amarus* Schum Thonn. (Euphorbiale: Euphorbiaceae), which are used by rural populations as a remedy for constipation, oral candidiasis, gastrointestinal and skin diseases, vomiting, diarrhea, heart palpitations, as well as for jaundice treatment (Adjanohoun 1989; N'Guessan, 1995). *Mitracarpus scaber* Zucc. (Rubiale: Rubiaceae) leaf extract has many effects on human and animal diseases and mosquito (*Culex quinquefasciatus*) larvae (Ali-Emanuel *et al*., 2003; Imam *et al*., 2008; Abdullahi *et al*., 2011). Unfortunately, there are few published works on the evaluation of *Mitracarpus scaber* effectiveness on stored food products insect pests. The aim of this study is first a laboratory screening of Ivorian medicinal plants with the objective to study their toxic potential effects against cultured and stock insects. In this context, investigations have been carried out with acetone and

* Corresponding Author:
Email: jan.klimaszewski@nrcan-rncan.gc.ca

ethanol total leaf extracts from *Mitracarpus scaber* Zucc. (Rubiale: Rubiaceae) and *Phillanthus amarus* Schum Thonn. (Euphorbiale: Euphorbiaceae).

MATERIALS AND METHODS

Insect rearing: The insects were chosen for their polyphagous habits (Agbaka, 1991). Wild strains of S*. zeamais*, *P. subdepresus* and *Cryptolestes* sp derived from maize stocks and *R. dominica* strain from infested sorghum stocks collected directly from Adjamé market traders (Central market in Abidjan, the Ivory Coast economic capital). The breeding of different species of insects was done separately on foods on which they were found. For mass insect rearing, glass jars were used containing 1 L of foodstuff with punctured lids and covered with 50 µm mesh kept in a room at temperature 30 ° C ± 2 and 70% ±5% r.h. Each insect species strain was purified during regular breeding by sieving to separate the grains with adults and spawning larvae. After the screening, the grains infested with larvae of each insect species were placed in specific jars for recovery of young insect adults that were used for toxicity tests. This practice allowed us to obtain individuals from the same generation for the tests.

Preparation of saturated ethanol and acetone extract solutions of *M. scaber* and *P. amarus* leaves: Ethanol and acetone were chosen as solvents because of their great dissolving power, their volatility, and their effectiveness against insects (Shepard, 1958; Busvine, 1971). For the preparation of ethanol and acetone saturated leaf extract solutions of the two plants, dried leaves under shelter in the shade were first finely crushed and sieved through a sieve with a 50 µm mesh. Subsequently, saturated solutions were prepared with the plant powder. The dilution was carried out with a minimum solvent volume that allowed us to obtain a homogeneous solution. The use of a saturated solution allows use of the product in its highest concentrated form. One gram of plant powder from each plant species was weighed using a Satorius scale balance with 10^{-4} g precision. The resulting sample was placed in a 50 mL glass flask with a flat bottom and then gradually the desired solvent was separated by a fraction of 5 mL. When the solvent was added, the flask was stirred until the total extract solution was homogenized. According to this methodology, the saturated solution of *M. scaber* was obtained by diluting 1 g of powder in 40 mL of ethanol or 80% acetone solutions of 0.025 g/mL. The saturated solution from *P. amarus* was obtained by diluting 1 g of powder in 50 mL of ethanol or 80% acetone solution of 0.020 g/mL.

Preparation of K-Othrine solutions (chemical insecticide used as reference): In order to have a basis for comparison of possible insecticidal preparations based on the plant extracts tested, the K-Othrine formulated as emulsifiable concentrate (25 g / L deltamethrin as active ingredient), was used as a reference product. In order to obtain solutions of 1%, a dose prescribed by the manufacturer, 0.5 mL of this commercial formulation was placed in a round bottomed flask and filled to 50 mL with distilled water.

