

Allelopathic potential and antifeeding activity of *Crassocephalum crepidioides* against native plants and *Spodoptera litura*

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ABSTRACT

Crassocephalum crepidioides is a new invasive plant in China. Bioassays were conducted with aqueous leachates of its fresh leaves to evaluate its suppressive activity against seedling growth of *Amaranthus retroflexus*, *Echinochloa crusgalli* and *Digitaria sanguinalis*. Aqueous leachates from fresh leaves at $\geq 0.25 \text{ gmL}^{-1}$ and the soil irrigated with aqueous extracts of *C. crepidioides* litters $\geq 0.025 \text{ gmL}^{-1}$ significantly inhibited the seedling growth of all three tested weed plants (*Amaranthus retroflexus* L., *Echinochloa crusgalli* (L.) Beauv. and *Digitaria sanguinalis* (L.) Scop). The essential oils were analysed by GC-MS. The dominant constituents of the essential oils were β -myrcene (27.4%), α -pinene (26.2%), germacrene D (5.81%), 1, 6, 10-pentadecatriene (5.54%) and α -caryophyllene (4.68%). In laboratory the antifeeding activity of *C. crepidioides* leaf extract and essential oils were evaluated against the third-instar larvae of *Spodoptera litura*. The leaf extract and essential oils of *C. crepidioides* at 4-concentrations (1.25, 2.5, 5, 10% and 0.625, 1.25, 2.5 and 5%, respectively) significantly reduced the leaf damage done by *S. litura*. The feeding deterrence during 48 h measured after treatment with leaf extract and essential oils at these concentrations ranged from 7.96 to 60.52% and 13.83 to 65.47%, respectively. Both laboratory and pot culture experiments showed the possibility of using allelopathic potential and insecticidal activity of this plant for sustainable pest management.

Key words: Allelopathic activity, *Amaranthus retroflexus*, antifeeding activity, bioassay, *Crassocephalum crepidioides*, *Digitaria sanguinalis*, *Echinochloa crusgalli*, essential oils.

INTRODUCTION

Crassocephalum crepidioides (Benth.) S. Moore (Asteraceae) is native to tropical Africa. It is an erect and slightly succulent annual herb growing about 180 cm tall (11). It was first recorded in Hainan province in 1974 and is now much spread in southern China (11). It grows abundantly as undercover in tree crop plantations (13). Although it has been listed as an invasive plant, it also cultivated as a vegetable in China (11).

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Spodoptera litura (Fabricius) is a polyphagous and serious pest causing enormous losses (26-100%) in many economically important crops (1,17). Its host range covers about 120 species. In the past, it was controlled by insecticides but now has become resistant to them making its control difficult (28).

Botanical compounds are used in agriculture since past 2000 years (12). Botanical insecticides' pest control is based on their degradability, efficacy and physiological activity. The plants have become the source to many insecticide, antioxidant, antiviral and bactericides (2,8). There is urgent need to develop alternate synthetic pesticides that are environment friendly, safe and efficient (4).

The phenomenon of allelopathy can be practically used for weed control as spray of allelopathic plant water extracts, intercropping, allelopathic mulches and crop rotations (30,31). Many plants (rice, sunflower, sorghum, wheat, eucalyptus etc.), have strong allelopathic potential, which may be used to manage weeds, diseases and insect pests effectively (11).

This study aimed to assess the allelopathic potential of *C. crepidioides* to control the common weeds (*Amaranthus retroflexus* L., *Echinochloa crusgalli* (L.) Beauv. and *Digitaria sanguinalis* (L.) Scop), analyze the chemical composition of its essential oils and evaluate its antifeeding activity against *S. litura*.

METHODS AND MATERIALS

I. Aqueous leachate preparation

The fresh leaves (50 g) of *C. crepidioides* collected from our Experimental Farm were soaked in 100 mL distilled water for 24 h at room temperature ($26 \pm 1^\circ\text{C}$) and filtered through two layers of Whatman No. 1 filter paper to get 0.5 gmL^{-1} aqueous leachate. The leachate was diluted with distilled water to prepare concentrations of 0.25 and 0.125 gmL^{-1} . The pH of all dilutions were adjusted to 7.0 with 1 M HCl or NaOH. The seeds of test species *Amaranthus retroflexus* L., *Echinochloa crusgalli* (L.) Beauv. and *Digitaria sanguinalis* (L.) Scop were harvested from the farm. Healthy 20 seeds were sown on two layers of filter paper (9 cm dia, No.1) in a glass Petri dish (9 cm dia) containing 5 mL of each aqueous leachate of *C. crepidioides*. The Petri dishes were incubated in a growth chamber at $25 \pm 1^\circ\text{C}$. The seedling root length and shoot height were recorded at 5d. All experiments were repeated three times independently.

