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NOTE

Identification of 'elemental sulphur (S_8) *in Boscia senegalensis* (PERS.) LAM ex POIR. leaves

by

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Summary

Studies were undertaken to identify the molecules responsible of *Boscia senegalensis* (PERS.) LAM ex POIR. (Capparaceae) insecticide (fumigant) properties ; the volatiles were isolated from the leaves by steam distillation and analyzed by Gas Chromatography - Mass Spectrometry. Only a few organic molecules were detected and identified. The main constituent was elemental sulphur (S_8), a compound rarely found as such in the plant kingdom.

Key-words : *Boscia senegalensis*, leaves, volatiles, elemental sulphur, GC-MS.

1. Introduction

Boscia senegalensis (PERS.) LAM ex POIR. (Capparaceae) is a little shrub which grows throughout West African countries principally in the Sahelian region [BOOTH, WICKENS, 1988]. Several parts of the plant are locally used for their medicinal properties [KERHARO, ADAMS, 1974] and

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sometimes the small cooked fruits are consumed by humans in case of famine [SALIH *et al.*, 1991]. The leaves exhibit a strong biocide activity against several stored grain insect species. A survey of the Senegalese practices revealed that *B. senegalensis* fresh leaves are traditionally added to stored grains in order to limit insect infestation and damages. Chemical investigations proved that methylisothiocyanate (MITC) enzymatically liberated from the methylglucosinolate (also called glucocapparine) precursor was responsible for the insecticidal properties [SECK *et al.* 1993]. Since other volatile molecules could act as MITC synergists, a characterization of *B. senegalensis* steam distillate was carried out. The results of this complementary study are reported herein.

2. Experimentals

B. senegalensis leaves were harvested in Thies (Senegal). They were kept in the dark at subambiant temperature (-20°C) until use. Hundred grams of freshly ground material were subjected to steam distillation for 1 hour. The aqueous distillate was extracted three times with 100 ml peroxide-free diethyl ether. The ethereal phases were pooled, dried with anhydrous magnesium sulfate and the solvent was distilled at 40°C to about 10 ml. The extract was further concentrated to 4 ml at room temperature under a stream of pure nitrogen and analyzed by Cas Chromatography-Mass Spectrometry. A Nermag R10-10C Mass Spectrometer coupled to a Delsi DI 700 Gas Chromatograph was used.

The operating conditions were the following :

- column CP-Sil-8 CI3 (Chrompack-The Netherlands) 25 m length, *0.32* mm I.D., 0.2 µm film thickness,
- temperature program : cold 'on-column' injection at 30°C, T° rise at 5°C/min to 240°C then at 10°C/min to 290°C,
- carrier gas : helium at 1 ml/min,
- ionisation voltage : 70 eV,
- source T° : 200°C.

The recorded fragmentation data were compared with those of the EPA-NIH and WILEY-NBS mass spectra libraries (reference).

3. Results and discussion

The total ion current (chromatographic profile) of *B. senegalensis* volatiles is presented in figure 1.

