BIOLOGICAL ACTIVITY OF THE SHRUB *Boscia senegalensis* (PERS.) LAM. EX POIR. (CAPPARACEAE) ON STORED GRAIN INSECTS

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Abstract—Biological activity of leaves, fruits and extract of the African shrub *Boscia senegalensis* (PERS.) LAM. ex Poir. was evaluated against five stored-grain insects. When added to cowpeas at 2–4% (w/w), fresh ground fruits and leaves caused 80–100% mortality in *Callosobruchus maculatus* (F.) adults and significantly reduced both emergence and damage of the F_1 progeny. Acetone fruit extract exhibited a potent fumigant effect on *Prostephanus truncatus* HORN., *C. maculatus*, and *Sitotroga cerealella* OLIV.; with LT_{50} values of 3.8, 2.3, and below 1.5 hr, respectively. LC_{50} determination for *B. senegalensis* fruits and leaves as well as pure methylisothiocyanate (MITC) on *Tribolium castaneum* HERBST, *Sitophilus zeamais* MOTSCH. and *C. maculatus* showed a differential response of the insects to plant parts or MITC. Quantitative dosage of *Boscia* active components and LC_{50} values obtained for the plant tissues, compared to those of pure molecules, indicate that the biological activity of *B. senegalensis* is due to the liberation of MITC from a glucosinolate precursor glucocapparin contained in *Boscia* fruits and leaves.

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INTRODUCTION

Insect infestation of stored grain causes weight and quality losses that lead to a reduction of commercial value and seed germination. To reduce this damage, several control measures have been taken, which mostly involve chemical methods. Synthetic insecticides are not only a drain on the farmer's meager resources but are often frequently used beyond permissible safe limits. The incidence of insecticide resistance is also a growing problem. Resistance to one or more insecticide(s) has been reported in at least 500 species of insects and mites (Georghiou, 1990). This situation has increased the need for alternatives to synthetic pesticides. Botanical insecticides are a less expensive and biodegradable option. The effectiveness of raw parts and plant extracts against stored-grain insects has been described by many authors (Jotwani and Sircar, 1965; Singh et al., 1978; Jacobson, 1983; Golob and Webley, 1980; Grainge et al., 1985; Lognay et al., 1991; Seck et al., 1991; Haubruge et al., 1989).

We investigated the biological activity of *Boscia senegalensis* (PERS.) LAM. (Capparaceae), a plant that is found throughout north-central and northern Senegal (Bille and Poupon, 1972), where it is traditionally used by farmers against stored grain insects. In addition, we isolated and identified biologically active components of this plant.

METHODS AND MATERIALS

Plant material was randomly harvested in the region of Thies (Senegal). Our material has been identified and deposited at the Jardin Botanique National de Belgique (BR) as *B. senegalensis*. Leaves used for trial 1 were harvested in November 1990. Fruits and leaves used for trials 2 and 3 were collected in May 1991.

Four experiments were conducted with plant parts (trials 1 and 2), extracts (trial 3), and pure molecules (trial 4) using five stored-grain insect species. The test insects, *Callosobruchus maculatus* (F.) (Col., Bruchidae), *Sitotroga cerealella* OLIV. (Lep., Gelechiidae), *Prostephanus truncatus* HORN. (Col. Bostrychidae), *Tribolium castaneum* HERBST. (Col., Tenebrionidae), *Sitophilus zeamais* MOTSCH. (Col., Curculionidae), were reared under controlled conditions ($32° \pm 2°C$ and $80 \pm 10\%$ relative humidity). Bioassays were also carried out under these conditions. All chemicals and solvents were of analytical grade.

Trials 1 and 2. These first two trials were performed with fresh ground leaves (FGL), fresh entire leaves (FEL), dry leaf powder (DLP), and fresh ground fruits (FGF). The test materials were added to cowpea seeds, *Vigna unguiculata* (L) WALP (var. Black Eyed) at 1–32% for FGL, FEL, and DLP in trial 1; and 0.5–2% for FGF and FGL in trial 2. FGL and FGF were prepared

using a small laboratory mill (Moulinex) for 5 min. DLP was similarly ground and then passed through a 500-mesh laboratory sieve. Following this procedure, 20 g of cowpeas were thoroughly mixed with plant material at the desired concentrations in Petri dishes (ϕ 90 mm) and infested with 10 unsexed 1-day-old *C. maculatus* adults. Untreated cowpea seeds were similarly infested with 10 insects. Each treatment, including the control, was replicated five times. Mortality assessment was made after one to two days. Twenty-two days later when F_1 adults started to emerge, insects were removed daily and counted for 10 days. Numbers of holed and unholed seeds were also counted and percent damage was calculated.

