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Influence of *Xanthium indicum L.* water extracts on DNA and RNA contents of green gram (*Phaseolus radiatus L.*)

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Abstract

Nucleic acids are molecules which act as intelligent hinge and containing nitrogen compound, some sugar and an acid. Due to its structural stoichiometry, it is highly stable form among the all macromolecules. Inspite of its stable structure, influx of allelochemicals and other metabolites to cell matrix develop a stress condition which is susceptible to damage of nucleic acids and their internal metabolic processes. In order to find out the allelopathic effect Xanthium indicum L. allelochemicals on nucleic acids of green gram, a pot culture experiment was conducted with different concentrations of various types of aqueous leachate of test weed. The results showed that different concentrations of various types of aqueous leachate of test weed (5, 10, 15 and 20 %) were exhibited a significant negative correlations with increase in the various types of leachate concentrations of test weed and positive correlations with increase in growth period upto 10 DAS thereafter a negative correlation were marked. This indicates that allelopathic stress of various types of leachates of Xanthium indicum L. were phytotoxic to green gram, when incubation period increase (i.e. 10 days after sowing) the degree of toxicity was higher, this might have due to the increase of more allelochemicals by cellular absorption and it attribute to change the cell matrix. The changed internal milieu of cellular matrix with higher concentration of allelochemicals and other intermediate dynamic unstable molecular species generated by allelopathic stress are reduced or disorganized the DNA and RNA contents. This piece of investigation reveals the adverse effect of allelochemical of Xanthium indicum L. on DNA and RNA contents of green gram.

Keywords: Nucleic acids, Allelochemicals, Xanthium indicum, Leachate, DNA, RNA.

Introduction

Allelochemicals are produced by plants as end product, by product and metabolites which belongs to diversified group of chemical compounds such as phenolic acids, flavonoides, and other aromatic compounds viz. terpenoids, alkaloids, organic cyanides and etc. Allelochemicals are impact their effect by targeting important physiological and biochemical processes of plants. Allelochemicals namely volatile monoterpenes, eucalyptol, camphor and etc altered external and anatomical behavior of the cell as a result roots become broad with reduced length as well as persuading nuclear abnormalities and increasing vacuole numbers[1,2] . Cruz Ortega et al.[3] observed and noticed that corn pollen extract checked mitotic activity by more than 50%, induced nuclear irregularities and pyknotic nuclei, and inhibited radicle and hypocotyl growth in watermelon (*Citrullus lanatus* var. *lanatus*). Allelochemicals from *Convolvulus arvensis* L. and catmint (*Nepeta meyeri* Benth.) effect on DNA and modify the random amplification of polymorphic DNA (RAPD) profiles of receiver plants[4,5]. Citral is a volatile essential oil component of lemongrass (*Cymbopogon citrates*) and other aromatic plants act as catalyst for allelopathic activities[6] which altered and disrupted the orientation of microtubules in wheat and *Arabidopsis thaliana* L. roots and also noticed that cortical microtubules were affected more and reduced the cell multiplication[7,8]. In addition, citral is a potent chemical compound effect on the cell ultra-structure of *A. thaliana* seedlings, thickening the cell wall and reducing intercellular communication and the formation of root hairs[9]. The integrity and stability of the DNA is associated with potency of the allelochemicals and increased temperature of the DNA cleavage, a few allelochemicals are inhibit DNA polymerase I and prevent the transcription and translation of DNA as a result it may inhibit protein biosynthesis mechanism[10]. Ramos *et al.*[11] and Zhang *et al.*[12] reported that sesquiterpene lactones are allelochemical present in *Xanthium* act as cytotoxic substances and triggered mitochondrial membrane transition and release of pro-apoptotic mitochondrial proteins leading to caspase activation and apoptotic cell death[13]. Ahmed et al.[14] reported that the double bonds present in the α-methylene gamma lactone and cyclopentanone moieties confer to sesquiterpene lactones an affinity towards thiol groups of proteins and glutathione including oxidative stress in the cell. Inhibition of key enzymes of energetic metabolism (oxidative phosphorelation) and nucleic acid replication (DNA polymerase) and expression of NF-kB and caspase 3, which are pro-apoptotic and induce DNA damage, have been observed by sesquiterpene lactones[11,12,15]. Baziramakenga et al.[16] reported that the concentration of different dose of allelochemicals was increased protein content and this stimulation correlated with