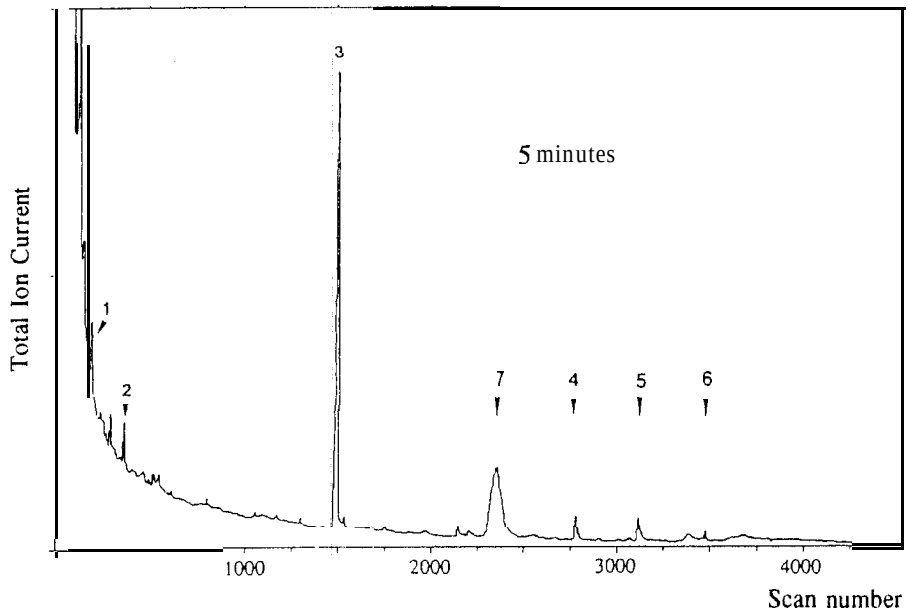

Figure 1. — Total ion current of *Boscia senegalensis* steam-distillated volatiles.

As shown, only a few peaks were observed. MITC (compound 1) liberated from methylglucosinolate during the distillation procedure was detected as a shoulder in the tail of the solvent. In previous analyses [SECK *et al.*, 1993] using polar GC columns, this molecule was completely resolved and unambiguously identified. Compound 2 corresponds to limonene whereas compound. 3 was identified as 2,6-di-*tert*-butyl-p-cresol. The latter compound came from the diethyl ether to which it is added as a stabilizer. Molecule 4 (M^+ = 312, other main fragments at m/z = 257, 256, 239, 227, 213, 129, 116, 71, 69, 57 and 56 [Base peak]) and its homologue 5 (M' = 340, other main fragments at m/z = 285,284, 267, 129, 116, 71, 69, 57 and 56 [Base peak]) were tentatively attributed to hexadecanoic and octadecanoic 2-methylpropyl esters. The phtalate 6 (Base peak at m/z = 149) eluted at 35.4 min is probably a contaminant from the plastic bags used to store the leaves. The broad peak 7 at

24.4 min exhibited a typical fragmentation pattem (Figure 2) with a molecular ion at 256 (33 %) and two isotopic ions p + 1 (1 %) and p + 2 (10 %) respectively at m/z = 257 and 258. This particular cluster (due to ^{33}S and ^{34}S natural isotopes) is indicative of a sulphur-containing product. The nature of such a molecule is fully confirmed by a series of similar clusters corresponding to a systematic loss of 32 atomic mass units. Compound 7 was unambiguously identified as elemental sulphur (S_8). The comparison of the recorded S_8 mass spectrum with those of the EPA-NIH and WILEY libraries supported our identification. The fact that *B. senegalensis* leaves samples came from trees not treated with S-containing pesticides is an argument confirming that elemental sulphur may not be considered as a contaminant.

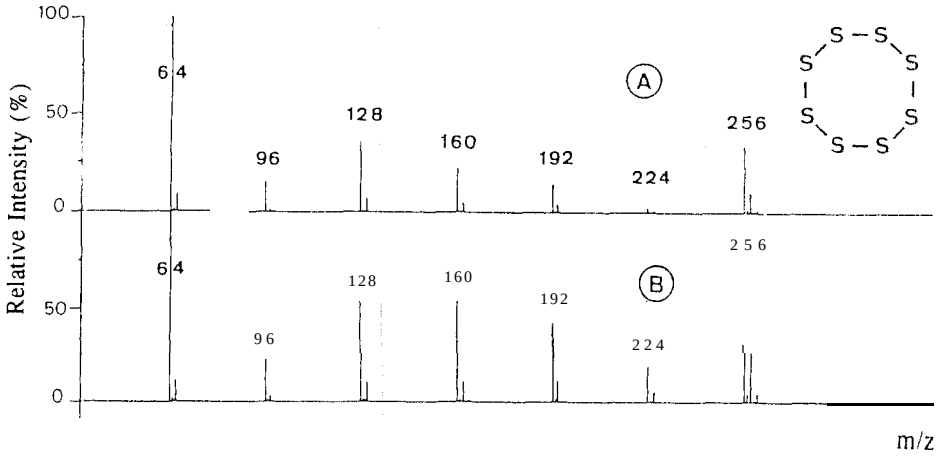

Figure 2. — Mass spectrum of the elemental sulphur (S_8).
A. from *Boscia senegalensis* ; B. reference.