Data were subjected to analysis of variance followed by a comparison of the means (Duncan's multiple-range test) at $P = 0.05$.

Trial 3. Fifty grams of *Boscia* fruits were ground with 200 ml acetone in a Waring blender and kept for 2 hr at room temperature. The supernatants were then filtered and concentrated under vacuum to a final volume of 50 ml to obtain a *B. senegalensis* acetone fruit extract (BAFE) containing approximately 30% by volume of water.

Bioassays were carried out in 825-ml sealed glass desiccators in which 5 ml of BAFE were deposited. Five milliliters of acetone were similarly pipetted in another desiccator used as control. After 3 hr at room temperature, the solvent evaporated completely in the control. One hundred adults of different species were then introduced in the desiccators, which were closed and placed under experimental conditions. After various time exposures, from 1.5 to 12 hr, mortality readings were recorded for each treatment. The time required to kill 50% (LT_{50}) of the insects was then determined by transforming mortality data to probits and calculating the lethal times (LT) (Snedecor and Cochran, 1967).

Analysis of volatiles from Boscia senegalensis *leaves.* Freshly ground leaves (100 g) were steam-distilled for 45 min and the aqueous distillate (900 ml) extracted three times with 100 ml diethylether. The ether solution was dehydrated with anhydrous sodium sulfate, concentrated to 4 ml by distillation of the solvent at 38°C, and finally analyzed by gas-liquid chromatography (GLC) using two types of columns under the following conditions: The polar column was a CP-Wax 52CB (50 m long, 0.32 mm ID, 0.2 μm film thickness) from Chrompack; carrier gas was helium at 100 kPa; temperature program: from 30 to 240°C at 10°C/min; cold "on-column" injector and FID detector maintained at 250°C; apparatus: Hewlett Packard HP 5880. The apolar column was a CP-Sil 8 CB (25 m long; 0.32 mm ID, 0.2 μm film thickness) from Chrompack; carrier gas was helium at 50 kPa; temperature program: from 30 to 240°C at 5°C/min; cold "on-column" injector and FID detector maintained at 300°C; apparatus: Carlo Erba Mega 5160.

Analysis of glucosinolates. The glucosinolates were analyzed in *B. senegalensis* FGF, FGL, and BAFE by reverse-phase HPLC after enzymatic desul-

fation according to the official EEC method (European Economic Community, 1990). Identification of BAFE glucosinolates was performed by GC-MS analyses of trimethylsilylated molecules and by examination of their degradation products liberated enzymatically under controlled conditions. For GC-MS investigations, glucosinolates were enzymatically transformed to desulfoglucosinolates and trimethyl-silylated for 20 min at 110°C with 50 μl of a reagent containing N-methyl-N-trimethylsilyltrifluorobutyramide, 5% methylimidazole in acetone and trimethylchlorosilane 30:15:3 (v/v/v). The chromatographic conditions were as follows: Column SE-52 (25 m long, 0.35 mm ID, 0.2 μm film thickness) was from Macherly-Nagel; carrier: helium at 50 kPa; temperature program: 50–280°C at 20°C/min; cold "on-column" injection. The mass spectra were recorded in the EI mode on a Nermag R10-10C spectrometer (70 eV, source at 130°C, interface at 280°C, mass range scanned from 100 to 800 amu) coupled to a Delsi DI-700 gas chromatograph.

For enzymatic degradation of B. senegalensis glucosinolates, 0.1 ml acetate buffer (pH 4.5) and 50 μl of 10 mg/ml buffered solution of thioglucosidase (EC 3.2.3.1), purified from Sinapis alba L. according to Appelqvist and Josefson (1967), were added to 0.1 ml of residual aqueous phase from BAFE or to a solution of pure methylglucosinolate (glucocapparin, Roth ref. 7485). After 24 hr, the degradation products were extracted with 2 ml diethylether and analyzed by GLC on polar and apolar stationary phases. Methylisothiocyanate (MITC) from BAFE and glucocapparin were identified by comparison of their retention times with that of a pure reference (Sigma ref. M8632).

