

ORIGINAL ARTICLE

**Allelopathic effect of *Solanum melongena* L. on  
*Vigna radiata* L.**

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The present study has been carried out to investigate the allelopathic effect of aqueous leaf leachate of *Solanum melongena* L. on *Vigna radiata* L. The effects of leachate on germination, radicle length, plumule length, protein content and cell division in root tip meristems of seedlings of *Vigna* were studied. The seeds of mungbean were soaked with leaf leachate of 10, 25, 50, 75 and 100% concentrations for 4h. Bioassay indicated that there was dose-dependent inhibition of germination and seedling growth. Protein content was found to be reduced by the leachate of different concentrations as compared with control. The study also revealed that antioxidative enzymes, viz. superoxide dismutase, catalase and peroxidase activities increased with the increase in concentration of aqueous leaf leachate. Mitotic activity in root-tip cells of mungbean was found to be reduced and the impact was dose-dependent. However, chromosomal abnormalities, viz. fragment, precocious separation, sticky chromosome, disturbed metaphase and bridge were found to be increased with increasing concentrations of leachate.

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*Key words:* Allelochemical, antioxidative enzyme, mitotic activity, *Solanum melongena*, sticky chromosome, *Vigna radiata*

*Abbreviations:* B, bridge; CA, chromosomal aberrations; CAT, catalase; DM, disturbed metaphase; Fr, fragment; MI, mitotic index; NBT, nitroblue tetrazolium; POX, peroxidase; PS, precocious separation; ROS, reactive oxygen species; SC, sticky chromosome; SOD, superoxide dismutase

Allelopathy is a phenomenon where results of allelochemical action can be considered at different levels of plant organization: molecular, structural, cytological, biochemical, physiological and biological system (Malheiros and Peres, 2001). The ecological (Reigosa *et al.*, 2004). The most common

effect of all environmental stresses is the oxidative damage to the plant tissue (Smirnoff 1998). In plants, reactive oxygen species (ROS) are produced continuously as by-products of various metabolic pathways, but under stressful conditions, their formation might be enhanced which leads to lipid peroxidation (Chen *et al.*, 2000), protein degradation (Jiang and Zhang, 2002) and nucleic acid damage (Hagar *et al.*, 1996) and ultimately lead to programmed cell death. To control the level of ROS and to protect the cells, plants possess enzymatic (superoxide dismutase, peroxidase, catalase, etc.) and non-enzymatic (carotenoid, proline) cell mechanisms. The inhibition/arrest of germination and reduction of root and shoot growth in early stages affect the establishment of seedling and ultimately yield of the crop. Analysis of mitotic index and detection of abnormalities in mitotic arrangement are used to study the allelopathic effect of one plant on other (Akinboro and Bakare, 2007). The use of plant tissue primarily root tips cells for studying and induction of chromosomal aberration is one of the most reliable and efficient test systems for rapid screening of chemicals for mutagenicity and clastogenicity (Amer and Farah, 1974). *Solanum melongena* L. (egg plant) and *Vigna radiata* L. (mungbean) are important vegetable and pulse crops in north India. Brinjal is an allelopathic crop containing anthocyanin, phenol, glycoalkaloids (solasodine). In crop rotation system brinjal is followed by green gram. The leachate/residue released in the soil influences the growth and development of succeeding crop. Object of the present study is to investigate the

allelopathic effects of brinjal on greengram which follows eggplant in cropping system. Eggplant is an allelopathic crop which contains anthocyanin, phenol, glycoalkaloids (solasodine). In the present study the emphasis is also given on how alteration in cell division pattern under the influence of allelochemicals affects the biophysical and biochemical parameters of test crop.

## MATERIALS AND METHODS

The certified seeds of mungbean (*Vigna radiata* L.) were procured from the seed agency at Allahabad. The nursery of donor plants of *Solanum melongena* L. were raised in March in the Department of Botany, University of Allahabad, Allahabad located at 24° 47' and 50° 47' N latitude and 81° 9' and 82° 21' E Longitude, 78 m above the sea level. Twenty one days old seedlings were transplanted at distance of 2 feet in experiment plot (size 10m<sup>2</sup>). After 45d the fresh and healthy leaves were collected and washed to remove the dirt and dust. The leaves were soaked in distilled water (1:4 w/v) and containers were kept in refrigerator at 7-8°C for the complete leaching of the allelochemicals and to avoid the degradation and decomposition. After three days, leachate was filtered through muslin clothes and Whatman No. 1 filter paper. The leachate was raised to initial volume by adding distilled water which was treated as 100% concentration. Different concentrations of leachate, i.e. 10, 25, 50 and 75% were prepared by diluting mother leachate with distilled water.