activation of nucleic acid content such as p-hydroxybenzoic, p-coumaric acids and increased incorporation of 35 S-methionine into protein, it may be attributed to the interference of allelochemicals with the cytoplasmic ribosomes and production of RNA, which in turn excites alternation of protein synthesis. The reduction in soluble protein, at the highest residual concentration, may be attributed to the effect of allelochemicals on DNA replication or translation by intercalation of ionic bonding with their negatively charged phosphate groups.

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 on allelochemicals of different plant negatively affect to crops but there is very no information about the effects of *Xanthium indicum* allelochemicals on the DNA and RNA content of 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 DNA and RNA content 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.[17]. 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 were *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.

All the trays of control and treated sets containing germinated seeds were transferred into the seedling growth chamber maintained at 30 ± 2^0C provided 12 hr photoperiod per day with illumination of 2 ± 0.8 Klux light intensity from two florescent electric tube lights from top of the seedlings. The seedlings were provided with equal volume of respective test leachates and distilled water as per the experimental design at an interval of 24 hours. The seedling growth parameters such as shoot and root lengths were recorded at an interval of 2 days from 6 days after sowing (DAS) till 12 DAS. Changes in total chlorophyll, total carbohydrates and protein content in the first pair of leaves during seedling growth were estimated as per the procedures described below.

Extraction and Estimation of nucleic acids (DNA and RNA): The first pair leaves of the seedlings collected from both control and treated sets separately at random on 5, 10, 15 and 20 DAS were washed with distilled water, blotted on blotting paper, cut into small pieces and weighed for 250 mg of such leaf-tissues were ground with 80% (v/v) ethanol with pre-chilled mortar and pestle. The homogenate was centrifuged at 5000 X g for 20 min at 28 ± 2°C and the supernatant was discarded and the pellets were again homogenized with little quantity of 80 % ethanol and centrifuged. The colorless pellets left over after ethanolic extraction from leaf materials were suspended in ethanol and ether mixture (2:1 and then 1:1) for 30 minutes, centrifuged at 5000 X g for 20 min. at 4 ± 1° C. Then the pellets were suspended in 5 % Trichloro-acetic acid (TCA) (w/v) at O^0C for 15 minutes, centrifuged at 5000 X g for 20 min at 4 ± 1° C. The procedure repeated twice and supernatants were discarded. The pellets were treated with 10 % Trichloro-acetic acid (TCA) (w/v) at 90 ± 1° C for 15 minutes, cooled and centrifuged at 5000 X g for 20 min. at 28 ± 1° C. The supernatants were considered as nucleic acids extract. The DNA and RNA content in the TCA extracts were determined spectophotometrically with diphenylamine and orcinol reaction medhods respectively and data are presented in mg/g fr. wt. of leaf tissues.

Estimation of DNA: DNA content in extract was estimated by Diphenylamine reaction method as suggested by Abraham et al.[18], using commercial sample of calf thymes DNA as standard.

To 2 ml of DNA extract, 4 ml of diphenylamine reagent [1% (w/v) diphenylamine in glacial acetic acid plus 2.5 ml of conc. H_2SO_4] was added, heated in a boiling water bath for 10 minutes, cooled and then O.D was measured at 595nm with help of spectrophotometer. Equal amount of extract media (10 % TCA solution) in place of DNA extract and Diphenylamine reagent were run parallel as blank. The data are expressed in mg / g fr. wt. of leaf tissues.