To the authors knowledge, it is only the second time that elemental sulphur is detected in a plant material. Indeed, recently, the same compound was identified in *Capparis spinosa* L. flower buds [BREVARD *et al.*, 1992].

The biochemical pathway leading to the accumulation of elemental sulphur remains unexplained.

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BIOLOGICAL ACTIVITY OF *CASSIA OCCIDENTALIS* L. AGAINST *CALLOSOBRUCHUS MACULATUS* (F.) (COLEOF'TERA: BRUCHIDAE)

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Abstract—In developing countries, traditional control methods are commonly used against stored-product insects and mites. In Senegal, the leaves of *Cassia occidentalis* L. (Caesalpiniaceae) are used to protect cowpea seeds (*Vigna unguiculata* L. (Walpers)) against *Callosobruchus maculatus* (Coleoptera: Bruchidae). The biological activity of the leaves, the seeds and oil of C. *occidentalis* was evaluated in controlled conditions (28 ± 2°C, 45 ± 5% r.h.) against C. *maculatus*. At the rate of 10% (w/w), both fresh and dry leaves as well as whole and ground seeds had no contact toxicity on the cowpea beetle. In contrast, seed oil induced an increase in mortality of *C. maculatus* eggs and first larval instar at the concentration of 10 ml/kg cowpea. The basis of the ovicidal and larvicidal activities are discussed in this paper. Several trials using pure compounds have highlighted that several fatty acids (linoleic, oleic and stearic) are responsible for *C. occidentalis* toxicity. C. *occidentalis* seed oil did not reduce the oviposition of C. *maculatus* at 10 ml/kg seed.

Key words—*Callosobruchus maculatus* (F.), *Cassia occidentalis* L., biological activity, stored cowpea seeds, traditional method control, *Vigna unguiculata* (L.) Walp.

INTRODZJCTION

In developing countries and especially on the African continent, the warm climate and vulnerable storage conditions are favourable to the proliferation of numerous insect species and therefore induce significant post-harvest losses.

At farm level where financial and technical means are limited, post-harvest losses in grain legumes can reach 100% in a few months (Labeyrie, 1981). In order to combat this major problem inexpensively, people often use selected indigenous plants exhibiting insecticidal properties by mixing them with the stored grains.

In Senegal, C. *occidentalis* L., a very widespread weed (Higgins et *al.*, 1985) possessing medicinal properties (Anon., 1987; Pandey, 1975) is used to preserve cowpea stocks. It is claimed that the leaves mixed with cowpea seeds are protected against *C. maculatus* attacks. Like many other traditional control methods, the mode of action of this plant is still unknown. The present study was therefore directed to improve the understanding of the effects of C. *occidentalis* on C. *maculatus*. In a first series of trials, the effects of seeds and leaves were evaluated. Some authors (Boughdad *et al.*, 1987; Credland, 1992; Hill and Schoonhoven, 1981; Ivbijaro, 1990; Messina and Renwick, 1983; Naik and Dumbre, 1984; Pereira, 1983; Schoonhoven, 1978; Singh *et al.*, 1978; Su, 1991; Su *et al.*, 1972) reported on the potentialities of vegetable oils to protect cowpea seeds. Therefore a second part of this work was focused on the biological properties of the oil of the seeds of C. *occidentah.*

The effect of vegetable oil on stored product insects involves two different but complementary mechanisms: a reduction of the respiratory activity and a direct toxicity of oil constituents on eggs (Credland, 1992; Don-Pedro, 1989b; van Huis, 1991). In a study about insecticidal activity of vegetable oils against C. *maculatus*, Don-Pedro (1990) demonstrated the toxic properties of several fatty acids. The presence of other toxic minor lipids in the food of one or many developmental instars of the insects could also enhance oil toxicity.

Miralles and Gaydou (1986) have shown that the unsaponifiable matter in *C. occidentalis* represents a relatively high proportion of the oil (8% w/w) in contrast to the other oils (for example, *Cassia alata*: 5% w/w) and that this lipid fraction contained at least 40% sterol. Knowing their

role in insect growth regulation (Svoboda and Feldlaufer, 1991; Svodoba *et al.*, 1991), we also assesscd their potential effect on cowpea weevil development.

