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Full Length Research Article

Phytotoxicity of Stem Aqueous Extract of *Urtica dioica* L. against *Zea mays* and *Cassia sophera*

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Abstract

Allelopathy is actually the biological warfare among the plants, in which one plant releases chemical substances allelochemicals to inhibit the growth, and affect the various physiological and morphological characteristics of plants in its vicinity. The allelopathic donor plants dominate the area by affecting the survival of other plant species in their areas. Investigations were made on the allelopathic influence of *Urtica dioica* stem aqueous extracts on *Zea mays* and *Cassia sophera.* Stem aqueous extracts showed a significant decrease on morphological characters root length, shoot length and dry weight as well as on the total chlorophyll content. At low concentration (0.5%), the growth parameters were increased. On increasing the concentration of extract, all the growth parameters were decreased. The total chlorophyll content of both the test plants was decreased on increasing the concentration of stem aqueous extract. Upon GCMS analysis of Ethanolic extract of Stem, 47 volatile compounds were identified. The compounds found in the major concentrations were STIGMAST-5-EN-3-OL, 3.BETA; Glycidyl palmitate; 1,8,11-Heptadecatriene, Z,Z-; 9-Octadecenoic acid, methyl ester, E-; 1,2-BENZENEDICARBOXYLIC ACID; HEXADECANOIC ACID, ETHYL ESTER; trans,trans-9,12-Octadecadienoic acid, propyl ester; Stigmast-5-en-3-ol, oleate; 9,12-OCTADECADIENOIC ACID Z,Z-, METHYL ESTER; ERGOST-5-EN-3-OL, 3.BETA.,24R- . These compounds may be responsible for imparting allelopathic effect on test species *Zea mays* and *Cassia sophera*. Our study revealed that stem of *U. dioica* possess a high concentration of water soluble allelochemicals, which are thought to have reduced test plants' growth and can be utilized as potential weedicides.

***Key words*:** Allelopathy, Allelochemicals, GCMS, Volatile compounds, Chlorophyll content, Ethanolic extract

Any plant which is at a wrong place is termed as weed. Weeds are responsible for an enormous loss in the crop productivity. It has been estimated that weeds are responsible for the loss of 33% crop productivity [12] and 20% of the cost of crop production is used for the removal of weeds [13]. Weeds compete with the crops for nutrition, space, light and moisture, hence affect the growth and yield. Some weeds, growing in pastures may not be palatable to grazing animals, as their poisonous nature could result in harmful effects [5]. Many weeds are disease carriers as they are hosts of numerous pathogens. *Lactuca scariola* and species of *Sonchus* are the hosts of downy mildew [20], wild *Brassica* species are the hosts of club root of cabbage [23]. In light of these characteristics, it becomes necessary to remove weeds from the areas where they unnecessarily grow. From the time when humans have endeavored to crop cultivation, they are dealing with the methods to remove them from the areas of cultivation. The techniques of removing weeds vary with the nature and place of existence of weed itself. The methods used for removing weeds from a crop field differ from removing them from a public garden and roadside areas. The methods vary because of ecological and financial reasons. Hand weeding in large fields become costlier [22], chemical sprays herbicide use in crop fields is largely preferred. But this method is not eco-friendly. The chemical buildup in food chains results in the biomagnification and disturbance in the food chains [25]. Allelopathy has reflective inferences in weed repressions and is taken in amongst momentous weed control measures.

Allelopathy is a chemical language between the plants in which one plant releases chemicals to inhibit the growth and development of other plants in its neighborhood [3]. It is basically a positive or negative interaction mostly negative among plants in which plants exude chemicals allelochemicals into the surroundings to affect the growth of nearby plants [14]. The discharged chemicals allelochemicals are basically the secondary metabolites which are formed as by-products of primary metabolic processes of plants [2]. These secondary metabolites are responsible for inhibiting the growth and metabolism of other plants in vicinity. These allelochemicals could be manipulated in such a way that their application could

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result in controlling of weeds in an ecologically safe manner. They have got the potential to be used as bioherbicides. Since the production of allelochemicals is selective, allelopathic effects vary from plant to plant. The variation exists in the amount and types of phytotoxins released. In our study, we have taken *Urtica dioica* L. as the main allelopathic donor plant. The part of the plant selected for the analysis was Stem. The test plants used for this study were *Zea mays* and *Cassia sophera.*

Fig 1 An image of mature plant of *Urtica dioica* L. captured in the month of October, 2022 from district Anantnag of Jammu and Kashmir

MATERIALS AND METHODS

Collection of plant material

The mature plants of *Urtica dioica* were uprooted and collected from the wastelands and roadsides of district Anantnag of Kashmir valley, India. Stems were separated and properly washed with tap water to get rid of dust and soil. The plant material stem was shade dried, and powdered with the help of an electric grinder. The powdered material was stored in air tight polythene bags for further use.