Estimation of RNA: RNA contents in the extract were estimated by Orcinol reaction method as suggested by Schneider[19], using commercial samples of yeast-RNA. To 2 ml of RNA extract, 3 ml of freshly prepared Orcinol reagent [0.1 gm of ferric chloride ($FeCl_3$ 6 H_2O) dissolved in 100 ml of conc. HCl plus 3.5 ml of 6%. (W/V) Orcinol in ethyl alcohol] was added. The mixture was heated in a boiling water bath for 20 minutes, cooled and their O.D. values were measured at 665 nm with the help of spectrophotometer. A reaction mixture containing 3 ml Orcinol reagent and 2 ml of extraction media (10 % TCA solution) was run parallel as blank. The data are expressed in mg / g fr. wt. of leaf tissues.

Results and discussion

In all living organisms, DNA is regarded as the director of all sort of metabolic activities which directly or indirectly regulate the growth, development and productivity. The change in DNA content in first pair of leaves of test seedling influenced by *Xanthium indicum* leachate of various types of different concentrations on DNA contents are described below.

All concentrations of *Xanthium* whole-plant-leachate considerably checked the DNA synthesis in the first pair of leaves of green gram seedling as a result maximum amount of 0.65 ± 0.05 mg/g fr. wt. DNA was estimated from control sets on 10 DAS and during same period of growth the least value of 0.39 ± 0.05 mg/g fr. wt. was assayed from seedling raised in 15 % leachate. Other concentrations correspondingly caused decrease in the DNA content as a result intermediate values were recorded (Table-1). The DNA content in the first pair of leaves of different seedlings showed negative correlations with increase in leachate concentrations of *Xanthium indicum* and positive correlations with increase in seedlings upto 10 DAS during period of observation.

In case of leaves-leachate, all concentrations considerably checked the DNA synthesis resulting decrease in DNA content in the first pair of leaves of treated seedling compared to seedling of respective controls. The maximum amount of 0.65 ± 0.05 mg/g fr. wt. DNA was estimated from the first pair of leaves of the seedling of control set and minimum amount of 0.38 ± 0.07 mg/g fr. wt. DNA was assayed from seedlings grown in 15 % leachate on 10 DAS. Other concentrations showed intermediate values (Table-1). The DNA content in leaves of green gram seedling exhibited negative correlation with increase in the leachate concentrations and positive correlations with increase in growth period upto 10 DAS.

Impact of different concentrations of fruits leachate of test weed on green gram showed maximum amount of DNA (0.65 ± 0.05 mg/g fr. wt.) was assayed from the first pair of leaves of the seedling of control set on 10 DAS whereas the minimum amount of DNA (0.39 ± 0.05 mg/g fr. wt.) was assayed from seedlings influenced by 15 % leachate on 10 DAS. Other concentrations of leachate exhibited intermediate values on different DAS during the period of observation (Table-1). The DNA content of the leaves showed similar correlations with advance of growth upto 10 DAS and increase of leachate concentration throughout the period of observation as were noticed in whole-plant and leaves leachate.

RNA play second major role next to DNA in various activities in biological system which are also influenced by both internal genetic and external environmental factors as a result their quantity, quality and functions vary depending upon the degrees of effectiveness of the factors. In this investigation, the RNA contents were gradually decreased with increases of concentrations of leachate and gradually increased with increase of seedling age upto 10 DAS. The results on the effect of various types of different concentrations of leachate (whole-plant, leaves and fruits) of test weed on RNA content in the first pair of leaves of all seedlings of green gram are described below.

All the concentrations of whole-plant-leachate *Xanthium indicum* considerably checked the synthesis of RNA resulting reduction of RNA content in the first pair of leaves of green gram seedling. The maximum amount of 3.22 ± 0.06 mg/g fr. wt. RNA was estimated from first pair of leaves of seedling of control sets on 10 DAS whereas the minimum amount of 0.78 ± 0.02 mg/g fr. wt. RNA was estimated from seedling raised in 15 % leachate during same period of growth. Data of intermediate values were recorded for seedlings grown in other concentrations of leachate at different developmental stages (Table-2). The RNA content in leaves exhibited positive correlations with advancement growth period upto 10 DAS only and negative correlations with increase in leachate concentration throughout the period of observation.

