Repellent and phagodeterrent activity of *Sphaeranthus indicus* extract against *Callosobruchus chinensis*

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Abstract

A secondary plant metabolite isolated from petroleum ether extract of *Sphaeranthus indicus* showed repellent and feeding deterrent activities to the stored-grain pest *Callosobruchus chinensis* (Coleoptera: Bruchidae) at 0.001–0.05% concentrations. The isolated fraction showed strong repellent activity at 0.01% concentration on filter paper, while complete deterrent activity was noted at 0.05% concentration when the beetles were exposed to treated seeds of green gram (*Vigna radiata*). All experiments were conducted in the laboratory at 28 ± 2°C and 60–70% r.h.

Introduction

Almost all the insect pests of stored grains have a remarkably high rate of multiplication and within one season they may destroy 10–15% of the grains and contaminate the rest with undesirable odours and flavours. Various plant products reported to possess chemicals preventing insect attacks have been used as stored grain protectants merit re-evaluation for their potential as environmentally safe and commercially viable compounds for pest control.

The effectiveness of many secondary plant metabolites for use against grain pests have been reviewed by Burnett et al. (1974), Jacobson (1975), and Ketkar (1976). Secondary metabolites belonging to the alkaloids, sesquiterpene lactones, steroids, phenyl propanes, coumarins, flavanoids and other class of compounds have been shown to deter feeding of insects (Levinson 1976; Nawrot et al. 1985; Jilani et al. 1988; Zehnder and Warthen 1988; Koul and Isman 1990). Apparently some of the compounds are not only strong as antifeedants but also disturb insects as repellents due to their strong odoriferous nature. Since Rodriguez et al. (1976), Kielezewski et al. (1979), Nawrot et al. (1982a, 1986) and Srivastava et al. (1990) have reported that plant extracts containing constituents of sesquiterpene lactones possessed the greatest feeding deterrent and repellent and phagodeterrent activity of *Sphaeranthus indicus* L. (Asteraceae) extract against *Callosobruchus chinensis* L., a stored-grain pest.

Materials and methods

Collection and extraction of plant material

Green aerial parts of the plant, *S. indicus*, were collected from Raisen district of Madhya Pradesh, India during the post monsoon season. The collected plant material was washed thoroughly with tap water and air dried in the shade for about a month. The dried plants were powdered and extracted for 48 hours with petroleum ether (60–80) by Soxhlation. The solvent was removed by vacuum distillation and the extract was evaporated to dryness under reduced pressure. The extract was used for chromatographic procedures.

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Chromatographic separation

For chromatographic procedures the method reported by Singh et al. (1988) were employed as follows: The biologically active compound was separated by chromatography on columns (10 × 62 cm) of silica gel (60–120 mesh). Successive elution with hexane, petroleum ether (40–60) and benzene removed fatty materials, carotenoids and phytosterols respectively. Further elution with ethyl acetate yielded fraction I contaminated with chlorophylls. Chlorophyll was removed by treatment with active charcoal and the concentrated extract (Fraction II) rechromatographed on a small (3 × 30 cm) column of silica gel. Elution with benzene: ethyl acetate (9:1) yielded Fraction III from which the biologically active principle was separated by preparative layer chromotography (PLC). TLC was performed on 0.25 cm layers of silica gel G using benzene: ethyl acetate: methanol (90:10: one drop Me OH) as developing solvent. The fraction III thus obtained were used for experimental bioassay.

Biological assay methodology

Rearing of Callosobruchus chinensis

The seeds of green gram, *Vigna radiata* (L.) Wilczek, were cleaned, washed with tap water, air dried and subjected to a temperature of 60°C for 6 hours to elimate any insect infestation. Later, they were conditioned for 24 hours at 55–65% r.h. and 24 ± 1°C temperature. A culture of pulse beetle *C. chinensis was developed on conditioned green gram seeds, from a sin*gle gravid female. All experiments were conducted at a temperature of 28 ± 2°C and 60–70% r.h.

Repellency

The experiments were conducted in petri dishes containing filter paper (Whatman No. 1) divided into two equal halves, A and B sections. Section A was treated with Fraction III at four different concentrations: 0.001, 0.005, 0.01 and 0.05%. The treatments were replicated three times and 20 freshly-emerged adult beetles were released in each trial. Repellency was measured by visual observations at every 2-hour interval for a period total 52 hours.