Collection of seeds of test plants

Vigorous, viable and standardized seeds of test plant, *Zea mays* were procured from the IARI, New Delhi, and of *Cassia sophera* from ICAR-Directorate of Weed Research Jabalpur.

Preparation of aqueous extract

100 ml of double distilled water were used to steep 4 g of powdered *Urtica dioica* stem. The mixture was left over night for solubilization of chemical compounds in the stem powder at room temperature 21-22ºC. After 24 hours, the mixture was filtered through muslin cloth and then again through filter paper Whatman no.1 and the filtrate was marked as 4%. Following filtration, the mixture was further diluted to produce an aqueous extract that ranged from 0.5 to 4%, respectively.

Experimental design

The surface sterilized seeds of test plants with 95% ethanol and 10% chlorax for 5 minutes were placed in autoclaved petri dishes. The petri dishes were lined with a single layer of Whatman No. 1 filter paper. 5ml of test extract for each concentration 4%, 2%, 1%, and 0.5% was poured in each petri dish. As a control, petri dishes that had been treated with pure water were used. Ten seeds of each variety were placed in each prepared petri dish. Five replicas of the sets with the previously described doses were left undisturbed in the lab for 15 days at room temperature 22-25ºC. After 15 days, the root length, shoot length, dry biomass, chlorophyll content were measured. Following a 24-hour period of oven drying at 80ºC, the dry biomass was calculated by weighing the plant specimens on an electric balance.

Determination of chlorophyll content

The chlorophyll content was determined by following the method of Lichtenthaler and Buschmann [17]. 10 ml of 80% acetone was used to smash 100 mg of freshly washed leaves. The material was filtered, and the absorbance of filtrate was read at 663 and 645 nm for the estimation of chlorophyll. The following formula was used to determine the total amount of carotenoid and chlorophyll.

$$\text{Total chlorophyll} = 20.2\ \text{OD}\ 645 + 8.02\ \text{OD}\ 663 \times \frac{V}{W} \times 1000$$
$$\text{mg}^{-1}\ \text{FW}$$

GCMS analysis of ethanolic extract of U. dioica stem

With the help of the GCMS-QP-2010 Plus with Thermal Desorption System TD, Shimadzu, Japan with built-in programmable head space auto-sampler and auto-injector, an Ethanolic extract of the *Urtica* stem was chemically evaluated. As a capillary column, DB-1/RTX-MS 30m was employed using helium as a carrier gas. Researchers [21], [24], performed the GCMS analysis of ethanolic extract of plant materials. The use of ethanol was employed because of its high affinity towards phenolic compounds [7], [11]. By comparing the mass spectrum of the analyte at a certain retention time to a reference standard from the National Institute of Standards and Technology NIST collection, the chemicals were identified. Total time for the GCMS experiment was 45 minutes. An important similarity index was one of 80% or more.

RESULTS AND DISCUSSION

Seedling growth radicle and plumule length

The results of *Zea mays* and *Cassia sophera*'s plumule length suggest that allelopathic chemicals have a deleterious impact on seedling development (Fig 1-2). At highest concentration 4%, the plumule and radicle length was dramatically decreased. The value of plumule length in *Z. mays* increased from 18.20. cm at control to 19 cm at the concentration of 0.5% stem aqueous extract. The retardatory allelopathic activity was observed at doses of 1, 2 and 4% of stem aqueous extract, which significantly decreased both radical as well as plumule length. The extract did not fully prevent plumule elongation in *Zea mays* however in *Cassia sophera*, the germination was totally inhibited at 4% concentration. The shoot length of *Zea mays* in the control series was 18.20 and that of *Cassia sophera* was 8.8 cm. Inhibition was seen at doses 1, 2, and 4%. Highest inhibitory effect was seen at 4% of SAE germination was totally inhibited. Similarly, root length of both the test species was significantly affected at higher concentrations.

