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FULL LENGTH ARTICLE

Allelotoxic effect of parthenin on cytomorphology of broad bean (*Vicia faba* L.)

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Abstract Parthenin is a natural constituent of *Parthenium hysterophorus* with phytotoxic and allelopathic properties. The present experiment was undertaken to determine the allelotoxic effect of parthenin on cytomorphology of *Vicia faba* L. The seeds were treated with different concentrations (100, 200, 300 and 400 μM) of parthenin for 8 h. The higher concentrations significantly reduced germination and seedling growth. A significant reduction in mitotic index was observed in seeds exposed to parthenin compared to control which decreased with an increase in concentration of parthenin. On the basis of these results, it was concluded that all parthenin concentrations significantly affect the cytomorphology of *V. faba*, while higher concentrations of parthenin were found to be more mutagenic and cytotoxic.

1. Introduction

Allelopathy is defined as any direct or indirect positive or negative effect of one plant on the other (including the microbes) through the release of chemicals into the environment (Rice, 1984). It plays a significant role in agroecosystems, and affects the growth, quality and quantity of the product (Kohli et al., 1998; Singh et al., 2001). *Parthenium hysterophorus* is an obnoxious, aggressive, neotropical composite weed that has spread very fast in India and other parts of the world. It exerts negative effects on agriculture, animal husbandry and the environment (Kohli and Rani, 1994; Evans, 1997). Allelopathic properties of this weed have been well demonstrated (Wakjira et al., 2009; Singh et al., 2003). Due to the action of allelochemicals, a large number of physiological functions and biochemical reactions are affected, such as seed germination, cell division, cell elongation, membrane permeability and ion uptake (Ortega et al., 1988; Tomita-Yokotani et al., 2005; Setia et al., 2007).

In recent decades many researchers, such as Einhellig (2002), Yang et al. (2002), Batish et al. (2002), Setia et al. (2007), Jabeen et al. (2011) Raoof and Siddiqui (2012a,b), Sisodia and Siddiqui (2010) have reported the effect of various allelochemicals of different plants on physiological and biochemical processes but reports regarding effects of allelochemicals on cytology of plants are still scanty. So, in the present experiment an attempt has been made to evaluate the effect of parthenin from *P. hysterophorus* on the cytology and morphology of *Vicia faba*, broad bean.

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2. Materials and methods

2.1. Collection of plant material and seeds of *V. faba*

Leaves of *P. hysterophorus* L. were collected locally from wild growing stands. These were shade-dried and powdered. Seeds of *V. faba* were obtained from the Department of Agronomy, Indian Agricultural Research Institute.

2.2. Extraction of parthenin

Parthenin was extracted from the shade-dried leaves of *P. hysterophorus* following the method of Saxena et al. (1991). For growth experiments, solutions of parthenin were prepared by dissolving the requisite amount of parthenin in 2 mL of absolute alcohol and made the final volume with distilled water.

2.3. Growth experiments

In this experiment, the effect of four concentrations of parthenin, i.e. 100, 200, 300, and 400 μM was studied on the early growth of *V. faba*. Seven seeds of *V. faba* were allowed to germinate and grow in a 15 cm diameter Petri dish lined with Whatman No. 1 filter paper moistened with the respective parthenin solutions. For each treatment there were three replicates. Treatment with distilled water in a similar manner served as control. The entire set-up was kept in a growth chamber at 25 ± 2 °C temperature, 74 ± 2% relative humidity and 16-h light:8-h dark photoperiod. After 7 days, seedling growth in terms of radicle length and shoot growth were measured. The experiment was repeated twice.

2.4. Treatment of *V. faba* with parthenin

Dry and healthy seeds of *V. faba* were surface sterilized with 0.5% sodium hypochlorite for 20 min, washed thoroughly with distilled water and soaked in distilled water for 2 h. Seeds were then soaked in test tubes containing 25 mL of parthenin solution of different concentrations (0, 100, 200, 300, and 400 μM) for 8 h. Control was treated with distilled water. Each group comprised of 21 seeds each. The seeds were thoroughly washed in running tap water 2–3 times. The seeds were then spread over moist cotton kept in Petri-dishes (15 cm diameter) and placed in a Biological Oxygen Demand incubator (BOD) at 25 ± 2 °C for further observation. The root tips of germinated seeds were used as a source of mitotic cells. The root tips were washed in water, fixed in a solution of glacial acetic acid and absolute alcohol in the ratio of 1:3 for 24 h and stored in 70% alcohol until further use. The fixed roots were washed and hydrolyzed at 60 °C for 10 min, in 1 N HCl. Root tips were stained with 2% acetocarmine and squashed on a slide, mounted and observed under microscope.

2.5. Statistical analysis

Germination and seedling growth bioassays were calculated in a complete randomized design with three replications. The data were subjected to one-way analysis of variance, and treatment means separated from the control at $P < 0.05$ applying post hoc Duncan test. Statistical analysis was done with SPSS 16.0 for Windows statistical software package.

Table 1 Effect of parthenin on germination, root length and shoot length of *Vicia faba*.

Concentration (μM)	Germination (%)	Root length (cm)	Shoot length (cm)
0	98.33 ± 0.88[a]	9.83 ± 0.20[a]	6.73 ± 0.09[a]
100	91.33 ± 0.67[b]	6.43 ± 0.12[b]	4.30 ± 0.12[b]
200	83.00 ± 0.58[c]	4.46 ± 0.09	2.60 ± 0.11[c]
300	76.00 ± 0.57[d]	3.23 ± 0.13[d]	2.13 ± 0.03[d]
400	65.33 ± 0.88[e]	1.73 ± 0.12[e]	1.56 ± 0.03[e]

Data are mean (±S.E.) of three replicates; different superscripts in a column indicate significant difference at $P < 0.05$.

3. Results

3.1. Effect of parthenin on germination

Parthenin exerted a phototoxic effect on germination of the seeds of *V. faba*. The germination on the control was 98.33%. The degree of inhibition increased with increasing the parthenin concentration. Maximum reduction (34.67%) was observed in 400 μM. It is followed by 300, 200 and 100 μM. At the highest parthenin concentration the seed germination was significantly inhibited compared with control (Table 1)

3.2. Effect of parthenin on root length and shoot length

Parthenin at all concentrations inhibited the root length and shoot length of *V. faba* compared with the control and the degree of inhibition increased with increasing parthenin concentration. In control the root length and shoot length are 9.83 and 6.73 cm respectively. The highest concentration (400 μM) resulted in 82% (1.73 cm) and 76% (1.56 cm) reduction in root length and shoot length over the control respectively (Table 1). The degree of inhibition was in the following order 400 > 300 > 200 > 100 μM.

3.3. Effect of parthenin treatment on mitotic chromosomal aberration assay

The mitotic aberrations were positively correlated with the treatment concentration. The highest value of mitotic index (40.42%) was recorded in 100 μM parthenin concentration. The lowest value of mitotic index (30.79%) was observed in 400 μM (Table 2). Mitosis was perfectly normal in the control plant showing 12 chromosomes at metaphase which segregated into 12:12 at anaphase and telophase was normal. However a

Table 2 Effect of parthenin on mitotic index of *Vicia faba*.

Concentration (μM)	Total no. of observed cells	Total no. of dividing cells	Mitotic index (%)
0	1200	564	47.00
100	1227	496	40.42
200	1342	521	38.82
300	1172	394	33.62
400	1246	386	30.79