MATERIAL AND METHODS

Experimen tal conditions

The strain of *C. maculatus* came from the Department of Nioro du Rip (Senegal). It was reared on cowpea seeds (*Vigna unguiculata* (L.) Walpers, variety Black-eyed no. 5) under controlled conditions of temperature, 28 ± 2°C, and relative humidity, 45 ± 5%. Seeds and dried leaves of C. *occidentalis* came from the department of Nioro du Rip (Senegal) while fresh leaves were grown in greenhouses at Gembloux (Belgium) using seeds from Senegal.

Four lots of experiments were performed in the same controlled conditions (T = 28 ± 2°C; r.h. = 45 ± 5%).

1. Biological activity of leaves and seeds

Four experiments were undertaken in 90 mm dia petri dishes containing 25 g of cowpea seeds. Each was replicated 5 times. As a control treatment, 25 g of untreated cowpea seeds were similarly infested with *C. maculatus* and placed in the same experimental conditions. Five replications were also made. The females from bath the "flightless" and "flight" forms were used for the experiments 1, 2 and 3. For the experiment 4, only females from the "flightless" form were used.

—*Experiment 1.* Whole seeds were mixed with cowpea seeds at 10% (w/w).

—*Experiment* 2. Seed powder was mixed with cowpea at 10% (w/w). *To* obtain the *Cassia* powder, seeds were ground for 2-3 min using a small laboratory mill. The powder was then passed through a 500 dia sieve to separate fine particles from the pericarp which was not ground.

—*Experiment* 3. Dry leaves harvested in Senegal and dried outside in the shade were mixed with cowpea seeds at 10% (w/w).

—*Experiment* 4. Fresh leaves were ground with a small laboratory mill and mixed with cowpea seeds at 10% (w/w).

One day after the treatment of cowpea seeds with different forms of *C. occident&,* all petri dishes were infested with 10 adults (5 males + 5 females) of *C. maculatus* less than 24 h old.

When the Fl population began to emerge 18 days after infestation (DAI), a daily count of adults emerged was made until 34 DAI, one day beiore F2 adults began to emerge.

2. Biological activity of C. occidentalis seed oil

2.1. Oil extraction. Seeds of C. *occidentalis* were ground and extracted with n-Hexane for 8 h in a Soxhlet apparatus. The solvent was evaporated at 40°C under reduced pressure. The lipid extract was kept at 4°C until use.

2.2. Bioassay. Ten grams of c:owpea seeds contained in 55 mm dia petri dishes were treated with *C. occidentalis* seed oil at 10 ml/kg. *Cassia* oil was uniformly distributed on cowpea seeds using a "turbula" mixer at speed setting 2 for 10 min. After the treatment, the seeds were put in an incubator maintained at 28 ± 2°C, 45 ± 5% r.h. and infested one day later with four *C. maculatus* adults (2 males + 2 females) of the "flightless" form (Taylor and Agbaje, 1974; Utida, 1972) that were less than 24 h old. Untreated cowpea seeds were similarly infested and placed in the same experimental conditions. 12 replicates of each procedure were conducted. 18 days later, dead adults were taken out of the petri dishes and the eggs were counted. Thirty seeds were randomly sampled from each replicate of treatment and the numbers of dead and hatched eggs as well as dead first instar larvae were counted. The daily count of Fl emergence was done from day 21 to day 40 after infestation.

3. Biological activity of fatty acids

This experiment was undertaken to confirm or refute the hypothesis of Don-Pedro (1990) who linked the toxicity of some vegetable oils to fatty acids contained in the oil.

3.1. Analysis of' thefatty acids of C. occidentalis. The fatty acid composition of *Cassia* oil was determined by gas chromatography after transesterification with methanol-BF3 according to the

AOCS standard (1983). The chromatographic conditions were as follows: column CPWAX 52 CB (25 m long, 0.32 mm i.d., 0.2 m film thickness) from Chrompack; the carrier gas was helium at 0.7 bar; temperature program heating from 50 to 150°C at 30°C/min and then to 240°C at 5°C/min; gas chromatograph or Hewlett Packard 5880a fitted with an "on-column" injector and an FID detector maintained at 250°C. The fatty acids were identified by comparison of their retention times with that of pure acid.