Antifeedant activity

Fraction III at four concentrations was poured into glass vials of equal volume containing 20 conditioned gram seeds. The solvents were allowed to evaporate completely before the release of five pairs of freshly emerged adults in each vial. Two controls were maintained, one in solvent treated vials and the other without any treatment. The numbers of damaged seeds were recorded daily for 7 days.

Table 1. Repellent action of Fraction III of petroleum ether extract of *S. indicus* against *C. chinensis*.

Concentration	Average % repellency at indicated hour after treatment				
	10	20	30	40	50
0.001	46.6	45.0	40.0	36.6	26.6
0.005	56.7	51.8	53.3	45.0	38.3
0.01	91.7	85.0	81.6	75.0	65.0
0.05	98.3	96.7	96.7	91.7	83.3

20 freshly emerged adults were taken in each test in replicates of three.

Table 2. Extent of feeding by adults of *C. chinensis* on treated gram seeds with Fraction III for *S. indicus*.

Concentration	Damaged (%)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7
0.05	-	-	-	-	-	-
0.01	-	-	-	2	6	10
0.005	-	-	-	3	7	12
0.001	-	-	-	6	11	18
Control (solvent-treated)	27	30	32	38	42	42
Control (without any treatment)	29	31	33	35	40	44

Experimental values are significantly different from control at the 5% level according to analysis of variance.

Results and Discussion

Fraction III petroleum ether extracts of *S. indicus* isolated through different chromatographic techniques exhibited strong repellent activity at 0.001 to 0.05% concentration against the adults of *C. chinensis* in the laboratory experiments. More than 50% of *C. chinensis* adults were repelled from filter paper sections treated with 0.01 and 0.05% concentrations for the entire 52-hour period, whereas at the lower concentrations (0.001 and 0.005%) repellency ranged from 26–56% at the end of 52-hour period. During the observation period, almost complete repellency was observed on the filter paper sections treated with 0.01 and 0.05% concentrations of the fraction indicating that these concentrations are relatively more persistent than the lower concentrations (Table 1). It was also noticed that *C. chinensis* avoided going into the treated section and once there, quickly returned to the control solvent-treated section of the filter paper. Chander and Ahmed (1986), reported that oils from medicinal plants showed repellent activity against the pulse beetle *C. chinensis* at doses of 0.25 and 0.50 mL/kg seed. The repellent activity of citronella oil was found to be promising against *Tribolium castaneum* (Herbst), *C. chinensis* and *Periplanta americana* (L.) (Saraswathi and Rao 1987). Similarly, Jilani et al. (1988) using oils of turmeric (*Curcuma longa* L.), sweetflag (*Acorus calamus* L.), and neem (*Azadirachta indica*) against the red flour beetle (*Tribolium castaneum*), reported that in addition to growth inhibiting effect, the oils from these plants possess significant repellent activity. Shukla et al. (1989) have also described the repellency of volatile constituents of *Foeniculum vulgare Pimpinella anisum* and Anithole against *T. castaneum*.

The results of the present study show that all concentrations of the extract had strong antifeedant activity to adults of *C. chinensis* (Table 2). There was no acceptance of food in the first 3 days for all the four concentrations. The highest concentration (0.05%) prevented feeding for all 7 days, but some feeding did occur between days 4 and 7 for the three lower concentrations of extract. This feeding was always much less than the control. Wisdom et al. (1983) described toxic and deterrent activity of secondary metabolites isolated from plants in the family Asteraceae. Nawrot et al. (1986) have also observed feeding deterrent activity of compounds isolated from plant origin in the beetles of *Sitophilus granarius* (L.) and *Tribolium confusum* (Jacquelin du Val) as well as in the larvae of *T. confusum* and *Trogoderma granarium* (Everts). Liu et al. (1990) reported that sesquiterpene alkaloids isolated from plants exhibited strong deterrent activity against several insects. Further, the results of the present investigation were found to be consistent in recent reports of Saxena et al. (1992) who stated complete deterrent activity of *Lantana camara* (L.) extracts in *C. chinensis*.

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