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Testing Fractionated Extracts Gained from the Ethnobotanical *Pachypodanthium Staudtii* (*Annonaceae*) for Bruchid Insect Control (*Coleoptera: Bruchidae*)

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Abstract: Hexane, acetone and ethanol extracts of *Pachypodanthium staudtii* were tested for their potential in protecting stored legume seeds against infestation by the bruchid beetles *Acanthoscelides obtectus* Say and *Callosobruchus maculatus* F. Seeds were uniformly coated with the extracts and infested with adult insects in Petri-dishes placed randomly on the shelves of the laboratory. While acetone and ethanol fractions were ineffective except for a significant but low effect on *A. obtectus*, the hexane extract significantly reduced adult survival of all two bruchid species within seven days, as well as the numbers of eggs laid and of F1 progeny produced, resulting in seed damage averaging 2.4% compared to 99.8% in the untreated controls. The potential for using this hexane fraction for post-harvest protection of legume seeds is discussed.

Key words: *Acanthoscelides obtectus*, *Callosobruchus maculatus*, *Pachypodanthium staudtii*, solvent extracts, seed damage.

INTRODUCTION

Ethnobotanicals play an important role in natural resource-based protection of stored legumes in Africa[1,2]. They may represent easily accessible tools for postharvest protection of legume seeds from attack by the major bruchid insects throughout the tropical belt, namely *Acanthoscelides obtectus* Say on common bean (*Phaseolus vulgaris*) and *Callosobruchus maculatus* F. on cowpea (*Vigna unguiculata*)[3,4]. These two bruchid species can cause seed losses up to 20-100% at the farm level if left untreated[5]. Adult bruchids do not feed and make damage themselves. Females deposit eggs and the newly hatched larvae bore into the legume seeds. All preimaginal stages develop unnoticed within the seeds. The first visible signs are the holes made in the seed by the emerging adults. This is perceived as damage[5,6].

We hypothesized that *Pachypodanthium staudtii* Engl. & Diels (*Annonaceae*), a plant commonly used in traditional medicine[7,8], had an effect on the target insects based on its constituents identified in different samples and extracts which include a styrene derivative, polyphenols and alkaloids [9,10,11], all of which are known to interfere with insect physiological processes and that fractionation of the plant powder might be a useful approach to concentrate efficacy in a single extract.

MATERIALS AND METHODS

Plant material and extraction: Fresh stem-bark of *Pachypodanthium staudtii* was collected around Edea, Littoral Province of Cameroon and its identification confirmed at the Cameroon National Herbarium, Yaoundé, where a voucher specimen is kept. This plant is a small tree widespread in the dense and humid tropical forest of Central and West Africa, its bark being used in traditional medicine, usually as an infusion in water to cure a wide range of ailments[7, 8]. This bark was cut into pieces, dried at 40ºC for 7 days and ground to powder. 50 g of this stem-bark powder was sequentially extracted in a Soxhlet with 400 ml of hexane (purity 99.5%) at 7ºC, followed by acetone (purity 99.5%) at 5ºC, then ethanol (purity 99.8%) at 8ºC. Extraction lasted 12 h for each fraction. Concentration of the hexane extract yielded a sticky light-brown deposit (2.2 g), while that of the acetone and ethanol fractions afforded thick dark-brown pastes (8.2 and 2.1 g, respectively). Each fraction was kept in a small screw-capped glass tube in a freezer at -18ºC for use in insect bioassays within two weeks.

Test insects: Adult *A. obtectus* and *C. maculatus* were obtained from stock cultures at the entomology laboratory of the IRAD Station in Dschang, Cameroon. *Acanthoscelides obtectus* was maintained on common bean (var. "GLP 190"), while *C. maculatus* was maintained on cowpea (var. "Black Eye Beans") in a climatic chamber (27±2_C, 60-80% R.H., 11:13 L:D). Prior to the bioassays, seeds from the rearing stock were sieved, adults were removed and the seeds containing immature stages were placed into jars and covered with a nylon net to avoid escape of emerging adults. The newly emerged adult bruchids were collected after three days and used for the bioassays.