### Germination and seedling bioassay

The experiment was performed in Petri plate culture. The seeds were soaked for 4 hours in

different concentrations of leachate. Seeds soaked in distilled water were taken as control. Ten seeds of each treatment were evenly placed on acid washed sand in sterilized Petri plates (diameter 9cm, depth 1.5cm). Ten ml of leachate were added to each Petri plate and distilled water was used for control. The experiment was performed in replicates of three. One extra set of all treatments were arranged for cytological analysis. Germination was determined by counting the number of germinated seeds at 24h intervals over a 5 days period and expressed as total percentage germination. Length of radicle and plumule was recorded at the interval of 24h upto 5 days. Biochemical analysis was done on 5<sup>th</sup> day after sowing.

#### **Cytological analysis**

For cytological observations root tips of germinated seeds from extra set were cut after 48 h and fixed in Carnoy's solution for 24 h and then transferred to 70% alcohol. Root tips were hydrolyzed in 1N HCl for 20 min at room temperature and then stained with 2% acetocarmine solution for 1 h (Qian, 1998). Chromosome spreads were prepared by squash technique as suggested by Savaskan and Toker (1991). A total of 500 cells were scored from each preparation to study the mitotic index (MI) and expressed in percentage. Various types of chromosomal aberrations (CA) such as fragment (Fr), precocious separation (PS), sticky chromosome (SC), disturb metaphase (DM) and bridge (B) was studied in minimum of 100 metaphase-anaphase plates.

#### **Measurement of protein contents**

Protein content was determined following Lowry *et*

*al.* (1951). The amount of protein was calculated with reference to standard curve obtained from bovine serum albumin.

#### **Extraction and assay of antioxidant enzymes**

Superoxide dismutase (EC 1.15.11) activity was determined by the nitroblue tetrazolium (NBT) photochemical assay method following Beyer and Fridovich (1987). About 0.2 g fresh root tissue was homogenized in 0.1 M potassium phosphate buffer (pH 7.0) and centrifuged at 10,000 rpm at 4°C for 30 minutes. The reaction mixture contained 0.05 M potassium phosphate buffer (pH 7.0), 2 ml solution 2b (methionine, NBT, ethylene di-amine tetra acetic acid) and 0.5 ml solution 3b (riboflavin) and SOD activity was determined spectrophotometrically against blank at 560 nm. One unit of enzyme was defined as the amount of enzyme which caused 50% inhibition of NBT reduction.

Catalase (EC 1.11.1.6) activity was assayed according to the method of Sinha (1972). Fresh root tissue (0.2 g) was homogenized in 0.1 M potassium phosphate buffer (pH 7.0) and centrifuged at 10,000 rpm at 4°C for 30 minutes. The reaction mixture contained 0.5 ml enzyme extract, 1.25 ml 0.2 M H<sub>2</sub>O<sub>2</sub>, 3.2 ml potassium phosphate buffer. After 3 min the reaction mixture was mixed with potassium dichromate acetic acid reagent. Absorbance was recorded at 570 nm.

Peroxidase (EC 1.11.1.7) activity was assayed following the method of Mc Cune and Galston (1959). Fresh root tissue (0.2 g) was homogenized in 0.1 M potassium phosphate buffer (pH 6.0) and centrifuged at 10,000 rpm at 4°C for 30 minutes. Reaction mixture

contained 2.0 ml enzyme extract, 2 ml potassium phosphate buffer (0.1 M, pH 6.0), 1.0 ml 0.1 N pyrogallol and 0.2 ml 0.02% H<sub>2</sub>O<sub>2</sub> was shaken and kept at 37°C and determined spectrophotometrically at 430 nm.

### Statistical analysis

Treatments were arranged in a randomized block design with three replications and experiments were repeated for two successive cropping years. Data were statistically analyzed using analysis of variance (ANOVA) by using GSPS software 3.0 (GRAPHPAD, California, USA). Appropriate standard error of means ( $\pm$ SE) was calculated for presentation with tables and graphs.