In case of leaves-leachate, all concentrations significantly reduced the RNA synthesis resulting decrease in RNA content in the first pair of leaves of treated seedling compared to seedlings of respective controls. The maximum amount of 3.22 ± 0.06 mg/g fr. wt. RNA was estimated from the first pair of leaves of the seedling of control set on 10 DAS whereas the minimum amount of 0.83 ± 0.03 mg/g fr RNA was estimated from leaves of seedlings raised in 15 % concentration leachate during same period of seedling growth. Data of intermediate values were recorded for seedlings grown in other concentrations of leachate at different developmental stages (Table-2). The RNA content of the leaves showed similar correlations with advance of growth upto 10 DAS and increase of leachate concentration throughout the period of observation as were noticed in whole-plant leachate.

Table-1: Effect of different concentrations of various types of aqueous leachate of *Xanthium indicum* on DNA content in the first pair of leave of green gram seedlings at different developmental stages. (Each value is mean of 5 replicates ± S.E.M. expressed in mg/g fresh wt.)

Types of leachates	Leachate concentration (%)	6 DAS	8 DAS	10 DAS	12 DAS
Whole-plant	Control	0.53 ± 0.03	0.60 ± 0.04	0.65 ± 0.05	0.55 ± 0.06
	5	0.39 ± 0.04	0.45 ± 0.02	0.56 ± 0.01	0.41 ± 0.03
	10	0.29 ± 0.03	0.35 ± 0.02	0.46 ± 0.01	0.31 ± 0.05
	15	0.21 ± 0.01	0.30 ± 0.03	0.39 ± 0.05	0.23 ± 0.03
Leaves	Control	0.53 ± 0.03	0.60 ± 0.04	0.65 ± 0.05	0.55 ± 0.06
	5	0.40 ± 0.03	0.46 ± 0.01	0.57 ± 0.05	0.42 ± 0.03
	10	0.30 ± 0.04	0.36± 0.02	0.47 ± 0.01	0.32 ± 0.05
	15	0.22 ± 0.03	0.28 ± 0.02	0.38 ± 0.07	0.24 ± 0.01
Fruits	Control	0.53 ± 0.03	0.60 ± 0.04	0.65 ± 0.05	0.55 ± 0.06
	5	0.41 ± 0.04	0.47 ± 0.02	0.57 ± 0.01	0.43 ± 0.04
	10	0.31 ± 0.02	0.37 ± 0.04	0.47 ± 0.01	0.33 ± 0.04
	15	0.23 ± 0.03	0.29 ± 0.01	0.39 ± 0.05	0.25 ± 0.03

Table-2: Effect of different concentrations of various types of aqueous leachate of *Xanthium indicum* on RNA content in the first pair of leave of green gram seedlings at different developmental stages. (Each value is mean of 5 replicates ± S.E.M. expressed in mg/g fresh wt.)

Types of leachates	Leachate concentration (%)	6 DAS	8 DAS	10 DAS	12 DAS
Whole-plant	Control	1.79 ± 0.03	2.44 ± 0.04	3.22 ± 0.06	2.21 ± 0.07
	5	1.24 ± 0.02	1.74 ± 0.04	2.17 ± 0.03	1.57 ± 0.02
	10	0.69 ± 0.03	0.94 ± 0.05	1.05 ± 0.02	0.77 ± 0.01
	15	0.58 ± 0.03	0.64 ± 0.05	0.78 ± 0.02	0.63 ± 0.07
Leaves	Control	1.79 ± 0.03	2.44 ± 0.04	3.22 ± 0.06	2.21 ± 0.07
	5	1.39 ± 0.02	1.89 ± 0.04	2.22 ± 0.03	1.62 ± 0.02
	10	0.74 ± 0.01	1.19 ± 0.05	1.45 ± 0.03	1.02 ± 0.01
	15	0.63 ± 0.01	0.79 ± 0.02	0.83 ± 0.03	0.75 ± 0.05
Fruits	Control	1.79 ± 0.03	2.44 ± 0.04	3.22 ± 0.06	2.21 ± 0.07
	5	1.45 ± 0.04	2.09 ± 0.03	2.42 ± 0.01	1.84 ± 0.03
	10	0.85 ± 0.01	1.36 ± 0.06	1.50 ± 0.02	1.12 ± 0.01
	15	0.77 ± 0.02	0.88 ± 0.01	0.93 ± 0.03	0.84 ± 0.04