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Insect bioassay: Bioassays with *A. obtectus* were made on common bean (var. "GLP 190") and with *C. maculatus* on cowpeas (var. "Black Eye Beans"), as in previous studies [5]. Fifty uninfested legume seeds were placed for each treatment inside a glass Petri dish with a diameter of 9 cm. There were nine treatments for each bruchid species: hexane, acetone and ethanol extracts (10 g/kg), the three solvent controls (10 ml/kg), the botanical standard neem (used as the commercial product NeemAZAL-T/S® EC 10 g/l purchased from Andermatt Biocontrol, Grossdietwil, Switzerland and applied at the dose of 5 ml/kg), the synthetic standard pirimiphos-methyl (used as Actellic® EC 50 g/l and applied at the dose of 4 mg active ingredient/kg as recommended against stored product pests) and the untreated control. Three replicates were made per treatment. Undiluted extracts and standards were thoroughly mixed with the legume seeds by shaking the Petri dishes to ensure uniform coating. Solvents were sprinkled with a pipette on to the seeds in the control Petri dishes and allowed to evaporate. Twenty sexed (10 pairs) 1 to 3-days-old adult insects of the same species were collected from the stock jar and introduced into each Petri dish with a glass lid to prevent escape of the insects. The dishes were arranged in a completely randomized design on a laboratory shelf (27±2_C, 60-80% R.H., 11:13 L:D) and not moved throughout the test period to avoid a negative impact on the insects. The experiment was repeated twice.

Dead insects were counted every 24 h for 7 days and then immediately discarded. The remaining living adults were removed from the Petri dishes after the 7 days and the number of eggs on the seeds was counted. Petri dishes were then kept under the same conditions to monitor the F1 generation emerging from the seeds. The number of F1 progeny produced was recorded daily for 30 days from the time of first adult emergence. The number of seeds with and without exit holes was counted to assess the percentage of damaged seeds. The solvent controls were included in the trials because the deposits might still have contained traces of solvents. As no significant differences were found between the solvent controls and the untreated control, presentations in the "Results" and "Discussion" sections are confined to the untreated control.

Data analysis: Survival analysis was carried out using the Logrank (Mantel-Cox) test to detect differences in survivorship among insects subjected to the different treatments. Data for percentage seed damage and for the percentage of adult beetle mortality after 24 and 48 h, when female beetles start to lay eggs[12], were arcsine-transformed, while a $\log_{10}$ (x+1) transformation was performed on the number of eggs laid and the number of F1 progeny produced, following guidelines given by Gomez and Gomez[13]. These data were then analyzed by one-way ANOVA[14] and the means were compared by Student Neuman-Keuls test, whereas paired t-test was used in the case of comparison of a given treatment with the control only.

RESULTS AND DISCUSSIONS

The acetone and ethanol extract fractions had no significant effect on the mortality of the two test bruchid species. Mortality of *A. obtectus* and *C. maculatus* within 24 and 48 h when exposed to the hexane extract was low but significantly higher ($P<0.05$) than that with the untreated controls (Table 1). Adult survivorship within 7 days was reduced significantly ($c^2= 5.65$, df = 1 and $P = 0.0175$ for *A. obtectus*. $c^2= 15.09$, df = 1 and $P = 0.0001$ for *C. maculatus*) from approximately 25% in the untreated controls (Figure 1) to 1.90 and 1.67% in the two bruchid species.

The acetone and ethanol extract fractions had no significant effect on the number of eggs laid by females of the two test bruchid species (Table 2), in contrast to the hexane fraction which significantly reduced ($P<0.05$) this number in comparison to untreated controls. This hexane fraction was less effective than the two standards (synthetic and botanical) in reducing the number of eggs laid. With respect to the number of F1 progeny produced, acetone and ethanol fractions were ineffective except for a significant but low effect on *A. obtectus* (Table 2). After one generation of *C. maculatus*, the number of F1 progeny produced from hexane extract treated seeds was not significantly different from that from pirimiphos or neem treated seeds. It was only marginally higher for *A. obtectus* (Table 2).

Table 1: Effect of stem-bark extracts of *Pachypodanthium staudtii* on percentage of adult mortality of *Acanthoscelides obtectus* and *Callosobruchus maculatus*, after 24- or 48-hour continuous exposure of the initially 20 beetles per replicate to treated legume seeds compared to a botanical (neem), a synthetic (pirimiphos-methyl) standard and to the untreated control.