Bioassays: Adult insects of different species aged 1 to 14 days were removed from the mass rearing cultures after screening a day before testing. These individuals were then placed in plastic boxes with dimensions of 10 x 20 x 7 cm, covered with muslin and contained the substance on which the insects developed to achieve the bioassays. At the time of the bioassays, product topical application on each individual was done by means of a micro-syringe of 50 µL mounted on a microinjector set so as to deliver at each step an amount of 2 µL. During the application, the insect is immobilized under a stereo-microscope lens at a magnification of 40 x, and receives on its pronotum 2 µL solution of extract or the test substance. Treated individuals were then put in lots of 20 in a Petri dish of 90 mm diameter. Control lots of 20 individuals each were also treated under the same conditions with ethanol or acetone (total ethanol or acetone extracts) or distilled water (control reference product, the K-Othrine). After product application, testing boxes were placed in the conditioning chamber. For each product tested and for each insect species, five repetitions were carried out. In the same treatment, the results of mortality observations in the course of the second count were added to those of the first count to give a cumulative mortality after 5 days. For the first time, we counted the correct mortality using Abbott's formula (1925).

Statistical analysis: The insect mortality for different testing was calculated by ANOVA analysis of variance for each insect species for 1 day and 5 days separately using Statistica 7.1. (StatSoft, 2005).

RESULTS

The analysis results (Table 1) show that topical

application of total ethanol and acetone leaf extracts from *M. scaber* on different insect species tested causes significant mortality of individuals of *Cryptolestes* sp, *P. subdepressus*, and *R. dominica.* Extracts from *P. amarus*, did not show toxicity on the tested insects. No toxic action of the extracts from the two plants was observed on *S. zeamais*, regardless of the nature of the solvent extraction used. The analysis of test results (Table 1) revealed that insects were more sensitive to acetone extractions which lead to a higher mortality than to ethanol solutions of the same plant. The differential biocid activity between the two solutions is very clear in the corrected mortality analysis after a day of testing determined using Abbott's formula for *M. scaber* (Table 2). We discovered for the first time that the plant *M. scaber* has insecticidal properties affecting *Cryptolestes* sp, *P. subdepressus*, and *R. dominica* species, and that the plant *P. amarus* does not have these properties.

Table 1. Mortalities after 1 day and cumulative mortalities after 5 days (mean ± standard error) of *S. zeamais*, *R. dominica*, *P. subdepressus* and *Cryptolestes* sp treated by topical application test with ethanolic and acetone total leaf extracts of *M. scaber* and *P. amarus*.

Insect species		Products tested									
		K-Othrine (Reference chemical)		*M. scaber* ethanolic extract		*M. scaber* acetone extract		*P. amarus* ethanolic extract		*P. amarus* acetone extract	
		1 day	5 days	1 day	5 days	1 day	5 days	1 day	5 days	1 day	5 days
S. zeamais	Products	20 ± 0.0	20 ± 0.0	0.0± 0.0	0.0 ± 0.0	0.2 ± 0.2	3.6 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.3	5.4 ± 1.2
	Control	0.2 ± 0.2	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.8 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.4	4.0 ± 1.2
	F	9801***	9801***	0 NS	0 NS	1 NS	0.1 NS	0 NS	0 NS	0 NS	0.7 NS
R. dominica	Products	20 ± 0.0	20 ± 0.0	4 ± 0.7	4.8 ± 0.6	17.4±0.5	19.6 ± 1	0.0 ± 0.0	0.4 ± 0.3	0.8 ± 0.4	1.4 ± 0.3
	Control	0.8 ± 0.4	2.4 ± 0.2	0.6 ± 0.2	0.8 ± 0.4	0.0± 0.0	0.2± 0.2	0.2 ± 0.2	0.6 ± 0.3	0.0 ± 0.0	0.8 ± 0.2
	F	2633.1 ***	5162.7***	20 ***	33.3***	2407.9***	2630.7***	1 NS	0.3 NS	4.6 NS	3.6 NS
P. subdepressus	Products	20 ± 0.0	20 ± 0.0	3 ± 0.9	8.6 ± 1.1	17.0±0.4	18.8±1.2	0.4 ± 0.3	0.6 ± 0.3	0.8 ± 0.4	2 ± 0.7
	Control	0.4 ± 0.4	2.8 ± 0.6	0.6 ± 0.4	1.8 ± 0.6	0.0 ± 0.0	1.2 ± 0.4	0.6 ± 0.3	0.8 ± 0.4	0.6 ± 0.3	1± 0.4
	F	2401***	870.1***	6*	30***	2403***	94.5***	0.3 NS	0.2 NS	1.4 NS	1.4 NS
Cryptolestes sp	Products	20 ± 0.0	20 ± 0.0	9.4 ± 0.9	15 ± 1.1	19.6±1.5	20 ± 0.0	5.8 ± 1.4	6.2 ± 0.9	6.6 ± 2	7.4 ± 0.6
	Control	0.6 ± 0.2	3.6 ± 0.7	0.6 ± 0.6	0.6 ± 0.6	0.4 ± 0.4	1.8 ± 0.4	2.6 ± 0.8	3.6 ± 1.1	3.4 ± 0.2	4.4 ± 1.3
	F	6272.7***	584***	63.5***	124.9***	2633.1***	1750.7***	4.10 NS	4 NS	4.10 NS	3.9 NS

