Assessment of Phytotoxicity of Parthenin

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Phytotoxicity of parthenin, a sesquiterpene lactone, was evaluated against four weedy species (*Amaranthus viridis, Cassia occidentalis, Echinochloa crus-galli,* and *Phalaris minor*) through a series of experiments conducted under laboratory or greenhouse conditions to assess its herbicidal potential. Under laboratory conditions, parthenin (0.5–2 mM) severely reduced seedling growth (root and shoot) and dry weight of test weeds. However, the effect was greater on root growth. Parthenin (1 mM) suppressed the mitotic activity in the onion root tip cells that could possibly be responsible for the reduction in seedling growth. Both pre- and post-emergent application of parthenin caused a significant loss of chlorophyll pigments and affected photosynthesis. Parthenin ($\geq$1 mM) caused an excessive electrolyte leakage in the plant tissues which was light-dependent. The root inhibition was associated with swelling and blackening of the root tip, shriveling and damage to the epidermal tissue and non-formation of root hairs. The study concludes that parthenin possesses weed-suppressing potential (both pre- and post-).

Key words: Root Growth Inhibition, Mitotic Activity, Weed Suppressing Ability

Introduction

Worldwide efforts are made to search novel chemicals of herbicidal interest owing to toxicological and environmental concerns linked with the use of synthetic herbicides. Such chemicals therefore must possess properties that cause little or no residual toxicity in the environment and to the non-targets. Natural plant products fit well in such models since these are of biodegradable nature and possess novel sites of action (Dayan *et al*., 1999a). To search suitable chemicals of herbicidal interest, scientists collect clues from various sources such as structure-activity relationships, ethnobotanical background and chemical ecology of plants (Duke *et al*., 2000). Of these, the last one involves exploitation of allelopathic plants or allelochemicals (chemicals responsible for allelopathy), as these possess phytotoxicity, species selectivity and are environmentally benign. In this direction, a number of allelochemicals has been screened (Singh *et al*., 2003). Among various classes of allelochemicals screened for weed suppressing ability, sesquiterpene lactones form an important group because of their wide spectrum of biological activities and structure-activity relationship (Fischer *et al*., 1989; Macias *et al*., 2001; Singh *et al*., 2003). These include artemisinin, cnicin, guaianolides, maculosin, *C*-dehydrozaluzanin, annuolide E and leptocarpin, heliannuols, bisnorsesquiterpene, annuionone E, helibisabonols, germacranolides, and parthenin, which possess phytotoxicity against a variety of plants (Singh *et al*., 2003 and references cited therein). Parthenin – a sesquiterpene lactone and a major constituent of the noxious weed ragweed parthenium (*Parthenium hysterophorus* L.) – is strongly allelopathic (Saxena *et al*., 1991; Batish *et al*., 2001, 2002; Singh *et al*., 2002) and possesses a number of biological activities including pesticidal (Datta and Saxena, 2001) and growth regulatory (Batish *et al*., 1997a). It is present in all parts of the weed and remains sequestered in the hairy trichomes to avoid autotoxicity (de la Fuente *et al*., 2000). Studies conducted in the past have shown that parthenin is a strong suppressant of aquatic weeds (Pandey, 1996). However, reports regarding its phytotoxicity towards terrestrial weeds are scanty. It is re-

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ported to inhibit the emergence and early growth of billy goat weed, *Ageratum conyzoides* (Batish *et al*., 1997b; Singh *et al*., 2002), and the germination of sicklepod, *Cassia tora* (Datta and Saxena, 2001). These studies pertain to the laboratory bioassay and the activity of parthenin towards mature weed plants remains unexplored. Therefore, in order to exploit its potential phytotoxicity for herbicidal activity, studies are required on both broad-leaved and grassy weeds (under both laboratory and greenhouse conditions) to find their differential sensitivity or tolerance level towards parthenin. With this objective in mind, four weedy species (two grassy and two broad-leaved) were chosen for the present study, and the effect of parthenin against them was studied in terms of a) early growth under laboratory conditions, and b) its pre- and post-emergent activity.

Material and Methods

Extraction of parthenin

Parthenin was extracted from the shade-dried powdered leaves of *Parthenium hysterophorus* L. (collected locally) by the method given by Saxena *et al*. (1991). For growth experiments under laboratory conditions, a stock solution of 2 mM parthenin was prepared by dissolving the requisite amount of parthenin in 2 ml of ethanol making the final volume with distilled water. It was further diluted to get solutions of 1 and 0.5 mM concentrations. The final content of ethanol in stock solution was only 0.2% and the same volume added to distilled water served as control.