Dry weight

Dry weight of both the test species was affected significantly by SAE. The observed percentage reduction in *Zea mays* was 82% and that of *Cassia sophera* was 100% seed germination was completely inhibited at 4%.

Chlorophyll content

When test species were exposed to various SAE doses, their chlorophyll content was considerably impacted. 0.5% SAE treatment showed a slight positive effect on the chlorophyll, whereas higher concentration 4%, reduced the chlorophyll levels by 79% in *Zea mays* and at 2% concentration, the chlorophyll content was decreased by 54% in *Cassia sophera*, in comparison to the control (Fig 1-2).

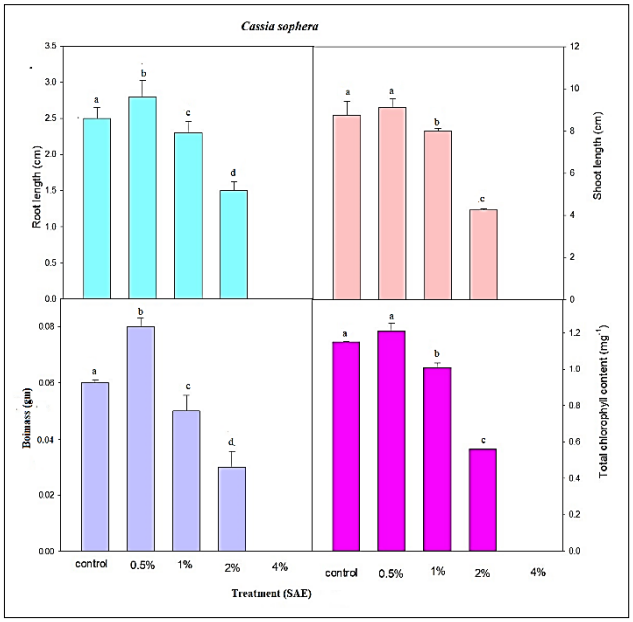

Fig 2 Effect of different concentrations of stem aqueous extract of *U. dioica* on root length, shoot length, dry biomass and chlorophyll content of *Cassia sophera*

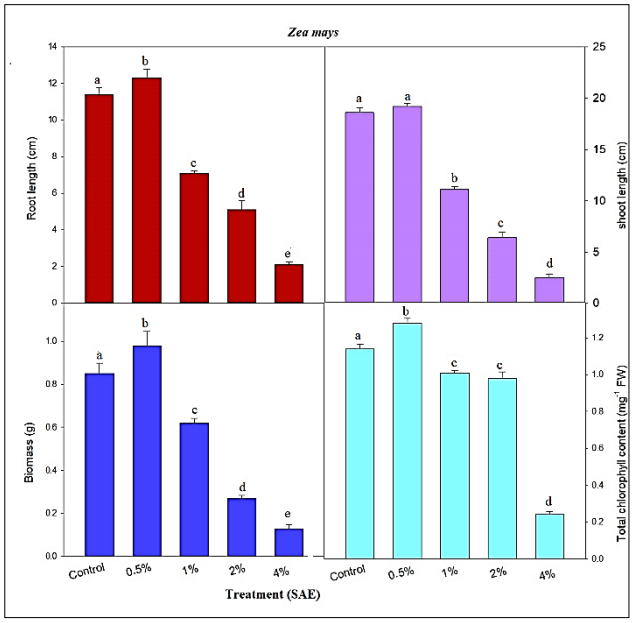

Fig 3 Effect of different concentrations of stem aqueous extract of *U. dioica* on root length, shoot length, dry biomass and chlorophyll content of *Zea mays*

GCMS of ethanolic extract of stem of Urtica dioica

47 volatile chemicals were found in the extract after GCMS analysis, accounting for approximately 99% of the overall composition. The compounds were identified on the basis of various peaks, retention times and peak areas (Table 1). According to their reducing peak areas, the top ten compounds were Stigmast-5-En-3-Ol, 3.Beta; Glycidyl Palmitate; 1,8,11-Heptadecatriene, Z,Z-; 9-Octadecenoic Acid, Methyl Ester, E-; 1,2-Benzenedicarboxylic Acid; Hexadecanoic Acid, Ethyl Ester; Trans,Trans-9,12-Octadecadienoic Acid, Propyl Ester; Stigmast-5-En-3-Ol, Oleate; 9,12-Octadecadienoic Acid Z,Z-, Methyl Ester; Ergost-5-En-3-Ol, 3.Beta.,24r-. Figure 3 displays a GCMS chromatogram of the stem of *Urtica dioica*.