Detection of MITC in Volatiles Liberated during Biotests. Acetone solutions of BAFE (5 ml) were introduced into conical flasks. After evaporation of acetone, the vessels were hermetically closed and left for 24 hr at 32 $\pm$ 2°C. Headspace sampling was performed by purging the apparatus with nitrogen for 30 min. The volatiles were collected in 3 ml diethylether maintained in a cryogenic trap at −20°C. Ether solutions were submitted to GC-MS in the aforementioned conditions except that the temperature program rate was fixed at 5°C/min. MITC was identified on the basis of retention and mass spectral data. The mass spectra were compared with EPA-NIH and Wiley libraries.

Trial 4. To study the dose–mortality responses to B. senegalensis tissues and MITC, various amounts of FGF and FGL from 0 to 8 g/liter (w/v) and pure MITC from 0 to 3 mg/liter (w/v) were deposited in 750-ml glass desiccators containing 25 adults of each insect species, in four replications. After 24 hr under the experimental climatic conditions, insects were transferred to clean Petri dishes and maintained in controlled conditions until the next day for mortality readings as indicated by Busvine (1981). Data were subjected to probit analysis (Finney, 1964). Log dose–probit line was analyzed for goodness of fit by the chi-square test (Busvine, 1981), followed by computation of LC_{50} values for each material.

RESULTS

Trial 1

Mortality. FGL at a concentration of 4% (w/w) caused 100% mortality after 24 hr. At 2%, 55.6% of the insects died within 48 hr, and at 1%, mortality ranged from 7.4 to 11.1% in one to two days. FEL scored 0–18.5% mortality and DLP 3.7–40.7% within 24–48 hr (Table 1).

Progeny. FGL completely inhibited the production of *C. maculatus* progeny at 2%, but at 1%, 21.6 adults emerged. At the same time, progeny ranged from 36.2 to 87.2 adults for FEL and from 40.2 to 53.2 for DLP (Table 1).

TABLE 1. BIOLOGICAL ACTIVITY OF *B. senegalensis* FRESH GROUND LEAVES (FGL), FRESH ENTIRE LEAVES (FEL) AND DRY LEAF POWDER (DLP) TO *C. maculatus* ADULTS, F_1 PROGENY, AND DAMAGE (MEANS)[a]

| Treatment | Conc (%, w/w) | Corrected mortality (%)[b] | | F_1 progeny | Damage (%) |
		24 hr	48 hr		
FGL	1	7.4c	11.1c	21.6ab	22.0b
	2	63.0b	55.6b	0.0b	0.0b
	4	100.0a	100a	0.0b	0.0b
	8	100.0a	100a	0.0b	0.0b
	16	100.0a	100a	0.0b	0.0b
	32	100.0a	100a	0.0b	0.0b
	Control			61.6a	70.3a
FEL	1	7.4a	18.5a	36.2b	49.1a
	2	0.0a	7.4ab	43.6ab	36.9a
	4	0.0a	7.4ab	69.0ab	59.8a
	8	0.0a	0.0b	87.2a	64.8a
	16	0.0a	0.0b	62.4ab	52.9a
	32	0.0a	0.0b	71.8ab	60.5a
	Control			61.6ab	56.3a
DLP	1	11.1a	37.0a	53.2a	67.5a
	2	3.7a	18.5a	47.6a	44.4abc
	4	11.1a	25.9a	51.0a	52.4abc
	8	11.1a	33.3a	49.8a	33.6bc
	16	14.8a	33.3a	40.2a	39.7abc
	32	11.1a	40.7a	45.8a	30.8c
	Control			45.0a	65.1ab

[a] Means followed by the same letter within a column of each treatment are not significantly different at the 5% level (Duncan's multiple-range test).
[b] By Abbott's (1925) formula.

FGL significantly reduced *C. maculatus* progeny, compared to FEL and DLP (Figure 1).

Damage. FGL gave 100% protection at 2% and 22% damage at 1%. In the same conditions, damage was 36.9–64.8% for FEL and 30.8–67.5% for DLP (Table 1).

Trial 2

Mortality. After 72 hr at concentration of 2%, mortality was 93.6% for FGF and 24.8% for FGL. After 48 hr, it ranged from 3.8 to 79.8% for FGF compared to 0–24.2% for FGL. After 24 hr, mortality ranged from 3.8 to 73.8% for FGF and from 0 to 8% for FGL (Table 2).