Laboratory growth studies

Four weed seeds – two broad-leaved (green amaranth, *Amaranthus viridis* L., and coffeeweed, *Cassia occidentalis* L.) and two grassy [little seed canarygrass, *Phalaris minor* Retz., and barnyard grass, *Echinochloa crus-galli* (L.) Beauv.] – were collected locally from the infested agricultural fields and used as bioassay species for studying the impact of parthenin. Fifty seeds of each weed species (except for *A. viridis*, where 100) were placed for imbibition in parthenin solutions of different concentrations (0.5, 1, and 2 mM) or distilled water (control) for 18 h. Imbibed seeds were then placed in a 15 cm diameter Petri dish on a Whatman No. 1 filter paper moistened with 7 ml of respective treatment solution. Five replicates were maintained for each treatment and each seed type. All the Petri dishes were placed in a completely randomized manner in a seed germinator at (22 ± 2) °C, (70 ± 2)% relative humidity, and a light cycle of 16 h/8h light/dark photoperiod of 150 μmol m^{-2} s^{-1} photon flux density. After 8 d, number of seeds germinated, root and shoot lengths of the germinated seeds and seedling dry weight were measured. The entire experiment was repeated twice.

Effect of parthenin on cell division in onion root tips

Effect of parthenin on mitotic activity was studied in onion (*Allium cepa* L.) root tips using the squash technique after Armbruster *et al*. (1991) with slight modifications. Onion root tips instead of weed roots were used since these are a widely used standard bioassay material for studying effects on cell division. Roots were raised from onion bulbs in water for 4 d, excised on the fifth day and treated with 1 mM parthenin solution or distilled water (control) for 24 h. They were then fixed in glacial acetic acid/ethyl alcohol (1:3, v/v) for 24 h, followed by rinsing with distilled water thrice and hydrolysis with 1 M HCl for 1 min at 25 °C. Thereafter roots were stained with Schiff's reagent for 30 min. After staining, two root tips were picked with forceps and macerated in a drop of 40% glacial acetic acid on a slide, covered with a cover slip and sealed with clear nail polish. Mitotic stages were observed under a bright field microscope (Getner, model 66475, Ambala, India). Five replicates were maintained per treatment and the experiment was repeated.

Determination of post- and pre-emergent activity of parthenin

The post-emergent activity of parthenin was tested against four-week-old plants of the weed species under study in a greenhouse experiment. Plants were raised in 15 × 15 cm polypropylene pots filled with 1500 g of garden soil (soil:sand, 3:1, w/w) and kept under greenhouse conditions with a light cycle of 14 h/10 h light/dark photoperiod of approximately 150 μmol m^{-2} s^{-1} photon flux density, day/night temperature of (24 ± 2) °C/(13 ± 2) °C, and a relative humidity of around 70%. Seven seeds of each weed species were sown per pot and there were five pots for each weed species for each treatment. 7 d after emergence, pots were thinned to five seedlings per pot. The

plants were watered every alternate day and on the 14th day after emergence pots were flushed with half strength Hoagland nutrient solution (Hoagland and Arnon, 1950). When four week old, they were spray-treated with 0 (control; water), 1.0 and 2.0 mM of parthenin solution applied (150 ml m^{-2}) with a hand-held sprayer. Next day after spraying leaves were plucked and their chlorophyll content was determined.

The pre-emergent activity of parthenin was tested by growing the plants as above except that the parthenin treatment was given 24 h before sowing of the weed seeds, and seedling length, dry weight and chlorophyll content of the emerged plants were determined two weeks after sowing.

Determination of chlorophyll pigment and photosynthetic activity

Chlorophyll pigment was extracted from leaves in dimethyl sulphoxide (25 mg per 4 ml) following the method of Hiscox and Israelstam (1979) and the amount determined spectrophotometrically according to Arnon (1949). It was expressed in terms of tissue dry weight as suggested by Rani and Kohli (1991). Further, effect on photosynthetic activity was measured through chlorophyll fluorescence in terms of quantum yield of photosynthesis (Y) and photochemical efficiency of PS II (F_v/F_m) using a pulse modulated chlorophyll fluorometer. F_v/F_m and Y were measured by dark and light adapting, respectively, of three leaves for 15 min using default parameters of the instrument for each test mode.