	Acanthoscelides obtectus		Callosobruchus maculatus	
Treatment	24 h	48 h	24 h	48 h
Hexane fraction	10.0±0.0 b	30.0±2.9 b	11.7±1.7 c	35.0±2.9 c
Acetone fraction	3.3±1.7 bc	8.3±3.3 dc	1.7±1.7 d	5.0±0.0 d
Ethanol fraction	5.0±2.9 bc	11.7±1.7 c	0.0±0.0 d	3.3±1.7 d
Neem	81.7±10.1 a	96.7±1.7 a	60.0±5.8 b	75.0±2.9 b
Pirimiphos-methyl	100.0±0.0 a	100.0±0.0 a	100.0±0.0 a	100.0±0.0 a
Untreated control	0.0±0.0 c	0.0±0.0 e	1.7±1.7 d	1.7±1.7 d

Data were arcsine-transformed before subject to ANOVA.
Means (± s.e.) in the same column followed by the same letter are not significantly different (P = 0.05, SNK test). n = 9.

Table 2: Effect of stem-bark extracts of *Pachypodanthium staudtii* on the number of eggs deposited by females of *Acanthoscelides obtectus* and *Callosobruchus maculatus* on treated legume seeds and on the number of F1 progeny produced in comparison to a botanical (neem), a synthetic (pirimiphos-methyl) standard, and the untreated control.

	Acanthoscelides obtectus		Callosobruchus maculatus	
Treatment	# Eggs†	# F1 progeny‡	# Eggs	# F1 progeny
Hexane fraction	13.0±2.0 b	2.3±0.9 c	18.3±4.3 b	1.0±0.6 b
Acetone fraction	180.7±16.5 a	54.0±10.1 b	142.0±21.7 a	97.3±19.3 a
Ethanol fraction	194.0±15.5 a	61.0±8.6 b	161.0±21.0 a	110.0±16.9 a
Neem	2.0±1.2 c	0.0±0.0 d	24.3±3.5 b	0.0±0.0 b
Pirimiphos-methyl	0.0±0.0 d	0.0±0.0 d	0.0±0.0 c	0.0±0.0 b
Untreated control	227.7±23.7 a	169.0±25.6 a	217.3±13.9 a	151.3±24.3 a

† Observation at 7 days after treatment.
‡ Observation during 30 days starting from first emergence.
Data were transformed using $\log_{10}$ (x+1).
Means (± s.e) in the same column followed by the same letter are not significantly different (P = 0.05, SNK test). n = 9.

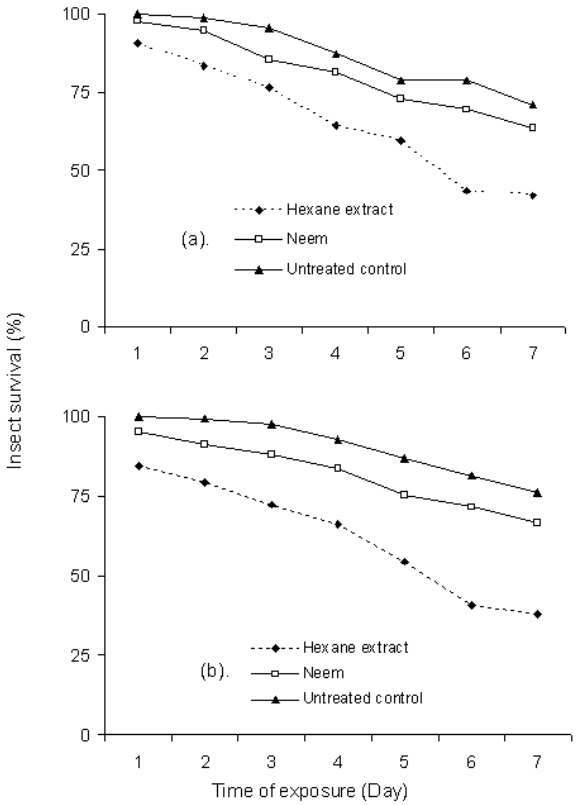

Fig. 1: Effect of stem-bark hexane extract of *Pachypodanthium staudtii* on survival rate of (a) *Acanthoscelides obtectus* and (b) *Callosobruchus maculatus*, on treated legume seeds compared to the botanical standard neem. (No survival with the synthetic standard pirimiphos-methyl). (n = 9; Logrank test).