3.2. Bioassay. Two sets of experiments were performed on fatty acids used either separately or together. Trials were conducted in 55 mm dia petri dishes containing 10 g of cowpea seeds. One kg of seeds was coated with the amount of fatty acid that would have been present in 10 ml of the *Cassia* oil, volume which was used in the experiment with C. ***occidentalis*** seed oil. Untreated seeds were used for the control.

The experiment was conducted following the same procedure as described in Section 2.2.

4. Biological activity of C. occidentalis unsaponifiable matter

4.1. Extraction. A sample of 2 g of oil was saponified for 1 h at 75°C with 100 ml of a 2N methanolic solution of KOH. The unsaponifiable matter was extracted 3 times with 100 ml of diethyl ether. The pooled ethereal extracts were washed 3 times with 40 ml of water and finally reduced to dryness at 35°C under reduced pressure.

4.2. Bioassay. The experiment was undertaken in 55 mm dia petri dishes containing 10 g of cowpea seeds and was replicated 6 times. Two treatments were considered:

-control treatment: 1 kg of seeds was treated with x ml of solvent,
—experimental treatment: 1 kg of seeds was treated with x ml of unsaponifiable solution, where x is the content of unsaponifiable matter contained in 10 ml of ***Cassia*** oil.

The F1 population began to emerge 18 days after the initial infestation. From that time to the 34th day after this infestation, newly emerged adults were counted daily.

Statistical analysis

Data were submitted to Student tests (Dagnelie, 1975) using the software package Minitab on the Vax 8250 at the calculation and data processing Center of the Faculty of Agricultural Sciences (Gembloux, Belgium).

RESULTS

1. Biological activity of seeds and leaves

The mean number of adults which emerged varied from 154.2 for the dry leaves treatment to 293.2 for the fresh leaves. In the same conditions 157.7 to 276.8 adults emerged from untreated cowpea seeds (Table 1).

2. Biological activity of seed oil

The mean numbers of eggs laid by ***C. maculatus*** females in the control treatment were 98.7 on cowpea seeds and 7.9 on the petri dishes. In the same time, 98.7 and 15.9 eggs respectively were laid in the presence of oil treated seeds. Statistical analysis of the data indicated that more eggs

Tabk 1. The biological activity of *Cassia occidentalis* leaves and seeds on *Callosobruchus maculatus*

	Mean number of adults emerged[1]	
Treatment	0 (control)	10
Whok seeds	*166.2 ± 61.8'*	196.0±*40.7'*
Seed powder	*235.6 ± 19.8'*	*229.0* ±77.4[a]
Dry leaves	*157.7* ± 43.6[a]	*154.2 ± 28.2'*
Fresh leaves[2]	*276.8 ± 16.6'*	*293.2 ± 17.3'*

[1]Within a line, means followed by the same ktter are not significantly different at the 5% level.
[2]Infestation with females from the "flightless" form.

Tabk 2. The effect of cowpea treatment with *Cassia occidentalis* seed oil on the oviposition of *Callosobruchus maculatus*

	Mean number of eggs laid on'		
Treatment	Cowpea seeds	Petri dishes	Total
Control	*111.7f8.6'*	*7.9* ±8.6[b]	*119.5* ±15.7[c]
H10	*98.7* ±21.5[a]	15.9 ± 9.7[b]	114.6 ± 17.1[c]

H10: seeds treated with 10 ml of seed oil/kg of cowpea.
'Within a column, means followed by the same letters are not significantly different at the 5% level.