Table 1 Identification of volatile compounds in the stem ethanolic extract of *Urtica dioica* through GCMS

Peak#	R. Time	Area	Area (%)	Name
1	4.371	230497	1.38	2-FURANCARBOXALDEHYDE
2	5.821	166815	1.00	5-ISOPROPYL-2-METHYLBICYCLO[3.1.0]HEX-2-ENE
3	6.586	98656	0.59	Tetraethyl silicate
4	7.509	139249	0.83	CYCLOHEXENE, 1-METHYL-4-1-METHYLETHENYL
5	7.981	32472	0.19	CYCLOHEXENE, 3-METHYLENE-6-1-METHYLETHYL
6	12.913	65861	0.39	1-UNDECENE, 9-METHYL-
7	13.400	12559	0.08	CYCLOHEXANE, 2-3-IODOPROPYL-1,1-DIMETHYL-3
8	14.427	131132	0.79	Phenol, 3,5-bis1,1-dimethylethyl-
9	16.664	23865	0.14	4-Heptanone, 2-methyl-
10	17.679	24910	0.15	2-Undecene, 9-methyl-, E-
11	18.131	77879	0.47	Neophytadiene
12	18.191	49563	0.30	Oxirane, decyl-
13	18.386	53495	0.32	Citronellyl butyrate
14	18.578	51712	0.31	BUTANOIC ACID, 3,7-DIMETHYL-6-OCTENYL ESTER
15	18.772	26428	0.16	Homosalate
16	19.038	552294	3.31	Hexadecanoic acid, methyl ester
17	19.706	660998	3.96	HEXADECANOIC ACID, ETHYL ESTER
18	20.672	582238	3.49	9,12-OCTADECADIENOIC ACID Z,Z-, METHYL ESTE
19	20.732	1146533	6.87	9-Octadecenoic acid, methyl ester, E-
20	20.786	50145	0.30	9-OCTADECENOIC ACID Z-, METHYL ESTER
21	20.968	148697	0.89	Methyl stearate
22	21.284	611511	3.66	trans,trans-9,12-Octadecadienoic acid, propyl ester
23	21.339	225051	1.35	Tricyclo[20.8.0.07,16]triacontane, 122,716-diepoxy-
24	21.537	196704	1.18	9,12-OCTADECADIEN-1-OL
25	21.746	48876	0.29	3,7,11,15-Tetramethylhexadec-2-en-1-yl acetate
26	22.375	148617	0.89	Carbonic acid, 2-dimethylaminoethyl neopentyl ester

27	22.505	1478562	8.86	Glycidyl palmitate
28	22.757	160871	0.96	Z-4-DECENAL
29	23.253	116327	0.70	E-2-Tetradecen-1-ol
30	24.214	202614	1.21	Benzedrex
31	24.434	1307416	7.83	1,8,11-Heptadecatriene, Z,Z-
32	24.833	99182	0.59	DECANE, 1,1'-OXYBIS-
33	25.292	715121	4.28	1,2-BENZENEDICARBOXYLIC ACID
34	30.734	130182	0.78	2-methyloctacosane
35	31.363	47030	0.28	7-2-HYDROXY-1-METHYLETHYL-1,4A-DIMETHYL-2
36	32.069	30153	0.18	.gamma.-Tocopherol
37	32.444	47467	0.28	Calcitriol
38	32.658	442467	2.65	STIGMAST-5-EN-3-OL, 3.BETA.-
39	33.003	276282	1.66	Vitamin E
40	33.365	66445	0.40	SOLANESOL
41	34.151	579474	3.47	ERGOST-5-EN-3-OL, 3.BETA.,24R-
42	34.811	82512	0.49	CYCLOPENTADECANONE
43	35.264	3979560	23.84	STIGMAST-5-EN-3-OL, 3.BETA.-
44	35.656	99792	0.60	1,1,4,7-TETRAMETHYLDECAHYDRO-1H-CYCLOPROP
45	36.575	323810	1.94	9,19-Cyclolanostan-3-ol, acetate, 3.beta.-
46	36.965	586784	3.52	Stigmast-5-en-3-ol, oleate
47	37.196	363283	2.18	Cholest-4-en-3-one
		16692091	100.00	