## RESULTS

The leaf leachate of egg plant influenced seed germination, seedling growth, protein content and antioxidative enzyme activities of mungbean. The leaf leachate exhibited adverse effect on seed germination. The germination successively decreased when concentration of leachate was increased. The leachate of 100% concentration caused maximum 19.65% inhibition of germination. The seedling growth declined under the influence of leachate. The radicle growth was severely affected in all concentrations of leachate but plumule growth was most affected at higher concentrations (75 and 100%) of leachate. Radicle and plumule length decreased by 57.82 and 65.70 % in T<sub>5</sub> treatment, respectively as compared with the control. Protein content of mung seedlings successively decreased when seeds treated with leachate. Maximum 54.11% inhibition of protein was recorded in 100% leachate concentration (Table 1).

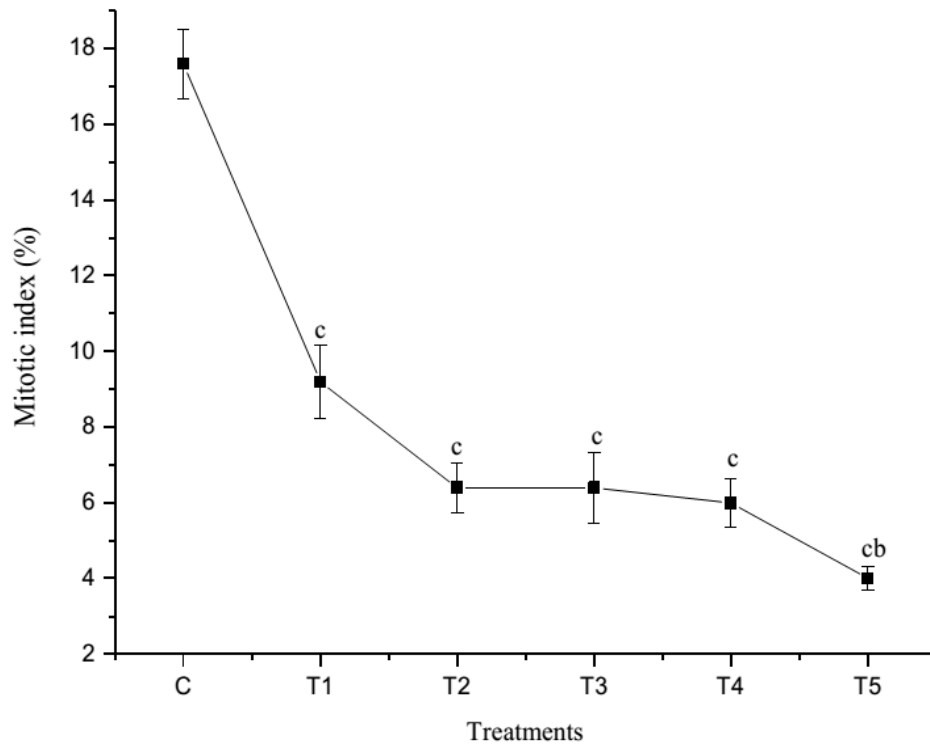
Antioxidative enzymes, secondary defense mechanisms, are induced by allelochemicals present in donor plant exhibiting tolerance against environmental/allelopathic stress. Superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) activities influenced by allelochemicals present in leaf leachate of egg plant. The antioxidants activities were increased and proportional to the concentration of leachate. The maximum activities of antioxidants were recorded at 100% concentration of leaf leachate. SOD, POX and CAT were increased approximately 5, 3 and 26 fold compared to the control, respectively, at the 100% leachate concentration (Table 1).

The effects of aqueous leaf leachate on cell division of mungbean are presented in figure 1. A progressive decline in MI of *V. radiata* root meristematic cells was observed at all concentrations of leachate as compared with control. The control group exhibited a MI of 17.6 $\pm$ 0.92. The lowest concentration of leachate used in this study resulted in 46% reduction in MI as compared with control which decreased further with increasing concentration of leachate. However, at highest concentration of leachate it resulted in a significant reduction in MI as compared to control ( $p < 0.001$ ).