Impact on ion leakage

Effect of parthenin on membrane integrity was determined by measuring ion/electrolyte leakage in one grassy (*P. minor*) and one broad-leaved weed (*C. occidentalis*) in response to parthenin treatment according to the method of Duke and Kenyon (1993). Fresh leaves (100 mg) were collected from 2-week-old plants of both the weeds (at 2-leaf stage) grown in the greenhouse under the same set of conditions as described earlier. They were dipped in 5 ml of 1 mM MES buffer [2-(*N*-morpholino)ethanesulfonic acid sodium salt, pH 6.5] containing 2% sucrose and parthenin (1 and 2 mM). A parallel control was also maintained but without parthenin. The conductivity of the bathing medium containing plant tissue with or without parthenin was measured (using an ECOSCAN CON5 instrument, Eutech Instruments Pte. Ltd., Singapore) in the darkness at regular intervals for 20 h followed by exposure to light for 10 h. Maximum electrolyte leakage was determined after boiling the plant tissue for 10 min. The experiment was repeated with five replicates each time.

Statistical analysis

For each experiment, five replicates were kept for each treatment and seed type in a completely randomized manner. All experiments were repeated and data presented are the mean of two. Data were analyzed by one-way analysis of variance followed by separation of means of control and treatment values at 1 and 5% level of significance. The statistical analysis was done by using SPSS/PC software version 10.0.

Results and Discussion

In response to parthenin though there was no effect on seed germination of test weeds (data not presented), yet seedling growth and dry weight were severely affected (Table I). Among the test weeds, the inhibitory effect was more on *P. minor* and *A. viridis* than on *C. occidentalis* and *E. crus-galli*. Inhibitory effect of parthenin was more on root than on shoot growth thereby indicating that parthenin is a potent root inhibitor. It was parallel

Table I. Effect of parthenin on percent decrease in root and shoot length and seedling weight of one-week-old weeds.

Weed species	Root length [cm]			Shoot length [cm]			Seedling weight [mg]		
	0.5 mM	1.0 mM	2.0 mM	0.5 mM	1.0 mM	2.0 mM	0.5 mM	1.0 mM	2.0 mM
A. viridis	79.7**	89.3**	95.2**	63.6**	72.1**	81.5**	14.8*	25.9*	59.3**
C. occidentalis	33.6*	58.1*	78.7**	43.6**	61.2**	72.9**	15.4*	23.4*	54.2**
E. crus-galli	25.5*	60.5**	88.4**	30.6*	38.7*	54.9**	32.6**	42.9**	57.0**
P. minor	51.6**	73.4**	89.9**	23.5*	44.2**	72.2**	1.3	19.7*	61.8**

* and **, significantly different from control at $p < 0.05$ and 0.01, respectively.

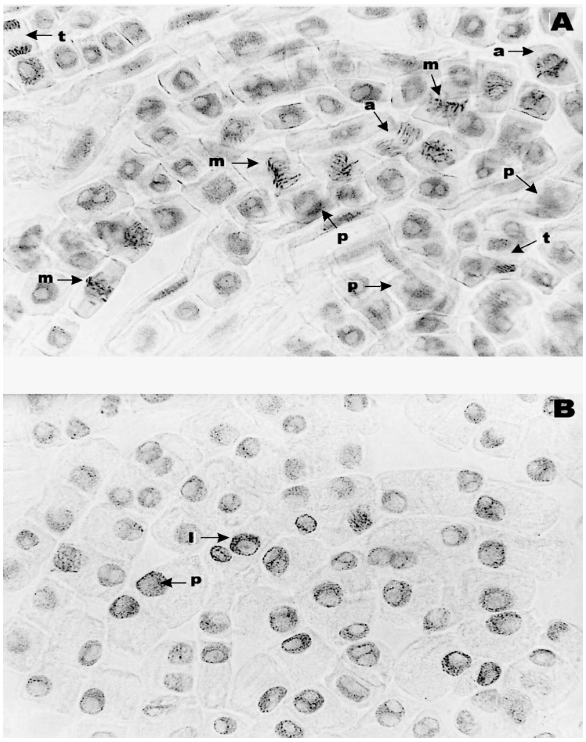

Fig. 1. Photomicrographs of control (A) and 1 mM parthenin-treated (B) cells of onion root tips. Arrows indicate different stages of mitosis; p, prophase; m, metaphase; a, anaphase; t, telophase.

to earlier reports that parthenin severely affects the rhizogenesis process in the hypocotyl cuttings of mung bean (Batish *et al.*, 2001). The root inhibition was associated with swelling and blackening of the root tips, shriveling and damage to the epidermal tissue and non-formation of root hairs. Further, parthenin (at 1 mM) completely suppressed the mitotic activity in the onion root tip cells (Fig. 1A, B). In parthenin-treated root tips, majority of cells was in the interphase with a few in the prophase showing early condensation (Fig. 1B). Such an inhibitory effect on mitotic activity has been reported for another sesquiterpene lactone – artemisinin (Dayan *et al.*, 1999b).