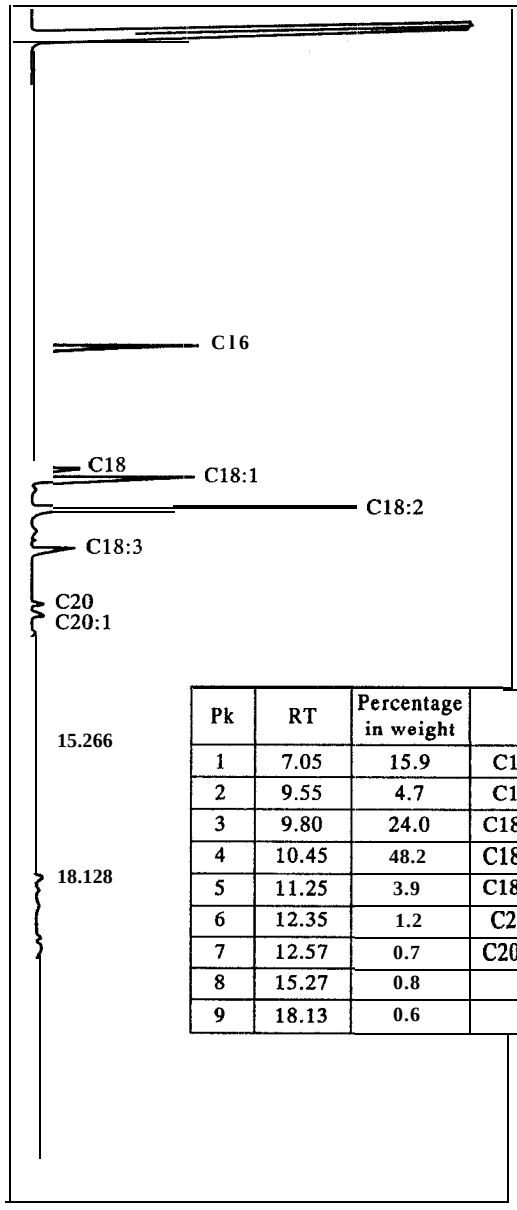
Table 3. The biological effects of *Cassia occidentalis* seed oil on *Callosobruchus maculatus* eggs, first instar larva and progeny

Treatment	Mean percentage mortality[1]		Mean number of F1 emerged[1]
	Eggs	Larvae	
Control	1.8 ± 1.8[1]	0.4 ± 2.4[a]	100.1 f0.8[1]
H10	28.4 ± 6.0[b]	51.6 ± 7.4[b]	10.9 ± 10.8[b]

H10: seeds treated with 10 ml oil/kg of cowpea.
'Within a column, means followed by the same letters are not significantly different at the 5% level.

were deposited on cowpea seeds compared to petri dishes even when seeds were treated with *Cassia* oil (Table 2).

The mean percentage mortality of eggs and larvae were respectively 1.8 and 0.4 in the control and 28.4 and 57.6 on oiled seeds. The mean number of C. *maculatus* which emerged was 100.1 insects in the control and was 10.9 on treated seeds (Table 3).



Pk	RT	Percentage in weight	Name	
1	7.05	15.9	C16	Palmitic acid
2	9.55	4.7	C18	Stearic acid
3	9.80	24.0	C18:1	Oleic acid
4	10.45	48.2	C18:2	Linoleic acid
5	11.25	3.9	C18:3	
6	12.35	1.2	C20	
7	12.57	0.7	C20:1	
8	15.27	0.8		
9	18.13	0.6		

Fig. 1. Chromatogram of dosage of different fatty acids from *Cassia occidentalis* seed oil.

Tabk 4. The effect of *Cassia occidentalis* oil fatty acids on the oviposition of *Callosobruchus maculatus*

Treatment	Mean number of eggs laid'		
	seeds	Petri dishes	Total
Control	116 ± 36.6'	20.2 ± 16.8'	136.2 ± 34.8'
linokic acid	138 ± 21.7'	9.2 ± 13.7'	147.3 ± 29.1[a]
control	95.2 ± 16.T	9.3 ± 9.5'	104.5 ± 21.7"
oleic acid	13.2 ± 25.5'	35.8 ± 19.6'	109 ± 27.9'
Control	155.8 ± 26.3'	8.8 ± 14.2[a]	164.7 ± 30.4[a]
palmitic acid	154.5 ± 36.6'	3.8 ± 7.0[a]	158.3 ± 42.4'
Control	146 ± 19.3[a]	6.7 ± 9.4'	152.7 ± 22.7
stearic acid	20.8 ± 19.0[b]	1.2 ± 1.3[a]	22.0 ± 19.4[b]
Control	129 ± 23.1'	23.8 ± 14.4'	152.8 ± 28.4'
bknd of the 4 acids	128.5 + 31.Y	14.7 + 11.1[a]	143.2 + 41.4[a]

'Within a column, means followed by the same letters are not significantly different at the 5% level.