In the present study, all the used concentrations of leachate induced a number of chromosomal aberrations (CA) in root tip cell of *V. radiata* L. The frequently seen abnormalities were fragment (Fr), precocious separation (PS), sticky chromosome (SC), disturbed metaphase (DM) and bridge (B). Among these abnormalities SC was the most frequently

observed CA (~ 4.2%). The occurrence of chromosomal aberration in control group was  $1.8 \pm 1.11$  and the CA observed was chromosomal Fr

and SC. The percentages of abnormal cells were seen to increase significantly ( $p < 0.05$ ) with increasing concentration of leachate (Table 2).



**Figure 1.** Effect of aqueous leaves leachate of *Solanum melongena* on the mitotic index of root tip cells of *Vigna radiata* at 48 h after the treatment.  $^{\circ}P < 0.001$  compared to control; C=control; T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> denote the 10%, 25%, 50%, 75% and 100% concentrations of leachate respectively.

**Table 1.** Effect of aqueous leaf leachate of *Solanum melongena* on seed germination, seedling growth and antioxidative enzyme activities in *Vigna radiata*

Treatments	Seed germination (%)	Length (cm)		Protein (mg/g)	SOD (EU/g FW/min)	POX (Act./g FW/min)	CAT (Act./g FW/min)
		Radicle	Plumule				
C	100.00±0.00	3.01±1.07	4.17±1.05	206.60±1.33	114.48±1.56	35.12±0.79	0.99±0.28
T <sub>1</sub>	95.32±0.32 <sup>c</sup>	2.06±0.40	4.21±0.16	203.30±0.95	156.20±1.53 <sup>c</sup>	42.02±0.42 <sup>c</sup>	1.15±0.01 <sup>c</sup>
T <sub>2</sub>	95.36±0.43 <sup>c</sup>	2.03±0.12	3.90±0.80	166.23±2.01 <sup>c</sup>	178.19±1.37 <sup>c</sup>	70.74±0.14 <sup>c</sup>	2.41±0.01 <sup>c</sup>
T <sub>3</sub>	90.38±0.34 <sup>c</sup>	1.48±0.40	3.60±0.34	165.26±2.20 <sup>c</sup>	317.01±1.42 <sup>c</sup>	82.64±0.62 <sup>c</sup>	8.24±0.02 <sup>c</sup>
T <sub>4</sub>	87.43±0.38 <sup>c</sup>	1.45±0.33	1.76±0.22	137.10±0.65 <sup>c</sup>	422.50±2.00 <sup>c</sup>	83.21±0.01 <sup>c</sup>	10.84±0.06 <sup>c</sup>
T <sub>5</sub>	80.35±0.20 <sup>c</sup>	1.45±0.40	1.43±0.37	94.86±1.90 <sup>c</sup>	512.23±1.27 <sup>c</sup>	108.93±1.60 <sup>c</sup>	26.42±0.30 <sup>c</sup>

Data are mean of three replicates  $\pm$  SEM.  $^{\circ}P < 0.001$  versus control. SOD= Superoxide dismutase, POX= Peroxidase, CAT= Catalase. C=control; T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> denote the 10%, 25%, 50%, 75% and 100% concentration of leachate respectively.

**Table 2.** Effect of aqueous leaves leachate of *Solanum melongena* on chromosomal aberrations in root tip of *Vigna radiata* at 48 h after the treatment

Treatments	Abnormal cells/500	Metaphase-anaphase aberrations (%)					Total aberrations
		Fragments	Precocious separations	Sticky chromosome	Disturbed metaphase	Bridge	
C	9	0.8±0.37	0.0±0.31	1.0±0.31	0.0±0.00	0.0±0.00	1.8±1.11
T <sub>1</sub>	30	1.4±0.54	1.2±0.58	1.4±0.40	1.2±0.48	0.8±0.37	6.0±0.54
T <sub>2</sub>	37	1.4±0.50	1.6±0.50	1.4±0.24	1.8±0.37	1.2±0.48	7.4±0.67
T <sub>3</sub>	58	2.2±0.86	2.4±1.12	2.6±0.50	2.4±0.92	2.0±0.70	11.4±0.50
T <sub>4</sub>	73	3.2±0.96	3.0±1.18	3.6±0.50	2.4±0.50	2.4±0.74	14.6±1.16 <sup>b</sup>
T <sub>5</sub>	94	3.8±1.06	3.4±0.50 <sup>a</sup>	4.2±1.28 <sup>a</sup>	3.6±0.74 <sup>b</sup>	3.8±1.15 <sup>b</sup>	18.8±0.66 <sup>c</sup>

Data are mean of three replicates ± SEM; <sup>a</sup>P <0.05, <sup>b</sup>P <0.01, <sup>c</sup>P <0.001 versus control. C=control; T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> denote the 10%, 25%, 50%, 75% and 100% concentration of leachate respectively.