Progeny. At 2%, FGF showed no adult emergence while 7.8 and 133.8 adults emerged, respectively, in FGL and the control treatment. At 1%, 12 adults emerged from FGF compared to 104 for FGL (Table 3).

Damage. At 1% concentration, damage was 8.7% for FGF and 63% for FGL. At 2% concentration, FGF gave 100% protection, while 6.1 damage was noted in the FGL treatment (Table 3).

Trial 3

LT_{50} values were 2.3 hr for *C. maculatus* and 3.8 hr for *P. truncatus* (Table 4). For *S. cerealella* all adults died within 1.5 hr.
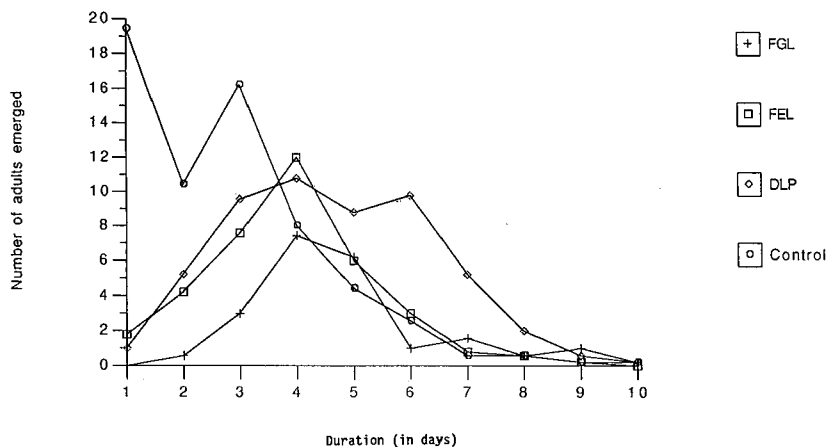

FIG. 1. Emergence pattern of *C. maculatus* from cowpea seeds treated with fresh ground leaves (FGL), fresh entire leaves (FEL), or dry leaf powder (DLP) of *Boscia senegalensis* at 1% conc. (w/w).

TABLE 2. COMPARATIVE TOXICITY OF *B. senegalensis* FRESH GROUND FRUITS (FGF) AND LEAVES (FGL) TO *C. maculatus* (MEANS)[a]

Treatment	Conc (%, w/w)	Corrected mortality after (%)[b]		
		24 hr	48 hr	72 hr
FGF	0.5	3.8b	3.8c	1.9c
	1	6.4b	24.9b	35.9b
	2	73.8a	79.8a	93.6a
FGL	0.5	0.0a	0.0b	23.4a
	1	3.8a	5.8ab	13.0a
	2	8.0a	24.2a	24.8a

[a] Means followed by the same letter within a column of each treatment are not significantly different at the 5% level (Duncan's multiple-range test).
[b] By Abbott's (1925) formula.

TABLE 3. EFFECT OF COWPEA TREATMENT WITH *B. senegalensis* FRESH GROUND FRUITS (FGF) or LEAVES (FGL) ON *C. maculatus* EMERGENCE AND DAMAGE IN COWPEAS (MEANS)[a]

Treatment	Conc. (%, w/w)	Number of emerged adults[b]	Percentage Damage (%)	Reduction (%)	
				Emergence	Damage
FGF	0.5	3.4a	22.7a	68.2a	73.0a
	1	1.6b	8.7a	90.7a	89.6a
	2	0.0c	0.0a	100a	100a
FGL	0.5	4.2a	46.0a	41.7b	45.2b
	1	4.6a	63.0a	22.3b	25.0b
	2	1.8b	6.1b	94.5a	92.7a
Control		4.9	84.0		

[a] Means followed by the same letter within a column of each treatment are not significantly different at the 5% level (Duncan's multiple-range test).
[b] Log (numbers).

Trial 4

Acute Toxicity of B. senegalensis *Fresh Ground Tissues and Pure MITC.* FGF, FGL, and MITC exhibited a differential acute toxicity against three stored-grain beetle species. LC_{50} values (in grams ground matter per liter volume) ranged from 1 to 4.23 for FGL and from 0.42 to 1.75 for FGF (Table 5). LC_{50}

Table 4. Lethal Time (LT) Values of Two Stored-Grain Insects Exposed to Vapors of B. senegalensis Fruit Extract (BAFE)

Species[a]	Slope ± SE	LT_{50} (95% FL^b), hr
C. maculatus	4.47 ± 1.71	2.31 (1.01–5.28)
P. truncatus	4.21 ± 1.16	3.80 (1.92–7.54)

[a]One-day-old C. maculatus; 2-week-old P. truncatus.
[b]Fiducial limits.