II. Pot Culture

The fresh leaves and stems of *C. crepidioides* were collected and air-dried, 10 g of each was soaked in 100 mL distilled water for 24 h at room temperature ($26 \pm 1^\circ\text{C}$) to get 0.1 gmL^{-1} aqueous leachates. The solutions were filtered twice through filter paper and further diluted with distilled water to prepare concentrations of 0.1, 0.025 and 0.005 gmL^{-1} . The prepared solutions were adjusted to pH of 7.0 with 1 M HCl and NaOH.

Two-hundred g soil (acidic red soil, pH 4.85) was filled into plastic pots. Five germinated seeds of each test plant were sown per pot. The pots were watered with aqueous extracts 5 mL ($0.1, 0.025$ and 0.005 gmL^{-1}) daily. Four replicates were used for each treatment. Shoot length and root length were measured 6 days after incubation.

III. Analysis of volatiles components

The fresh leaves of *C. crepidioides* (1 kg) harvested from our Experimental Farm were subjected to steam distillation for 3 h. The distillate was extracted using ether as solvent (1:1 v/v) and dried over anhydrous sodium sulfate. The essential oils were stored in dark at 4°C before going for GC-MS analysis and anti-feeding test.

The essential oils were analyzed using GC-MS (Finnigan Voyager, USA). The capillary column type was BPX5 (25 m × 0.22 mm × 0.25 μm). The carrier gas was He at constant flow rate of 1 ml min⁻¹. The initial temperature was 100°C. After 3 min, the temperature was increased to 250°C (gradient of 5°C min⁻¹). 1 μL essential oils were injected and analyzed using the above parameters (19). The compounds were identified by a peak matching library search that used authentic standards and NIST (National Institute of Standard and Technology) and data from literature.

IV. Antifeeding activity of *C. crepidioides*

S. litura were artificially grown in plastic containers (20 × 14 × 8 cm) in laboratory [at 26 ± 1°C, 50-60% relative humidity and a 16:8 light:dark photoperiod] without exposure to any insecticide.

Anti-feeding activity of fresh *C. crepidioides* leaf extract and essential oils was evaluated under laboratory conditions by the disc dipping method (24) using fresh leaf discs of *Brassica campestris*. The fresh leaves (500 g) of *C. crepidioides* were collected from our farm and soaked in 500 mL distilled water for 24 h. The extract was filtered through a muslin cloth and collected in a conical flask. This extract was considered as 100% solution (mother solution). Four different concentrations (10, 5, 2.5 and 1.25%) of the solutions were prepared with distilled water. Different concentrations of *C. crepidioides* essential oils (5, 2.5, 1.25 and 0.625%) were prepared with Tween 80 for proper miscibility.

Fresh *B. campestris* leaves were cut into uniform discs (8 cm dia) and were impregnated with different concentrations of test solutions. The leaf discs were dipped in different solutions and air-dried. Five third-instar larvae of *S. Litura* were released into petri dishes (9 cm dia), each of which contained a leaf disc treated with one specified concentrations. Leaves of *B. campestris* were changed daily and the unconsumed area of each leaf dices was measured after 24 and 48 h feeding using a leaf area meter (CI-203 Area-meter; CID, USA). Feeding deterrence was calculated as under:

Feeding deterrence (%) = $\frac{\text{Average leaf area consumed in control} - \text{Average leaf area consumed in treatment}}{\text{Average leaf area consumed in control}} \times 100$ (6)

RESULTS AND DISCUSSION

I. Aqueous extracts bioassay

The leaf aqueous leachates of *C. crepidioides* increased the shoot height and root length of *D. sanguinalis* by 21.6 and 35.5% at 0.125 gmL⁻¹, respectively (Fig. 1). The inhibition enhanced with increasing concentrations of leaf aqueous leachates. The root length of *A. retroflexus*, *E. crusgalli* and *D. sanguinalis* were inhibited by 53.8, 60.3 and 56.4% at 0.5 gmL⁻¹, respectively.