Pre-emergent application of parthenin strongly reduced the seedling growth and weight and chlorophyll content measured 2 weeks after sowing (Tables II, III). Post-emergent treatment also significantly reduced the chlorophyll content in the four-week-old weeds (Table IV). Chlorophyll loss was at maximum in *A. viridis* and at minimum in *C. occidentalis*. It indicates a possible interference of parthenin with either *de novo* chlorophyll synthesis or degradation of already present chlorophyll. Recently, Yang *et al.* (2004) reported that phenolic allelochemicals influence both degradative and synthetic pathways of chlorophyll. Though not ascertained in the present study, however, any reduction in chlorophyll is likely to affect the plant's photosynthetic ability. It becomes clear from the measurement of photosynthetic yield (Y) and photochemical efficiency (F_v/F_m) in response to parthenin. Parthenin decreased Y (significant at $p < 0.01$) that was more prominent at post-emergent application compared to pre-emergent treatment. The value of Y decreased from 0.75–0.77 in control to 0.67–0.71 in parthenin-treated plants. However, F_v/F_m values declined from 0.81–0.83 in control to 0.78–0.79 in parthenin-treated plants and were significant ($p < 0.05$) at 2 mM pre-emergent and ≥ 1 mM post-emergent application. Though not much is known about the possible interference of parthenin with other physiological attributes, however, some reports indicate that it

Table II. Effect of parthenin applied pre-emergent in soil on the initial growth of test weeds measured after 2 weeks.

Weed species	Seedling length [cm]				Seedling weight [mg]			
	0 mM	0.5 mM	1.0 mM	2.0 mM	0 mM	0.5 mM	1.0 mM	2.0 mM
A. viridis	6.92 ± 0.41 (0)	3.82 ± 0.17** (44.8)	1.52 ± 0.16** (78.0)	0.70 ± 0.07** (89.9)	3.19 ± 0.21 (0)	1.65 ± 0.18** (48.3)	0.87 ± 0.07** (72.7)	0.31 ± 0.06** (90.3)
C. occidentalis	6.37 ± 0.34 (0)	4.57 ± 0.31* (28.3)	3.84 ± 0.27** (39.7)	2.87 ± 0.19** (54.9)	7.32 ± 0.42 (0)	5.76 ± 0.29* (21.3)	4.31 ± 0.18** (41.1)	3.18 ± 0.24** (56.6)
E. crus-galli	9.04 ± 0.29 (0)	7.12 ± 0.34* (21.2)	5.94 ± 0.27** (34.3)	2.62 ± 0.37** (71.0)	0.81 ± 0.05 (0)	0.69 ± 0.08* (14.8)	0.59 ± 0.03* (27.2)	0.29 ± 0.07** (64.2)
P. minor	8.49 ± 0.13 (0)	6.01 ± 0.21* (29.3)	5.20 ± 0.35** (38.7)	2.26 ± 0.27** (73.4)	0.78 ± 0.07 (0)	0.60 ± 0.02* (23.1)	0.53 ± 0.04** (32.1)	0.19 ± 0.05** (75.6)

Values in parenthesis indicate percent decrease over control.
* and **, significantly different from control at $p < 0.05$ and 0.01, respectively.

Weed species	Chlorophyll content [μg/mg]			
	0 mM	0.5 mM	1.0 mM	2.0 mM
A. viridis	12.72 ± 0.32 (0)	4.80 ± 0.30* (62.3)	2.26 ± 0.42** (82.2)	0.69 ± 0.09** (94.6)
C. occidentalis	5.69 ± 0.65 (0)	4.53 ± 0.32* (21.7)	3.87 ± 0.29* (31.9)	2.32 ± 0.19** (59.3)
E. crus-galli	13.28 ± 1.38 (0)	4.67 ± 0.27** (64.8)	3.29 ± 0.18** (75.2)	0.88 ± 0.35** (93.4)
P. minor	10.24 ± 2.04 (0)	6.78 ± 0.89** (33.8)	4.21 ± 0.36** (58.9)	1.22 ± 0.20** (88.1)

Table III. Effect of parthenin applied pre-emergent on chlorophyll content (μg/mg) in leaves of two-week-old weed seedlings.