A decrease in the adherence of eggs on the seeds treated with the oil has been observed.

3. *Biological activity of fatty acids*

The fatty acid profile of C. ***occidentah*** seed oil is shown in Fig. 1. The analysed samples contained 48.2% of linoleic acid, 24.0% of oleic acid, 15.9% of palmitic acid and 4.7% of stearic acid. These results are in line with those of Miralles and Gaydou (1986).

The mean number of eggs laid by C. *maculatus* females in the control varied from 95.2 to 155.8 on cowpea seeds and from 6.7 to 23.8 on petri dishes. On treated seeds, their means varied respectively from 20.8 to 154.5 and from 1.2 to 35.8. The total, mean number of eggs laid by females varied from 104.5 to 158.3 eggs except to seeds treated with stearic acid (only 22 eggs) (Table 4).

The average mortality of eggs and first Iarva instars were respectively less than 3.4% and 2.6% on untreated seeds. On the other hand, mortality varied from 3 to 61.4% for the eggs and from 1.2 to 23.6% for the larvae on treated seeds (Table 5).

The mean numbers of C. *maculatus* emerged varied from 81.5 to 124.2 adults in the control and from 5.2 to 82.8 adults on treated seeds (Table 6).

4. *Biological activity of unsaponifiable part of oil*

The mean number of C. *maculatus* which emerged was 123.9 on untreated seeds and 141.2 on treated seeds (Table 7).

Table 5. Thc effect of *Cassia occidentalis* seed oil fatty acids on the mortality of *Callosobruchus maculatus* eggs and first instar larva

	Mean percentage of eggs[1]		Mean percentage of first instar larva dead[1]
	Dead	Hatched	
Control	2 ± 1.2'	91.4 ± 1.6'	0.6 ± 1.0[a]
linokic acid	25.4 ± 25.0[b]	73.6 ± 24.4[b]	1.2 ± 1.0[a]
Control	2.4 ± 3.0'	97 ± 3.8'	1.0 ± 1.6'
oleic acid	59.4 ± 8.6[c]	36 ± 7.0[c]	4.6 ± 3.8'
Control	3.4 ± 3.2'	94 ± 3.8'	2.6 ± 3.8'
palmitic acid	3 ± 4.6'	93.6 ± 5.0[a]	3.4 ± 3.8'
Control	1.5 ± 1.0[a]	98.5 ± 1.0[a]	0.0 ± 0.0[a]
Stearic acid	6.1 ± 1.0[d]	91.5 ± 3.3"	2.4 ± 2.8'
Control	4 ± 4.2'	93.4 ± 6.6'	2.6 ± 2.8'
blend of tbe 4 acids	61.4 ± 21.8[c]	15.4 ± 7.8[d]	23.6 ± 17.0[b]

'Within a column, mean percentages followed by the same letters arc not significantly different et the 5% level.

Table 6. The effect of four fatty acids extracted from *Cassia occidentalis* oil on the emergence of adults of *Callosobruchus maculatus*

	Mean number of Fl emerged[1]
Control	117.7 ± 25.2[a]
linoleic acid	68.7 ± 19.5[b]
Control	81.5 ± 19.5[a]
okic acid	20.5 ± 6.7[c]
Control	124.2 ± 22.0[a]
palmitic acid	82.8 ± 4.6[b]
Control	117 ± 13.7[a]
stearic acid	17.2 ± 4.6[c]
Control	83.4 ± 23.3[a]
bknd of the 4 acids	5.2 ± 3.1[d]

[1]Within a column, means followed by thc same ktter arc not significantly different at the 5% level.

Tabk 7. The biological effects of *Cassia occidentalis* unsaponifiable matter on *Callosobruchus maculatus* progeny

	Mean number of Fl emerged[1]
Control	123.9 ± 20.7[a]
Treatment	141.2 ± 15.6[a]

[1]Within a column, mean percentages followed by the same letters are not significantly different at the 5% level.