Table 5. Acute Toxicity of B. senegalensis Fresh Ground Fruits (FGF) and Leaves (FGL) to Adults of Three Stored-Grain Insect Species

Species[a]	FGF		FGL	
	Slope ± SE	LC_{50} (95% FL)[b]	Slope ± SE	LC_{50} (95% FL)[b]
Tribolium castaneum	8.60 ± 1.00	1.75 (1.63–1.86)	6.14 ± 1.38	4.23 (1.11–16.15)
Sitophilus zeamais	7.12 ± 0.79	0.87 (0.80–0.94)		
Callosobruchus maculatus	4.91 ± 0.75	0.42 (0.36–0.47)	6.15 ± 0.93	1.00 (0.88–1.09)

[a]Four replications of 20 insects were exposed to plant materials for 24 hr before they were transfered to Petri dishes and placed in controlled conditions (30°C, 70% relative humidity). Mortality was counted after 24 hr of pest exposure (Busvine, 1981).
[b]Grams per liter volume, with Fiducial limits.

for MITC ranged from 0.73 to 2.38 ppm according to insect species, with C. maculatus being the most susceptible (Table 6).

Chemical Investigations

GLC analysis of the volatiles from FGL showed one major peak (92% of the total area) at retention times of 9.3 min and 6.5 min, respectively, on polar and apolar columns, which corresponded to those of an authentic sample of MITC. The detection of MITC from B. senegalensis leaves (Figure 2) suggested that methylglucosinolate (glucocapparin) may be the main precursor of the insecticidal compound. Glucosinolate enzymatic degradation leads to several by-products among which isothiocyanates predominate (Tookey et al., 1980). To test the aforementioned hypothesis, we analyzed the residual water phase of BAFE following a three-step procedure (HPLC, identification of glucosinolate by-product, and GC-MS). The HPLC retention time of pure desulfomethylglucosinolate (2 min) corresponded to that of the major peak detected in BAFE. On the other hand, the elution profiles liberated enzymatically from BAFE

TABLE 6. ACUTE TOXICITY OF METHYL ISOTHIOCYANATE TO ADULTS OF THREE STORED-GRAIN INSECT SPECIES

Species[a]	Slope ± SE	LC_{50} (95% FL)[b]
Tribolium castaneum	7.05 ± 0.58	2.38 (2.24–2.52)
Sitophilus zeamais	7.22 ± 1.18	1.25 (1.14–1.34)
Callosobruchus maculatus	4.81 ± 0.59	0.73 (0.64–0.82)

[a] Four replications of 20 insects were exposed to plant materials for 24 hr before they were transfered to petri dishes and placed in controlled conditions (30°C, 70% relative humidity). Mortality was counted after 24 hr of pest exposure (Busvine, 1981).
[b] ppm, with Fiducial limits.

residual aqueous phase and methylglucosinolate are practically identical; MITC is distinguishable on the two chromatograms but is absent on the blank. The identification of glucocapparin in BAFE was finally validated by GC-MS. A typical mass spectra is shown in Figure 3. The mass fragments at $m/e = 103$, 117, 147, 169, 204, 243, 271, 361 (base peak), and 451 were generated by the glucidic moiety of the molecule and were not of interest for the identification of the aglycone. Nevertheless, the ions recorded at $m/e = 613$ $(M)^+$, 598 $(M\text{-}CH_3)^+$, 524 $(M\text{-}CH_3\text{-}TMS)^+$ and 508 $(M\text{-}CH_3TMSOH)^+$ indicated clearly a glucosinolate bearing a methyl radical. As for all other alkylglucosinolates, the intensities of these characteristic mass fragments were low. The methylglucosinolate content of *B. senegalensis* material tested in trial 4 represented 23.6 ± 0.8 μmol/g fresh leaves and 38 ± 1.2 μmol/g fresh fruits (HPLC determination with sinigrin as internal standard). Headspace sampling conducted under the same conditions as trial 3 and trapping the volatiles in diethyl ether at −20°C led to detection of MITC from the vapor phase. The total ion current (Figure 4) showed several peaks, among which was MITC ($R_t = 16.6$ min). The molecule was unambiguously identified by comparison of its mass spectrometric pattern with EPA-NIH and Wiley libraries and also on the basis of GLC retention time.