## DISCUSSION

The germination is resumption of metabolic activity of seeds initiated by absorption of water through imbibition and osmosis. The seed germination involves complex transformation leading up to the biochemical level. The allelochemicals present in plant leachates inhibit some physiological processes i.e. synthesis of enzymes, growth hormones, cell division and other metabolic activities responsible for germination of seeds. Plant growth is influenced by allelochemicals present in leachates. The reduction of growth may be due to inhibition of cell division and enlargement (Einhellig, 1950). The cell growth is the result of loosening of fibrils of cell wall and inhibited deposition of wall polymers. Growth reduction under allelochemicals stress is well studied (Singh *et al.*, 2009a; Singh *et al.*, 2009b). Increased concentration of leachate caused suppressed plant growth due to greater concentration of potential allelochemicals (Singh *et al.*, 2009a; Singh *et al.*, 2009b). Allelopathins present in leachate/extract cause reduction of protein. The reduction of protein may be due to inhibition of biosynthesis and/or increased

degradation of protein (Batish *et al.*, 2006). Allelopathins are known to generate reactive oxygen species which caused oxidative modification and/or degradation of proteins.

The increased activity of SOD, CAT and POX due to biotic and abiotic stresses (Mittler, 2002) enhanced the tolerance of plants in unfavorable environmental conditions. SOD catalyses the conversion of highly toxic singlet oxygen into equally toxic H<sub>2</sub>O<sub>2</sub> (Singh *et al.*, 2009a) which is further detoxified by CAT and POX. Increased activity of SOD under environmental stress prevents oxidative damage of membranes, proteins and lipid (Ueda *et al.*, 2008). Increase in POX and CAT activities is also evident from the allelopathic stress caused by donor plants. Increased level of H<sub>2</sub>O<sub>2</sub> due to SOD results in increased CAT and POX activities. CAT converts H<sub>2</sub>O<sub>2</sub> into nontoxic forms, viz. water and oxygen (Jiang and Zhang, 2002). POX activity also regulates the concentration of ROS in the cell (Apel and Hirt, 2004). Effective antioxidant enzyme system increases the tolerance of plants against environmental stresses (Singh *et al.*, 2009a).

The mitotic frequency decreased parallel to the

increase in doses of leachate agreed with earlier study (Batish *et al.*, 2006). The decline in cell division activity could be due to change in the duration of mitotic cycle. The inhibition of MI occurs due to the inhibition of DNA synthesis which was considered as one of prerequisites for a cell to divide (Badr *et al.*, 1992). The ATP demand of a dividing cell is more higher compared to a non-proliferating cell (Badr *et al.*, 1985). The ATP deficiency caused by leachate treatment may be one of the reasons for the decrease in MI.

Chromosomal abnormality like fragmentation is consequence from multiple breaks of the chromosome in which there is a loss of chromosome integrity (Grant, 1978). Furthermore, in the present study the incidence of precocious separation (PS) and sticky chromosome (SC) also increased with increasing concentration of leachate. PS could appear because of the disturbed spindle activity or breaking of the protein moiety of nucleoprotein backbone due to leachate while SC is a result of the improper folding of chromosome fibers into chromatids and thus there is an intermingling of the fibers and chromosomes become attached to each other by means of subchromatid bridge. Disturbed metaphase indicated by chromosome spreading irregularly all over the spindle apparatus and bridges recorded in the present studies are the result of chromosome breakage and reunion (Badr, 1983).

## CONCLUSIONS

The present study showed that the allelochemicals present in aqueous leaf leachate of *S. melongena* possess inhibitory, mitodepressive, turbagenic and

phytotoxic to germination and seedling growth of mungbean. Increased levels of antioxidant enzymes exhibited the tolerance of pulse crops as a secondary defense mechanism in response to various allelochemicals present in leachate.

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