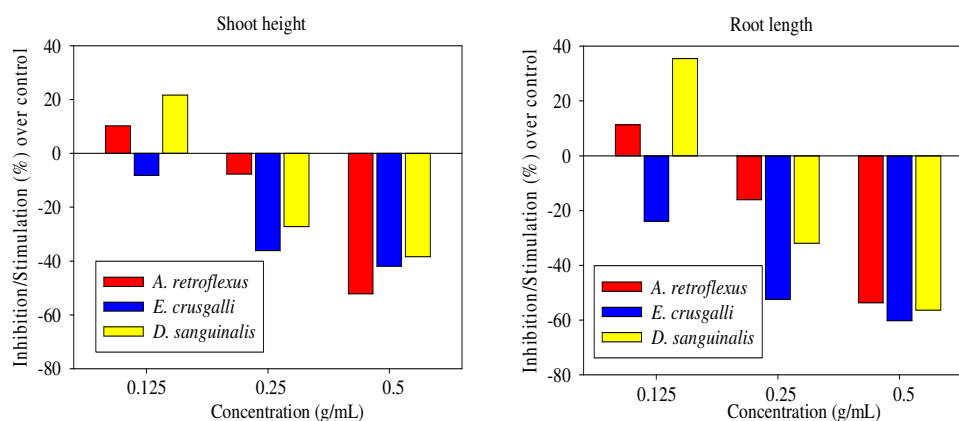


Figure 1. Effects of aqueous leachates from fresh leaves of *Lygodium microphyllum* on seed germination and seedling growth of *Amaranthus retroflexus*, *Echinochloa crusgalli* and *Digitaria sanguinalis*.

While all water extracts (0.04-0.10 gmL^{-1}) from *C. crepidioides* and *G. parviflora* significantly inhibited the seed germination and seedling growth of wheat and maize (14). The allelopathic activity of aqueous leachates from *C. crepidioides* was stronger than *G. parviflora* (14). The germination of *A. retroflexus*, *E. crusgalli* and *D. sanguinalis* was 38.46, 85.71 and 50.00%, respectively with 0.025 gmL^{-1} extract from *Allium macrostemo*. The germination and seedling growth of *A. retroflexus*, *E. crusgalli* and *D. sanguinalis* were strongly inhibited at 0.5 gmL^{-1} (35). The aqueous leachates (0.1 gmL^{-1}) of *Tephrosia vogelii* inhibits (32) the shoot height of *Festuca arundinacea*, *D. sanguinalis*, and *Cynodon dactylon* by 24.4, 31.5 and 21.6%, respectively.

II. Pot Culture

The shoot height and root length of *E. crusgalli* were significantly stimulated by 25.4 and 26.7% when the soil was irrigated with aqueous extract of *C. crepidioides* at 0.005 gmL^{-1} (Fig. 2). But the aqueous extract had no effect on the root length of *A. retroflexus* at the same concentration. The aqueous extract of *C. crepidioides* inhibited the seedling growth of three receptor plants at 0.1 gmL^{-1} . The minimum root inhibition was 44.3% against *E. crusgalli* and the maximum root was 62.3% against *A. retroflexus* at 0.1 gmL^{-1} . Our results suggested that the soil irrigated with aqueous extract of *C. crepidioides* enhanced its phytotoxicity on the target plants. The aqueous extracts of *Mikania micrantha* at 0.1 gmL^{-1} showed stronger inhibitory effects than the soil beneath *M. Micrantha*. The allelopathic potential of *M. micrantha* for soil properties is related to the aqueous extracts concentration (5). Invasive plants that alter soil ecosystems are often successful competitors due to their effects on nutrients cycling (9). *Lantana camara*, an invasive shrub species, makes the substratum ideal for its own growths, which explain the weed's ability to outcompete the native plants (18).

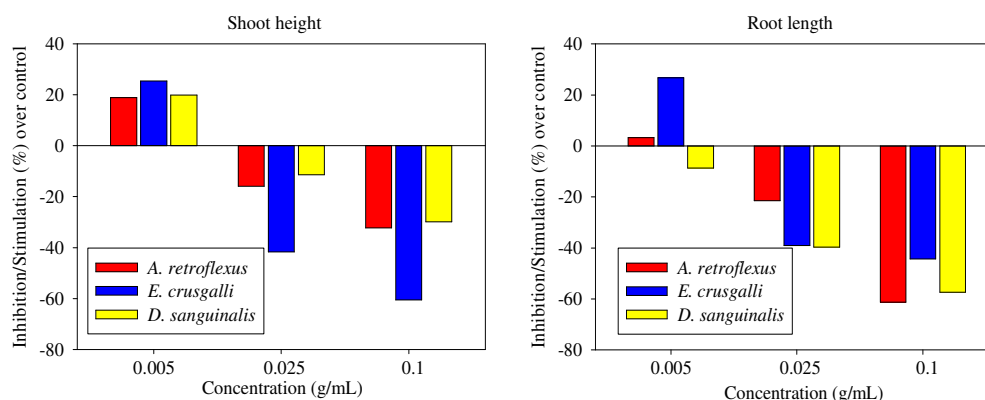


Figure 2. Phytotoxicity bioassays of the soil irrigated with aqueous extract of *Lygodium microphyllum* on seedling growth of *Sorghum sudanenses*, *Cynodon dactylon* and *Festuca arundinacea*.

