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Phytotoxic Effect of Cocklebur (*Xanthium Indicum L.*) Allelochemicals on Seed Germination and its Enzymatic Activities of Green Gram (*Phaseolus Radiatus* L.)

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Abstract: The purpose of this study was to assess the effect of different concentrations of various types of aqueous leachate of *Xanthium indicum L.* on seed germination and catalase and peroxidase activities in germinated seeds of green gram. These two enzymes are act as key indicator of phytotoxicity in different morphological and biochemical dynamics of plant body. Allelopathy, one of the stress factors generated by secondary metabolites, of one plant that influences the biochemical reactions, growth and development of neighboring plants. The experiment carried out to study the effect of various types of leachate concentrations on per cent of seed germination and analyzes the activities of catalase and peroxidase in germinated seeds. The results showed that different concentrations of various types of aqueous leachate of test weed (5, 10, 15, 20 and 25 %) were decreased the seed germination and activities of both enzymes. A positive correlation was noticed between increases of leachate concentrations with decrease in enzymes activities. This indicates that allelochemicals present in the leachates of *Xanthium indicum L.* might have increased the rate of production of reaction oxygen species (ROS) which are well known for causing degradation of different bio-macromolecules and ultimately decreased the percentage of seed germination.

Keywords: Allelopathy, *Xanthium indicum*, Catalase, Peroxidase, Germination.

INTRODUCTION

Basic plant process such as hormonal balance, protein synthesis, photosynthesis, chlorophyll production, plant water relations, permeability and etc. are mostly affected by allelochemicals. The chemical inhibition is brought by production of a group of phytochemicals through secondary metabolism. These secondary metabolites or phytochemicals have been considered as potential allelochemicals [1, 2]. The inhibition of one plant by another through the release of allelochemicals is very common practice in competition among plants. In nature, plant face various abiotic and biotic stresses and the negative effect of allelochemicals on recipient plants are considered as biotic stress and called allelochemical stress [3].

Catalase is a haemo-protein which catalyses the backdown of hydrogen peroxide into oxygen and water. Its role in plant is believed to be prevention of harmful hydrogen peroxide accumulation. The catalase activity is highly correlated with dormancy of Grapevine buds[4]. Peroxidases are also metalloprotein enzymes containing porphyrin bound iron and found to be associated with a wide range of substrates including phenol, aromatic amines, amino acids and inorganic compounds. Morphogenesis in the form of root and leaf initiation in cultured calli was found to be influenced by peroxidases [5]. Kagawa *et al* [6] reported that in sunflower cotyledons noticed the sharp decline of glyoxisomal marker enzymes activities during peroxisome transition and catalase is an expression of glyoxysome breakdown. Phenolics are one of the anti-nutritional factors which might have metabolized the seed nutritive value in presence of peroxisome enzymes during germination[7]. Effect of leachate from different plant parts and decomposed plant residue contain a large number of toxic compounds which are known to inhibit seed germination and seedling growth. Allelochemicals and its derivatives can inhibit the ATPase, catalase, peroxidase and cellulose activities [2]. The enzymatic oxidative system (SOD, POD, CAT, ascorbate oxidase and glutathione reductase) help in elimination of free radicals generated during oxidative stresses [8].

Cocklebur, *Xanthium indicum L.* commonly known as Bur weed. It is found that this weed grows profusely in field of green gram crop during winter season. Green gram (*Phaseolus radiatus L.*) has an important rabi crop in the Indian agricultural economy. It has high protein content and serves as main protein supplement nutrient for rural people. Approximately 25-30 % agricultural crop fields are cultivated with green gram after harvesting of rice. *Xanthium indicum L.* is a predominant weed in agricultural fields of Odisha. There are several reports that allelochemicals from this plant negatively affect crops but there is very no information about the effects of *Xanthium indicum* allelochemicals on the seed germination and enzymatic activities on green gram. Basing on the above facts and views, the main objective of this study was to evaluate the effect of different concentration of various types of leachate of *Xanthium indicum* on the seed germination and degree of change in activities of two antioxidant enzymes (catalase and peroxidase) present in germinated seeds of green gram (*Phaseolus radiatus L.*).