DISCUSSION

This research has demonstrated a significant biological effect of *B. senegalensis* plant parts and extracts. The evidence in support of these results was obtained from four experiments. First, *B. senegalensis* FGL (when added to cowpeas at 4% w/w) completely killed *C. maculatus* adults within 24 hr, inhibited the production of F_1 progeny and prevented bruchid damage. Under the same conditions, FEL and DLP had almost no effect. Second, comparative
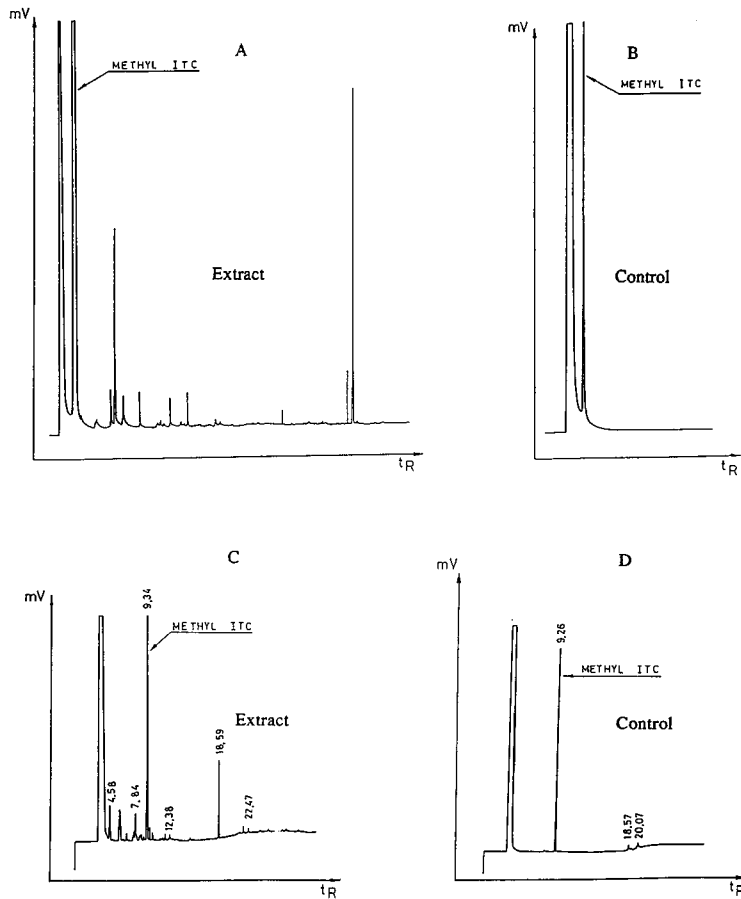
FIG. 2. Identification of methylisothiocyanate (MITC) from *Boscia senegalensis* leaves with apolar column (A and B) and polar column (C and D).

evaluation of FGF and FGL revealed that fruits were more toxic to *C. maculatus* and reduced both progeny and damage to a greater extent, than did leaves. Third, BAFE exhibited a high fumigant effect on three stored-grain insect species, which had a differential time–mortality response. Finally, we quantified the acute toxicity of *B. senegalensis* fresh ground fruits and leaves as well as pure MITC and obtained dose–mortality responses for three stored-grain beetles.