*** = very highly significant difference ($P < 0.001$); ** = highly significant difference ($P < 0.01$); * = significant difference ($P < 0.05$); NS = no significant difference ($P > 0.05$).

Table 2. Adjusted mortality rates (%) after 24 h of *S. zeamais*, *R. dominica*, *P. subdepressus* and *Cryptolestes* sp. treated by application test with ethanol and acetone saturated leaf extracts of *M. scaber*. For each column, the numbers with no letters in common are statistically different (P<0.001).

Products tested	Insect species tested			
	S. zeamais	*R. dominica*	*P. subdepressus*	*Cryptolestes* sp
K-Othrine (Reference chemical)	100a	100a	100[a]	100a
M. scaber ethanolic extract	0b	18b	12b	45b
M. scaber acetonic extract	1b	87a	85a	98a

DISCUSSION

The experiment proved to be very highly significant (P<0.001), and total ethanol and acetone leaf extracts from *M. scaber* were effective against three of the four insect species that underwent treatment (Table 1). The total ethanol and acetone leaf extracts from *P. amarus*, were not significant against tested insects. This study also revealed that acetone leaf extracts from *M. scaber* have a higher toxic impact against insects than ethanol extracts of the same leaves (Table 2). These results confirm Busvine's (1971) observations, indicating that the absorption power of acetone is greater than that of ethanol, which liberates many compounds at dilution time. However, no toxic impact of ethanol and acetone extracts from *M. scaber* was observed on *S. zeamais.* This could probably be linked to *S. zeamais* strains biological or genetic quirks which make this species less susceptible to the toxic effects of the plant. The experiments conducted in this study showed for the first time the insecticidal properties of *M. scaber*. *M. scaber* is also known as a medicinal plant used in the treatment of liver diseases (Germano *et al*., 1999). Bisignano *et al*., (2000) indicated that *M. scaber* has antimicrobial and antifungal qualities that justify its effective use in the treatment of "bovine dermatoses" in Benin (Ali-Emmanuel *et al*., 2003). According to several studies, *M. scaber* antimicrobial and antifungal properties are due to active chemical substances in the plant such as the naphthoquinones (Ogundaini, 1999), Azaanthraquinone (Okunade *et al*., 1999), gallic acid, 3,4,5-trimethoxybenzoic, 4-methoxyacetophenone and 3,4,5-trimethoxyacetophenone (Bisignano *et al*., 2000). It is therefore likely that these antimicrobial and antifungal substances identified in *M. scaber* are also involved individually or combined in the manifestation of its insecticidal properties observed in this study against selected insect species. Additional investigations are therefore necessary for a better understanding of the different roles played by the various *M. scaber* chemical components, acting as singulars or in synergy, in the fight against food pests. Future investigations should develop alternative methods of combatting coffee and cocoa insect pests, to comply with food products export requirements of the European Union.

The experiments conducted in this study show for the first time that *M. scaber*, besides having medicinal properties, also has insecticidal properties that were not observed in *P. amarus*. It would therefore be preferable, in terms of primary screening of African plants in search of possible insecticides, to conduct a preliminary investigation on medicinal plants with antimicrobial and antifungal properties known from traditional and African botanical medicine.

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