MATERIALS AND METHODS

In the morning hours Xanthium indicum plants collected at flowering and post flowering stage, from agricultural fields, were washed thoroughly with tap water followed by distilled water to remove the dust and other adhering particles from the surface of plants. Plant parts such as leaves, fruits were separated and allowed to dry-up in an incubator at 40 ± 2^0C. Different types of leachate from leaves, fruits and whole plant body were prepared as per the methods described below.

Different plant material i.e. whole plant, leave and fruits were chopped into pieces separately and 200 gm of such chopped materials were allowed to leach for 72 hr in 1 litre of distilled water at 30 ± 2^0C as per the method adopted by Padhy *et al* [9]. The leachates were filtered through 2 layer gauge cloth and then watman No. 1 filter paper were considered as 20 % concentration and different diluted leachetes (5, 10, 15, 20 and 25 %) with distilled water were prepared and used for seed germination studies.

Seed germination: In order to study the percentage of seed germination of green gram influenced by different concentration of leachate, visually selected seeds of test crop was *surface* sterilized with 0.03 % formalin solution for 10 minutes separately and then washed thoroughly with distilled water.

The surface-sterilized seeds were allowed to germinate in plastic trays (3 x 9 x12 cm size) at the rate of 20 seeds per tray containing equal volume of sterilized sand wetted with equal volume of 5, 10, 15, 20 and 25 % concentrations of leachate. The seeds were placed 0.5 cm below from the top sand level. Trays with equal number of seed of test cultivar placed in sand, wetted with distilled water, equal to the volume of different leachate, were served as control set. For accuracy of the experiments, the trays of both treated and control sets were divided into five replicates with 3 trays in each set for each type of leachate.

All the trays of both treated and control sets containing seeds were kept in a B.O.D. incubator maintaining 30 ± 1℃ for germination. To maintain the wetness of the sand care was taken to add distilled water and leachate as per experimental schedule. Appearance of sprouts from the seeds was considered as the criteria of germination. The germination was observed at an interval of 12 hrs from 12 to 72 hours after sowing (HAS) and per cent of germination was calculated.

Extraction of catalase and peroxidase: The germinated seeds of test cultivar of control and treated sets during germination were collected @ 2 seeds from each tray at random, at an interval of 24 hours between 24 HAS till 96 HAS, washed with distilled water to remove the sand particles adhered to it, blotted on filter paper, cut into small pieces and weighted for 10 mg, the weighed seed tissues were then thoroughly ground with 1.2 ml of 0.1 M phosphate buffer (pH 7.0) in a pre-chilled mortar and pestle. The homogenate were centrifuged at 15,000 × g at 3 ± 1°C for 30 minutes. The process was repeated thrice and the supernatants were pooled together and used at the source of enzymes for catalase and peroxidase.

Assay of Catalase: Catalase activity was assayed by a modified method of Braber[10]. To a thoroughly mixed solution of 2 ml of 0.005N H_2O_2, 3 ml phosphate buffer (pH 7.0) and 1.0 ml of the enzyme extract was added. The reaction was stopped by an addition of 10 ml of 0.7 N H_2SO_4 after an incubation of one minute at 20 ± 1℃. The acidified-reaction mixture was titrated with 0.01 $KMnO_4$ to determine the quantity of hydrogen peroxide utilized by the enzymes. A blank was simultaneously prepared by adding 1 ml of the extract to an acidified solution of the reaction mixture at zero time. The catalase activity was expressed as m mole H_2O_2 utilized per gram seed (wet weight) per minute.

Assay of Peroxidase: Peroxidase was assayed by following the method of Kar and Mishra [11] with the following modifications. Assay mixture for peroxidase contained 1 ml of 0.1 M pyrogallol, 2 ml of 0.01M phosphate buffer (pH 7.0), 1ml of 0.01 M H_2O_2 and 1ml of enzyme extract was added and the reaction mixure was stopped by adding 1 ml of 5 % H_2SO_4 after an incubation period of 5 minutes at 25 ± 1℃. The amount of purpurogallin was estimated by measuring the absorbency of the mixture at 420 nm and the enzyme activity was expressed in absorbency units.

RESULTS AND DISCUSSIONS

From **Table-1** it can be observed that all concentration of various types of aqueous leachate considerably checked the process of seed germination in green gram. The percentage of seed germination exhibited negative correlation with increase in the concentrations of different types of leachate whereas it was positive with increase of incubation period throughout the periods of observation. Changes in some enzymatic activities in germinating seeds of test cultivar are given below.