DISCUSSION

In our experimental conditions, the whole seeds, the seed powder, the dry leaves and the fresh leaves did not reduce the number of adult emerged and thus had no biological activity against C. ***maculatus.*** It is noteworthy that fresh leaves used in our trials were harvested in greenhouses in Belgium. Ermel ***et al.*** (1986), Levin and York (1978) and Singh (1986) reported the importance of the origin of the Neem tree (*Azadirachta indica* L.), a plant used as an alternative control method, as a source of variability for chemical composition and probably for biological activity also. Modification of growing conditions induced changes of azadirachtin content (the active principle of Neem tree) and therefore led to significant changes in the insecticidal properties.

Moreover, it is important to recall that females from both the "Aightless" and "flight" forms were used for the biological activity tests with whole seeds, the seed powder and the dry leaves. Sano (1967) and Utida (1972) observed that females from the "flight" form had a low fecundity and fertility compared to females from the "flightless" form. The use of these two forms explained the great variability in the results obtained in trials with whole seeds, seed powder and dry leaves. For all other tests, only females from the "flightless" form were used.

C. occidentalis seed oil did not significantly affect the number and the distribution (on cowpea seeds and on petri dishes) of eggs laid but did reduce the mean number of Fl adults which emerged. The present results indicate that C. ***occidentah*** seed oil is effective in reducing damage to cowpea seeds from C. *maculatus.* There is an increase in mortality of both eggs and first instar larvae which results in a significant reduction in C. *maculatus* progeny and damage. Our results with ***C. occidentah*** seed oil are in agreement with many other works on the use of oils against stored-products insects (Boughdad ***et al.***, 1987; Credland, 1992; Don-Pedro, 1989a, b, 1990; Hill and Schoonhoven, 1981; Ivbijaro, 1990; Messina and Renwick, 1983; Naik and Dumbre, 1984; Pereira, 1983; Schoonhoven, 1978; Singh ***et al.***, 1978; Su, 1991; Su ***et al.***, 1972; van Huis, 1991). Four different hypotheses have been given by these authors to explain the toxicity of vegetable oils:

(1) The toxicity to the eggs and first instar larvae is the consequence of the occlusion of the short funnel at the posterior end of the egg. This hypothesis can explain the ovicidal and larvicidal effects observed in our experiments;

(2) A reduction of egg adherence on the cowpea seed which prevents the first instar larva from penetrating the seed testa. This decrease of adherence has been observed in the experiments described and can explain mortality of C. ***macularus*** first instar larvae;

(3) A direct toxic effect of some oil constituents;

(4) The toxicity of different fatty acids contained in the oil.

In our experimental conditions, the four fatty acids used separately or all together reduced significantly the number of progeny. Oleic, stearic acids and the blend of the four fatty acids were the most effective. Both linoleic and oleic acids exhibit only ovicidal effects. This observation is in line with Don-Pedro's results (1990). Palmitic acid did not reduce oviposition and did not affect either fecundity or first instar larva survival. **Nevertlheless, stearic acid is the only lipid tested which**

reduced the mean number of eggs laid by C. *maculatus* females. Moreover, stearic acid has also an ovicidal effect, although the increase of mortality is less than with oleic and linoleic acids.

From the results (Table 7), it is clear that the unsaponifiable matter of *Cassia* oil did not significantly reduce the number of beetle progeny.

CONCLUSIONS

In the present experimental conditions, the fresh and dry leaves, whole and powdered seeds of *C. occidentalis* showed no contact toxicity. As modifications of growing conditions are known to induce modifications of the chemical composition of plant material, it would be interesting to test fresh harvested leaves from tropical areas to confirm or invalidate the aforementioned results.

The toxicity of *Cassia occidentalis* oil can be assigned to physical effects (suffocation of eggs and decrease of eggs adherence) and to direct toxicity of fatty acids of the oil.

In tropical Africa, *Arachis* oil is used by farmers to protect stored cowpea seeds (Boughdad *et al.*, 1987; Don-Pedro, 1989a; Messina and Renwick, 1983; Pereira, 1983) but this oil could be valorized for trade and export. It is also possible that other underexploited oils could have a potential role in grain protection.

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