III. Chemical analysis of volatile components

A total of 27-compounds were identified by GC-MS in the essential oils of *C. crepidioides* (Table 1). The essential oils of leaves contained β -myrcene (27.4%), α -pinene (26.2%), germacrene D (5.81%), 1,6,10-pentadecatriene (5.54%) and α -caryophyllene (4.68%) as major constituents. Thirty-two compounds were identified in the essential oils of the leaves of *C. crepidioides*. But the essential oil in stem yielded just five compounds (19). The major constituents of essential oils from the leaves of *C. crepidioides* contained β -cubebene (13.77%), α -farnesene (13.27%) and α -caryophyllene (10.29%), while the dominant constituents of essential oils from the stems of *C. crepidioides* were thymol (43.93%), 4-cyclohexybutyramide (20.94%) and α -caryophyllene (15.16%) (19). Thirty-seven volatile components were found in the fresh leaves of *C. crepidioides* and the main constituents were myrcene (61.61%), germacrene D (6.48%) and α -humulene (6.29%) (26).

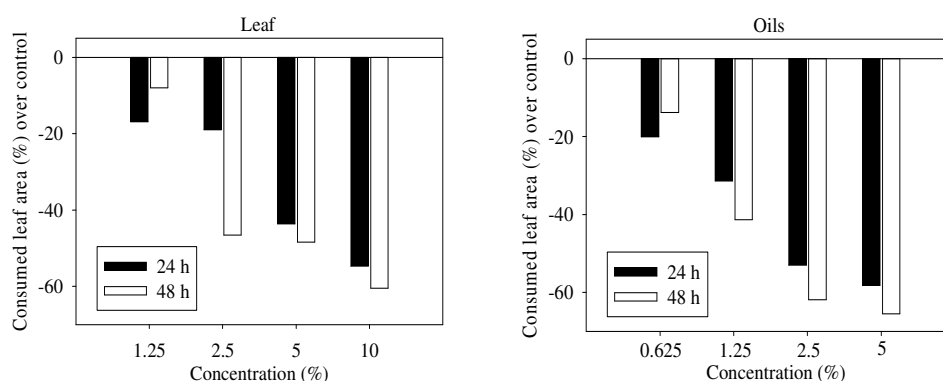
Volatile components of *Chrysanthemum coronarium* were extracted by solid phase microextraction and analyzed by GC-MS. Twenty-five compounds were separated and identified. The main compounds were α -pinene (38.6%), ocimene (19.3%), 2-hexenal (9.0%), 1,6, 10-pentadecatriene (6.4%), borneol acetate (4.3%) and germacrene (2.7%) (34).

IV. Antifeeding assay

During the first 24 h of feeding, both leaf extract and essential oils at all concentrations significantly decreased the leaf damage by *S. litura* larvae (Fig. 3). The rate of feeding detergency during the first 24 h of feeding ranged between 16.87 to 54.69% and 20.05 to 58.17% at various concentrations of leaf extract and essential oils, respectively. During the next 24 h, the extent of damage increased for all treatments, especially the essential oils at the highest concentration (5.0%). The results indicated that the leaf extract and essential oils of *C. crepidioides* has feeding deterrent activity on the larvae of *S. litura*.