Values in parenthesis indicate percent decrease over control. * and **, significantly different from control at $p < 0.05$ and 0.01, respectively.

Weed species	Chlorophyll content [μg/mg]			
	0 mM	0.5 mM	1.0 mM	2.0 mM
A. viridis	9.48 ± 0.24	7.87 ± 0.49* (17.0)	5.75 ± 0.26** (39.2)	4.97 ± 0.71** (47.4)
C. occidentalis	10.20 ± 0.19	9.84 ± 0.67* (3.5)	9.29 ± 1.26* (8.9)	7.13 ± 1.30** (30.1)
E. crus-galli	13.64 ± 1.60	12.34 ± 0.73** (9.5)	11.61 ± 0.34* (14.9)	9.27 ± 0.98** (32.1)
P. minor	13.74 ± 1.46	10.9 ± 0.49** (20.7)	6.88 ± 0.70** (49.9)	5.31 ± 1.21** (61.4)

Table IV. Changes in total chlorophyll content (μg/mg) in the four-week-old weed plants after post-emergent treatment of parthenin.

Values in parenthesis indicate percent decrease over control. * and **, significantly different from control at $p < 0.05$ and 0.01, respectively.

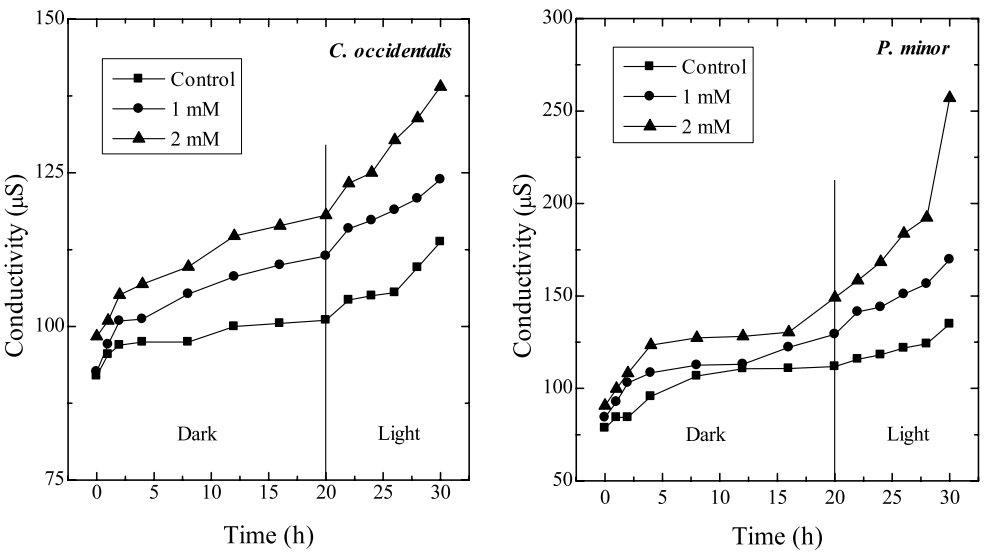

Fig. 2. Effect of parthenin on ion leakage in leaves of *C. occidentalis* and *P. minor*.

inhibits respiration (Kohli *et al.*, 1993), protein and carbohydrate contents, and activities of enzymes – proteases and amylases (Singh *et al.*, 2002). Parthenin caused a severe electrolyte leakage in *P. minor* and *C. occidentalis* leaves which was light-dependent (Fig. 2). It suggests a disruption of membrane permeability resulting in ion leakage. A similar effect on membrane leakage has been observed with another sesquiterpenolide, *C*-dehydrozaluzanin (Macías *et al.*, 2000).

The present study concludes that parthenin possesses phytotoxicity, inhibits root growth, causes

ion leakage, reduces the chlorophyll content and affects the photosynthetic activity in test weeds. However, its selectivity against crops remains largely unknown; though a preliminary study reported that parthenin suppressed the germination of billy goat weed (*Ageratum conyzoides* L.) without affecting the emergence of wheat (Batish *et al.*, 1997b). Further, it can serve as a template for the synthesis of derivatives for further exploration of herbicidal activity. In fact, earlier its photo-derivative has been reported to be more active than the parent compound (Batish *et al.*, 1997a).

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