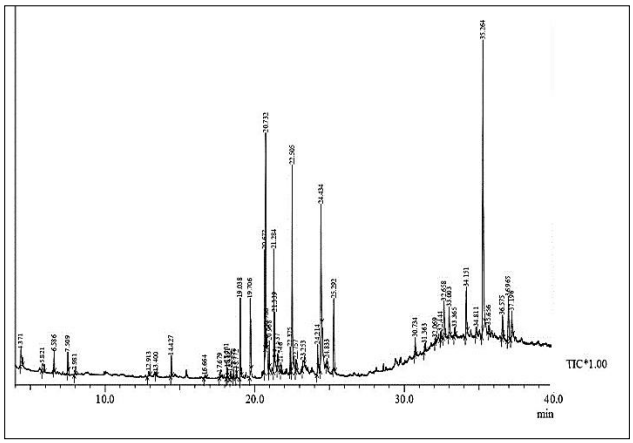

Fig 4 GCMS chromatogram of stem ethanolic extract of *Urtica dioica*

Statistical analysis

Every experiment was carried out using a totally random design. The SPSS/PC programme version 16 was used to analyze the data on sapling growth root length, shoot length and dry biomass. The standard deviation of the measurements is indicated by the bars in (Fig 1). Comparative analysis was conducted using DMRT Duncan 1955 and ANOVA, with the treatment means being separated from the control at $p < 0.05$.

The SAE treatment caused delayed germination and a low germination rate. The treatments reduced dry weight by delaying germination, lowering seed germination rate, slowing seedling growth, and reducing the root-to- shoot ratio. With higher concentrations, the inhibitory effects became stronger. This study demonstrates that different leachate concentrations had diverse allelopathic inhibitory effects on the test species. Lower concentrations 1 and 0.5% had low inhibitory or even beneficial effects hormesis, but higher concentrations 2% and 4% had more potent inhibitory effects. The effect on the root length is because root membranes are the main site of action for phenolics. The interaction of phenolic acids with the root cells causes depolarization, efflux of ions and decrease in hydrolic conductivity, water uptake, net nutrient uptake [4]. These phenomena may affect the shoot length of species as well.

As seen in the several researches, allelochemicals from Melia, extracted with ethanol and water, had reduced the germination and growth of recipient plants [1], [6], [15]. This is consistent with our studies. Decrease in chlorophyll content was significant in our study. On increasing the concentration of aqueous extracts, the chlorophyll content decreased significantly. The considerable decrease in chlorophyll content seen at all concentrations may be caused by either the activation of chemicals that degrade chlorophyll or the inhibition of its production [16]. By interactions with photosystem II components, allelochemicals can impair photosynthesis and O_2 evolution [9].

54 volatile phenolic allelochemicals were identified from the Ethanolic extract of *Urtica dioica* stem. The presence of phytochemicals in the stem extract of *Urtica dioica* indicates that they play a significant role in giving the aforementioned plat its cytotoxic/allelopathic nature. Nevertheless, phenolic acids are the main components of allelopathic plants, even though they are not required for the fundamental activities taking place in plants and are released as secondary metabolites. Allelochemicals with inhibitory effects act key elements in plant defense mechanisms against weed invasions. There are several organic pesticides that can potentially reduce weed growth in areas that have been manufactured [8], [10], [19]. For certain weeds, all phenolic acids had an inhibitory effect on seedling growth and germination. This possession of allelochemicals by the *Urtica dioica* stem depicts that it is an allelopathic plant, and the chemicals can be utilized as organic weedicides in future after further research.

CONCLUSION

According to our study, *Urtica dioica* Stem has a high concentration of water soluble allelochemicals, which are thought to have reduced test plants' growth. This offers significant evidence of its capacity to stop the growth of weed seeds. That knowledge could be applied further to create environmentally friendly herbicides for weeds and other undesirable plant growth, expanding the potential for organic farming.

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