Table 1. Volatiles components of *Crassocephalum crepidioides*

No.	Chemical composition	Formula	Retention time (min)	Relative content (%)
1	myrtenol	C ₁₀ H ₁₆ O	3.66	2.58
2	trans-2-Hexenal	C ₆ H ₁₀ O	4.49	1.64
3	β-elemene	C ₁₅ H ₂₄	5.47	2.19
4	artemisia triene	C ₁₀ H ₁₆	5.76	2.26
5	camphene	C ₁₀ H ₁₆	5.78	0.57
6	limonene	C ₁₀ H ₁₆	6.63	0.49
7	α-pinene	C ₁₀ H ₁₆	6.91	26.2
8	benzaldehyde	C ₇ H ₆ O	8.65	0.13
9	β-myrcene	C ₁₀ H ₁₆	9.43	27.4
10	caryophyllene oxide	C ₁₅ H ₂₄ O	10.27	3.42
11	z-Ocimene	C ₁₀ H ₁₆	10.32	2.34
12	trans-β-ocimene	C ₁₀ H ₁₆	10.52	3.03
13	β-caryophyllene	C ₁₅ H ₂₄	10.98	0.91
14	α-fernesene	C ₁₅ H ₂₄	12.01	1.05
15	1,6,10-pentadecatriene	C ₁₅ H ₂₄	12.23	5.54
16	hexadecane	C ₁₆ H ₃₄	13.05	1.78
17	α-cubebene	C ₁₅ H ₂₄	13.37	1.09
18	n-heptadecane	C ₁₇ H ₃₆	13.45	0.39
19	n-eicosane	C ₂₀ H ₄₂	13.88	0.25
20	3,7,11,15-tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	15.01	0.29
21	nonanal	C ₉ H ₁₈ O	16.02	0.12
22	n-hexadecanoic acid	C ₁₆ H ₃₂ O ₂	17.18	1.09
23	copaene	C ₁₅ H ₂₄	19.69	1.58
24	α-caryophyllene	C ₁₅ H ₂₄	21.46	4.68
25	germacrene D	C ₁₅ H ₂₄	23.77	5.81
26	δ-cadinene	C ₁₅ H ₂₄	25.31	0.83
27	n-tetracosane	C ₂₄ H ₅₀	41.43	2.34

Figure 3 Antifeeding activity of *Crassocephalum crepidioides* leaves on *Spodoptera litura*

The crude oils from roots of *Saussures lappa* Clanke and *Inula racemosa* Hook. f. at four concentrations (from 0.625 to 5%) significantly reduced the leaf damage done by *S.*

litura (6). Essential oils from *Satureja hortensis*, *Ocimum basilicum* and *Thymus vulgaris* have potential control agents against *Bemisia tabaci* Genn and *Tetranychus urticae* Koch in greenhouse conditions (4). Thirteen oils from aromatic plants (black pepper, verbena, camphor, thyme, amyris, lemon, helichrysum, myrtle, sandalwood, cedarwood, frankincense, dill and juniper,) induces 100% mortality of *Aedes aegypti* after 24 h at 50 ppm concentration (3). Jantan *et al.* (16) evaluated 17-methanol extracts and 9-essential oils of Malaysian plants for their larvicidal activity against *Aedes aegypti*. The essential oils of *Curcuma domestica*, *Cinnamomum microphyllum* and *Cinnamomum impressicostatum* showed significant larvicidal activity against *A. aegypti* effects, reaching LC₅₀ values of 20.9, 20.6 and 13.7 mgmL⁻¹, respectively (16). The essential oil of flower heads of *Chrysanthemum coronarium* possesses higher nematocidal activity on *Meloidogyne artiellia* (20). The extracts of fresh peels of grapefruit, lemon and orange showed significant nematostatic effect against *Meloidogyne incognita* second-stage juveniles after 48 h treatment (27).

At 50 and 100 % concentration of stock solution, essential oils prepared from leaves of *Zhumeria majdae* inhibited the seed germination and seedling growth of *Lepidium sativum* and *Echinochloa crus-galli* drastically (23). Takayuki *et al.* (25) investigated allelopathic effects of essential oils of *Cuminum cyminum* (100, 300, 500, 700 and 1000 ppm) and *Bunium persicum* (200, 500, 700, 1000 and 2000 ppm) on germination characteristics of *Descurainia sophia* *Bromus tectorum* and *Centura ovina* and the result showed that germination of all receptor weed species was reduced after treatment.

Plant invasion has caused serious challenges to the ecosystem, economy and environment all over the world (7,10,33). *C. crepidioides* growing in South Western Nigeria may be used as natural source for thymol which is known to possess the antimicrobial activity (19). As a pioneer species, *C. crepidioides* infests widely and is an important invasive plant in many countries (11,29). *C. crepidioides* has established steady breeding populations and caused a serious threat to biological diversity of nature reserves and do harm to native ecological environment (15).

Herbicides are a very effective method to control weeds (16,22), but their non-judicious and excessive use have lead to herbicide resistance in many weeds, crop injury and soil and water pollution (16,32). Combining allelopathic water extracts with lower doses of herbicides is highly economical than use of only herbicides (21). Our previous studies showed that plant extracts have numerous beneficial uses in applications ranging from pharmaceuticals to insecticides (3). The present findings indicated that extract and essential oils from *C. crepidioides* has potential to be used as insecticide for sustainable management of *S. Litura* in the laboratory.

The results suggested that the allelopathic potential and insecticidal activity of *C. crepidioides* might be used for sustainable pest management. These studies need to be further expanded to evaluate the efficacy and cost of the extract and essential oils in the field condition.

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