B. senegalensis is a shrub, growing up to 3 m high, that is frequently found on abandoned termite mounds and on barren and fire-scorched soil of the Sahel. It is distributed from Mauritania to Niger, northern Nigeria, the northwest Cameroons, and across Africa to Sudan and Ethiopia (Booth and Wickens, 1988).
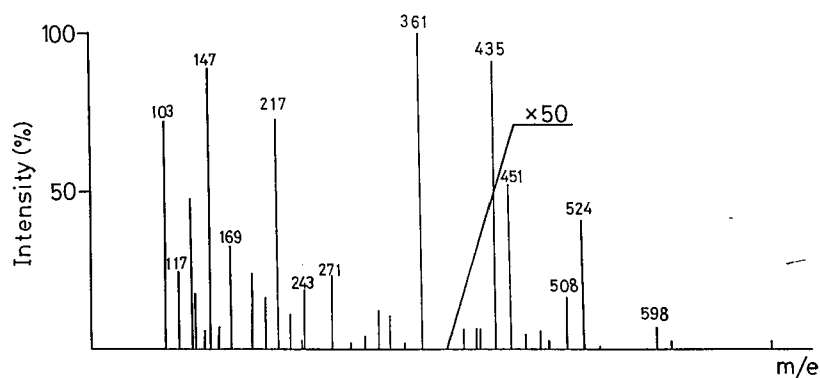
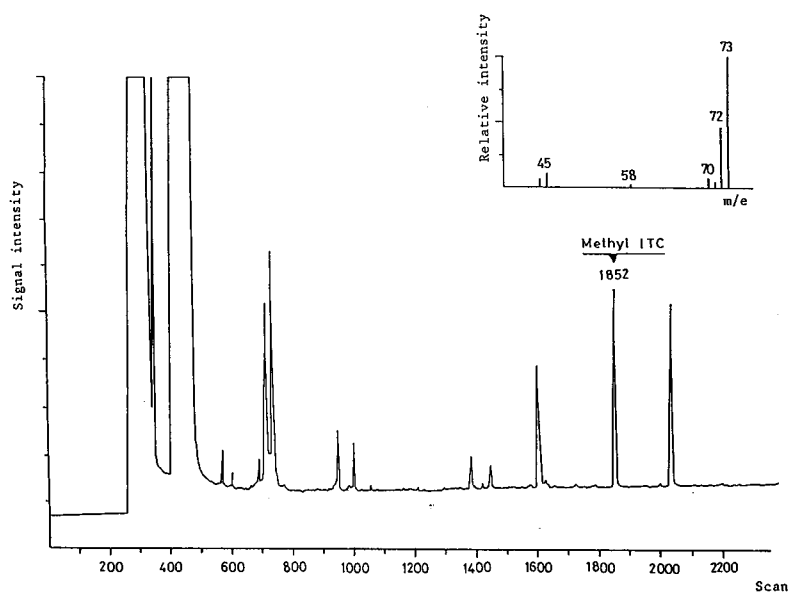FIG. 3. Mass spectrum of silylated desulfoglucocapparin from *Boscia senegalensis* fruit extract.

FIG. 4. GC-MS total ion current of the volatiles liberated during the bioassays of *Boscia senegalensis* fruit extract.

Leaves and fruits are used as human food and animal fodder (Bernus, 1979; Baumer, 1981; Maydell, 1983; Burkill, 1985; Becker, 1983). The leaves, bark, and roots are widely used in northern Senegal for their medicinal properties (Kerharo and Adam, 1974; Booth and Wickens, 1988).

Alzouma and Boubacar (1985) reported on the toxicity of *B. senegalensis* leaves from Niger, which also reduced *Bruchidius atrolineatus* Pic. and *C. maculatus* oviposition, but they gave no details about the active components. Kjaer et al. (1973) reported that *B. senegalensis* leafless twigs contained methyl and isopropyl glucosinolates.

Our bioassays, performed by comparing *Boscia* tissues and MITC, indicate that *B. senegalensis* fruits, leaves, and MITC were toxic to insects at various levels, according to the species and the plant tissue. They also indicate, considering LC_{50} values for FGF, FGL, and pure MITC on the one hand, and the amounts of glucocapparin found in the plant tissues on the other hand, that *Boscia* fruits and leaves contained sufficient glucocapparin to liberate MITC at levels comparable to the LC_{50} of the pure molecule.

These results indicate that in addition to its medicinal properties (Dalziel, 1948) and utilization as a famine food (Becker, 1986; Salih et al., 1991), *B. senegalensis* also has potential in stored-grain protection due to a potent fumigant effect on different insect species. *B. senegalensis* has been traditionally used by African farmers as a grain protectant, but the basis of its effectiveness has never been explained. We have shown that *B. senegalensis* biological activity is linked to the liberation of methyl isothiocyanate from a glucosinolate precursor, glucocapparin, contained in its fruits and leaves. As the plant grows spontaneously in some of the poorest areas of the world (mainly in the arid sahelian regions), this research suggests a natural insecticide from *B. senegalensis* as an alternative to synthetic pesticides in developing countries.

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