Impact of different concentrations of fruits leachate of test weed on green gram showed maximum amount of RNA (3.22 ± 0.06 mg/g fr. wt.) was estimated from the first pair of leaves of the seedling of control set on 10 DAS whereas the minimum amount of RNA estimated from seedling of green gram grown on 15 % leachate on 10 DAS was 0.93 ± 0.03 mg/g fr. wt. All other concentrations test leachate showed intermediate values at different DAS in other seedlings of both control and treated sets (Table-2). The RNA content in leaves of green gram seedling exhibited negative correlation with increase in the leachate concentrations and positive correlations with increase in growth period upto 10 DAS.

The synthesis of nucleic acids directly or indirectly depends on protein, carbohydrate, and other metabolites contents in the cells and tissues. The declined trends in the nucleic acids content in the leaves of test seedlings influenced by different concentrations of various lachates might be due to presence of lower state of precursor of nucleic acid-synthesizing materials. Baziramakenga et al.[16] have suggested that nucleic acids and protein metabolism were influenced by phenolic acids, major allelochemicals of leachate, reduced seedling growth of soybean. Neil Towers *et al.*[20] have reported that hadalin related sesqiterpene lactones, an allelochemic, inhibited DNA synthesis and DNA polymerase activity. Svenson [21] and Wink and Twardowski[22] reported that a number of allelochemicals interfere with DNA and RNA metabolism in the roots of maize and wheat. The growth and expansion in shape of multiplication of cell and DNA synthesis in plant meristems are influenced by different allelochemicals of monoterpenoids viz. camphor, 1,8-cineole, betapinene, alpha-pinene, and camphene[23] and sorgoleone reduced the number of cells in each cell division period, damaging tubulins and resulting in polyploid nuclei[24]. Li et al.[25] observed the interaction of the allelochemicals with basic biological processes (such as DNA, RNA, protein biosynthesis and related processes) and found that all biochemical reaction and their integrity are disrupted. Fang *et* al.[26] noticed that plant growth hormone's signal transduction, p53 signaling pathways, nucleotide excision repair and the peroxisome proliferator-activated receptor were highly coupled with allelochemical present in barnyard grass co-cultured with allelopathic rice. Padhy et al.[27] and Pattnaik[28] also reported such inhibitory actions of allelochemicals present in leaf-leachate of Eucalyptus on nucleic acids content in ragi seedlings. From the above reports, it is clear that the synthesis of nucleic acids might have affected or influenced by various phytotoxins present in the leachate. In the present investigation, the declined trend in DNA and RNA content might have occurred due to direct or indirect influence or interference of various phytotoxic compound present in the leachate. Similar findings were reported by Tripathy[29] in rice seedlings influenced by phyllode/leaflet-and/or bark leachate of Acacia *auriculaeformis* and *A. nilotica.* The findings of Gantayet[30] on impact of Eucalyptus globulus leaf-leachate on change in nucleic acids content in legume seedlings corroborate with present findings.

Conclusion

Since no sufficient information's regarding the allelochemic effect of *Xanthium indicum* on various crop-plants are available, the present findings on effect of leachate on changes in nucleic acids contents seems to be first of its kind. In order to draw any definite and concrete conclusions and postulations in this regard a depth study and research at molecular level are highly needed.

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