Table-1

Effect of different concentrations of various types of aqueous leachate of *Xanthium indicum* on germination (%) of green gram seeds at different hours after sowing (HAS).

(Each value is mean of 5 replicates ± S.E.M.)

Types of leachates	Leachate concentration (%)	12 HAS	24 HAS	36 HAS	48 HAS	60 HAS	72 HAS
Whole-plant	Control	50.0 ± 0.3	86.4 ± 0.8	90.2 ± 0.4	95.4 ± 0.3	98.6 ± 0.3	100.0 ± 0.8
	5	30.4 ± 0.1	60.2 ± 0.6	60.4 ± 0.3	72.4 ± 0.1	82.2 ± 0.2	90.2 ± 0.6
	10	16.4 ± 0.4	32.8 ± 0.3	32.2 ± 0.2	66.2 ± 0.3	70.2 ± 0.4	80.2 ± 0.3
	15	10.2 ± 0.2	20.6 ± 0.2	20.6 ± 0.3	35.2 ± 0.2	41.4 ± 0.2	50.4 ± 0.2
	20	*	*	03.0 ± 0.5	05.6 ± 0.2	08.0 ± 0.1	10.8 ± 0.3
	25	*	*	*	*	*	*
Leave	Control	50.0 ± 0.3	86.4 ± 0.8	90.2 ± 0.4	95.4 ± 0.3	98.6 ± 0.3	100.0 ± 0.8
	5	37.4 ± 02	65.2 ± 0.4	75.4 ± 0.3	82.6 ± 0.1	85.4 ± 0.3	95.2 ± 0.4
	10	21.2 ± 0.6	43.8 ± 0.3	61.4 ± 0.3	67.2 ± 0.4	71.6 ± 0.4	88.4 ± 0.2
	15	16.8 ± 0.8	24.0 ± 0.4	30.4 ± 0.3	44.6 ± 0.4	52.8 ± 0.2	68.8 ± 0.1
	20	08.4 ± 0.2	11.4±0.5	16.8 ± 0.5	24.6 ± 0.6	29.8 ± 0.1	31.7 ± 0.3
	25	*	*	03.4 ± 0.2	05.3 ± 0.2	08.2 ± 0.2	10.4 ± 0.3
Fruits	Control	50.0 ± 0.3	86.4 ± 0.8	90.2 ± 0.4	95.4 ± 0.3	98.6 ± 0.3	100.0 ± 0.8
	5	40.8 ± 0.6	72.2 ± 0.6	83.4 ± 0.3	88.2 ± 0.4	94.2 ± 0.6	96.6 ± 0.2
	10	27.2 ± 0.8	54.0 ± 0.4	68.0 ± 0.4	78.0 ± 0.4	82.0 ± 0.8	90.2 ± 0.4
	15	21.0 ± 0.2	29.8 ± 0.6	43.2 ± 0.6	52.6 ± 0.6	60.2 ± 0.4	70.2 ± 0.2
	20	10.4 ± 0.1	15.2± 0.3	22.8 ± 0.6	29.8 ± 0.4	31.1 ± 0.2	35.2 ± 0.3
	25	*	*	05.2 ± 0.2	08.4 ± 0.5	10.2 ± 0.1	11.6 ± 0.2

At all concentrations of whole-plant leachate considerably checked the catalase activities in germinating seeds of green gram compared with their respective control seeds. Maximum catalase activity noticed in seeds of control set at 96 HAS was 0.99 ± 0.04 mμ mol of H_2O_2 utilized /g wet wt. of seed / minute, while it was only 0.12 ± 0.03 in seeds soaked in 25 % leachate at 96 HAS. Other concentrations exhibited intermediate values (**Table-2**). The catalase activities exhibited negative correlations with increase of concentrations of leachates and were positive with incubation period. It was further noticed that 25 % concentration of leachate completely checked the cayalase activity as a result no germination was noticed.

Table-2

Effect of different concentrations of various types of aqueous leachate of *Xanthium indicum* on change in catalase activities (mμ mol of H_2O_2 utilized /g wet wt. of seed/min) in germinating seeds of green gram at different hours after sowing (HAS).