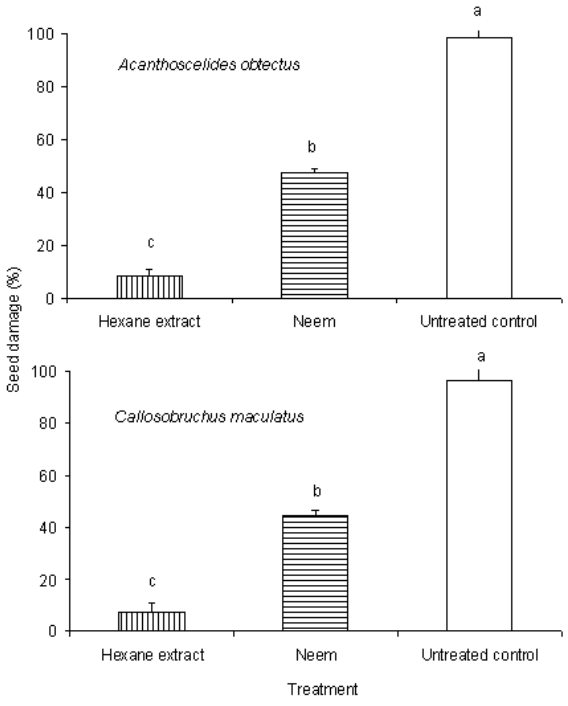

Fig. 2: Damage to legume seeds treated with stem-bark hexane extract of *Pachypodanthium staudtii* after infestation with *A. obtectus* and *C. maculatus*. No seed damage recorded for the two standards (neem, pirimiphos-methyl). Data were transformed to $\log_{10}$ (x+1) before analysis. Bars with the same letters are not significantly different (P = 0.05, SNK test). n = 9.

The acetone and ethanol extract had no effect on damage to seeds by the two test bruchid species, except for a significant although small reduction of damage by *A. obtectus* when compared with the untreated controls. In contrast, the hexane extract reduced damage by all two bruchid species to an average of 2.4% compared to an average of 99.8% in the untreated controls (Figure 2). Seeds treated with the synthetic and botanical standards were free of damage (0.0%).

The hexane extract from powdered stem-bark of the tropical tree Pachypodanthium stautii showed significant insect control efficacy, as successfully exemplified by post-harvest treatment of legume seeds against the two bruchids *A. obtectus* and *C. maculatus*. In contrast, little or no insect control efficacy was found in the more polar fractions gained with acetone and ethanol. To our knowledge, this is the first report on arthropod control by a bioactive fraction from this plant.

Seed damage within one bruchid generation was reduced by the P. staudtii hexane extract to a level below 2.5%, while nearly a complete seed loss was noted for the untreated control. In contrast to this considerable long-term effect, the short-term effect on adult bruchids was relatively low compared to the standards neem and pirimiphos-methyl.

Adult bruchid survivorship after 7 days in the untreated control reflects the relatively short adult lifespan of the bruchids under bioassay conditions in Petri dishes (e.g.[15]). The relatively high contact toxicity of the botanical standard (neem) to the two bruchid species in this study is comparable to the mortality reported by Ivbijaro[16] for *C. maculatus* exposed to the seed oil of this plant. However, the physico-chemical properties of the neem preparation used in this study may influence the level of lethal effect, as neem powder lacked contact toxicity on this bruchid species[17].

The number of eggs laid on seeds treated with the hexane extract was reduced to less than 12% of those deposited on the untreated (control) seeds, reaching the effect of the neem treatment. Neem is known to exhibit an effect on insect reproduction[18], manifested in reduced numbers of eggs deposited by bruchids exposed to treated seeds[17] and a similar effect on oviposition seems to occur with P. staudtii hexane extract. The preimaginal mortality

occurring between egg deposition and emergence of the F1 progeny, shown in the low proportion of F1 progeny emerging from the eggs, is another indication of a long-term effect of the hexane extract. It is remarkable that after one generation, the effect of hexane extract was not statistically different from that of neem on *C. maculatus* and the population size was only marginally higher in *A. obtectus*. Neem interferes with the synthesis and release of ecdysteroids, causing disruption of larval moulting, with pupation and eclosion of adults and finally also with reproduction[18] as confirmed above.

In conclusion, the hexane extract from the stem-bark of P. staudtii exhibited high insect control efficiency on all two bruchid species tested. A major constituent of the hexane extract from P. staudtii was described as 2, 4, 5-trimethoxystyrene[19, 20], This compound is structurally similar to b-asarone, which was isolated from a different plant, Acorus calamus L. (Araceae) and found to be toxic against several major storage pests[21, 22]. This structural similarity strongly suggests that the hexane extract from P. staudtii could also be used as a space treatment or fumigant against storage insect pests as reported for b-asarone by Risha *et al*.[23] and El-Nahal *et al*.[21].

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