(Each value is mean of 5 replicates ± S.E.M.)

Types of leachates	Leachate concentration (%)	24 HAS	48 HAS	72 HAS	96 HAS
Whole-plant	Control	0.82 ± 0.03	0.90 ± 0.02	0.92 ± 0.01	0.99 ± 0.04
	5	0.71 ± 0.02	0.79 ± 0.01	0.84 ± 0.02	0.89 ± 0.04
	10	0.61 ± 0.03	0.73 ± 0.02	0.81 ± 0.01	0.84 ± 0.02
	15	0.46 ± 0.02	0.63 ± 0.01	0.73 ± 0.02	0.78 ± 0.01
	20	0.36 ± 0.02	0.43 ± 0.03	0.55 ± 0.02	0.63 ± 0.04
	25	*	0.04 ± 0.01	0.08 ± 0.04	0.12 ± 0.03
Leave	Control	0.82 ± 0.03	0.90 ± 0.02	0.92 ± 0.01	0.99 ± 0.04
	5	0.69 ± 0.02	0.77 ± 0.01	0.82 ± 0.03	0.87 ± 0.01
	10	0.59 ± 0.02	0.76 ± 0.02	0.79 ± 0.01	0.82 ± 0.02
	15	0.44 ± 0.04	0.61 ± 0.02	0.71 ± 0.03	0.76 ± 0.01
	20	0.34 ± 0.02	0.41 ± 0.03	0.53 ± 0.01	0.61 ± 0.03
	25	*	0.06 ± 0.01	0.10 ± 0.02	0.14 ± 0.04
Fruits	Control	0.82 ± 0.03	0.90 ± 0.02	0.92 ± 0.01	0.99 ± 0.04
	5	0.68 ± 0.02	0.76 ± 0.01	0.81 ± 0.03	0.86 ± 0.02
	10	0.58 ± 0.03	0.70 ± 0.02	0.78 ± 0.04	0.81 ± 0.03
	15	0.43 ± 0.03	0.60 ± 0.02	0.70 ± 0.02	0.75 ± 0.02
	20	0.33 ± 0.01	0.40 ± 0.03	0.52 ± 0.02	0.60 ± 0.04
	25	*	0.08 ± 0.04	0.12 ± 0.02	0.16 ± 0.04

The different concentration of leave leachate checked the catalase activities in germinating seeds of green gram compared with the seeds of control set. Maximum activity noticed at 96 HAS in seeds of control set was 0.99 ± 0.04 mμ mol of H_2O_2 utilized /g wet wt. of seed / minute, while it was minimum(0.14 ± 0.03) in seeds incubated in 25 % leachate. Intermediate data were recorded in seeds of other sets during different HAS. The catalase activities exhibited positive correlations with increase in the incubation period and negative concentrations with leachate concentrations throughout the period of observation.

Table-2 indicates that all concentrations of fruits leachate considerably checked the catalase activities as a result minimum activity (0.16 ± 0.04 mμ mol of H_2O_2 utilized /g wet wt. of seed / minute) on 96 HAS in seeds soaked in 25 % leachate whereas same period of incubation maximum values of 0.99 ± 0.04 was noticed in seeds of control set. Data of intermediate values were recorded in seeds of other treated and control sets at different HAS. The catalase activities exhibited positive correlations with increase in the incubation period and negative concentrations with leachate concentrations.

Increased concentration of various types of leachate considerably checked the peroxidase activities in germinating seeds of green gram compared with their respective control seeds. Maximum peroxidase activity noticed in seeds at 96 HAS of control set was 0.80 ± 0.01 (O.D. value at 420 nm), while it was only 0.14 ± 0.01 in seeds soaked in 25 % leachate at 96 HAS. Other concentrations exhibited intermediate values (**Table-3**). The peroxidase activities exhibited negative correlations with increase of concentrations of leachates and were positive with incubation period throughout the period of observation.

During the process of various metabolic activities hydrogen peroxide (H_2O_2) is released in plant and animal tissues by transfer of electrons to molecular oxygen by different oxidative enzymes[12]. Inspite of decomposition and utilization by the enzymes catalase and peroxidase, respectively, a steady state of hydrogen peroxide amount is usually maintained in tissues [13, 14]. Such a balanced level of H_2O_2 indicates that catalase and peroxidase are not involved in complete destruction and utilization of H_2O_2, rather a balance is maintained between synthetic and decomposing enzymes involved in H_2O_2 metabolism. Parida *et al* [15] reported that the increase in the level of endogenous H_2O_2 is considered as one of the factors that lead to destruction of tissues. In the present investigation it was observed that peroxidase activity decreased with increase of leachate concentration in seeds of the test cultivar during germination, as a result of which there was higher accumulation of H_2O_2 by oxidative break down of various reserve food material present inside the seed. On the other hand, in principle, catalase enzymes are supposed to utilize instant H_2O_2 released and accumulated during the process of oxidation to maintained H_2O_2 level in seeds. But the present findings reveled that all concentrations of various leachates checked the catalase activity as a result the amount of H_2O_2 liberated and accumulated by peroxidase activity was not completely utilized as a result exerting toxic effect on seed germination. Mishra and Choudhury [16] reported that increase in level of H_2O_2 in cells and /or tissues cause membrane damage in rice plant. The allelochemicals such as phenolics, alkaloids, steroids, etc. present in the leachate might have caused lower rate of seed germination mediated through suppressing the catalase activity. Similar inhibiting findings were reported by Pattanaik [17] and Padhy *et al* [18] in ragi; Gantayet [19] in legumes influenced by leaf leachates of *Eucalyptus globulus* and Tripathy [20] in rice seeds influenced leaf/phyllode and bark-leachates of *Acacia auricuaeformis* and *Acacia nilotica*. Since no authentic literature are available on effect of phytochemicals present in Xanthium plants at molecular level in plant systems, it difficult to draw any definite conclusion on the role of allelochemics on enzyme activities during growth and development of plants in general and seed germination in particular.

Table-3

Effect of different concentrations of various types of aqueous leachate of *Xanthium indicum* on change in peroxidase activities (O.D. value at 420 nm) in germinating seeds of green gram at different hours after sowing (HAS).

(Each value is mean of 5 replicates ± S.E.M.)

Types of leachates	Leachate concentration (%)	24 HAS	48 HAS	72 HAS	96 HAS
Whole-plant	Control	0.58 ± 0.02	0.64± 0.01	0.72 ± 0.03	0.80 ± 0.04
	5	0.44 ± 0.01	0.52 ± 0.02	0.63 ± 0.01	0.72 ± 0.01
	10	0.35 ± 0.03	0.41 ± 0.02	0.51 ± 0.01	0.62 ± 0.02
	15	0.28 ± 0.02	0.35 ± 0.01	0.46 ± 0.03	0.54 ± 0.04
	20	0.20 ± 0.04	0.28 ± 0.03	0.39 ± 0.01	0.42 ± 0.02
	25	0.04 ± 0.01	0.06 ± 0.02	0.08 ± 0.04	0.14 ± 0.03
Leave	Control	0.58 ± 0.02	0.64± 0.01	0.72 ± 0.03	0.80 ± 0.04
	5	0.42 ± 0.01	0.50 ± 0.03	0.61 ± 0.01	0.72 ± 0.04
	10	0.33 ± 0.01	0.36 ± 0.02	0.49 ± 0.03	0.60 ± 0.02
	15	0.26 ± 0.03	0.33 ± 0.01	0.44 ± 0.08	0.52 ± 0.01
	20	0.18 ± 0.03	0.26 ± 0.05	0.37 ± 0.07	0.40 ± 0.08
	25	*	0.08 ± 0.01	0.12 ± 0.02	0.14 ± 0.01
Fruits	Control	0.58 ± 0.02	0.64± 0.01	0.72 ± 0.03	0.80 ± 0.04
	5	0.41 ± 0.04	0.49 ± 0.03	0.60 ± 0.01	0.71 ± 0.01
	10	0.32 ± 0.02	0.37 ± 0.05	0.48 ± 0.02	0.59 ± 0.03
	15	0.25 ± 0.05	0.32 ± 0.06	0.43 ± 0.02	0.51 ± 0.03
	20	0.17 ± 0.03	0.25 ± 0.05	0.36 ± 0.06	0.39 ± 0.07
	25	*	0.10 ± 0.02	0.14 ± 0.02	0.16 ± 0.03

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