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Application of nutsedge (*Cyperus rotundus* L.) extracts for weed suppression and identification of allelochemicals

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ABSTRACT

Selection of allelopathic plants is a commonly used approach to identify plants with biologically active natural products. Laboratory experiments were conducted to determine the allelopathic effects of purple nutsedge (Cyperus rotundus L.) extracts on common upland weeds of Kerala like Chromolaena odorata (L.), Synedrella nodiflora and Gomphrena decumbens with a view to explore its weed seed inhibition potential. Aqueous nutsedge extracts inhibited germination and growth of Gomphrena decumbens while it had no significant influence on the germination of other two weed seeds. In Gomphrena, nutsedge extracts taken after flowering caused greater growth inhibition compared to extract taken before flowering indicating differential inhibition at various growth stages of nutsedge as well as increased allelochemical production after flowering stage. Suppression of plumule growth and significant reduction in radicle growth and vigour index was observed for all the three weed seeds tested. The study revealed greater inhibition rate for extracts collected after flowering, which indicated that allelochemical production is more after flowering. The tuber extracts of nutsedge as identified by HPLC technique revealed the presence of phenolic compounds viz. p-hydroxybenzoic acid and p-coumaric acid.

***Keywords*:** Allelopathic potential, nutsedge extract, phenolic compounds, weeds seed inhibition

The current agricultural practices involving heavy use of synthetic herbicides to control weeds may create a number of environmental hazards. Researchers are now searching for new alternatives of synthetic herbicides which are bio-degradable and environment friendly. Allelopathy is known as the harmful or beneficial effects of one plant on another through the release of allelochemicals (Molísch, 1937). The allelochemicals are released into the environment through exudation, decomposition, leaching and or volatilization, and may be toxic or stimulatory to the plant itself or other plant species (Islam and Kato-Noguchi, 2013). Allelochemicals refer mostly to the secondary metabolites produced by the plants and are bye-products of primary metabolic processes. The use of natural plant products particularly the allelochemicals for the management of weeds is a logical strategy and has been suggested by some earlier workers (Duke *et al.,* 2000).

In order to identify plants with biologically active natural products, selection of allelopathic plants is a good and commonly used approach. Nut sedge *(Cyperus rotundus* L.) is an important weed in the world that is distributed widely in all tropical and sub-tropical area. Allelopathy of *C. rotundus* is not only to suppress crop growth and production, but also to suppress several weeds growth. Some literatures reported that allelopathy of *C. rotundus* is able to suppress the growth of crop or other plant including weeds (Elrokiek, 2010). However, specific and systematic studies regarding the use of allopathy of *C. rotundus* as agent for controlling weed growth in an environmentally friendly agricultural system is still lacking. Keeping this in view, an attempt has been made to study the allelopathic effect of nutsedge on some of the most prominent seed propagated upland weeds of Kerala. It is also envisaged to identify the allelochemicals present in purple nutsedge plant to explore its allelopathic potential for bio herbicide formulation.

MATERIALS AND METHODS

Preparation of nutsedge extracts

Laboratory experiments were undertaken to examine the allelopathic influence of nutsedge extracts on some of the important seed propagated annual weeds and the test plants were *Chromolaena odorata* (L.), *Synedrella nodiflora* and *Gomphrena decumbens.* The design of the experiment was Completely Randomised Design with 6 treatments in 4 replication. The treatments of the study consisted of aqueous extract of dry whole plant taken before (T_1) and after flowering (T_2) along with ethanol extract of whole plant taken before (T_3) and after flowering (T_4). Distilled water (T_5) and ethanol (T_6) were taken as control treatments. For preparing the extract, purple nutsedge (*Cyperus rotundus* L.) plant samples were collected from infested fields at the respective growth stage. The plants were then cleaned off dirt and soil. It was then shade dried for one week. One hundred gram shade dried plant samples were immersed in 200 ml distilled water separately and kept at room temperature for 48 h. There after, it was stirred manually for few minutes and filtered through whatman no.1 filter paper. It was considered as a leachate of 50 per cent concentration and was further diluted with distilled water

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to 10 per cent concentration. Ethanol extract was prepared in the same way with ethanol as the extractant. Glass petri dishes (9 cm diameter) were sterilized in auto clave at an atmospheric pressure of 15 lb $inch^{-2}$ for one hour and later dried in hot air oven at 120^0C. Seeds of test plants were sterilized by dipping in 0.1 per cent Hg Cl_2 solution for five minutes followed by repeated washing under tap water to remove residues of Hg Cl_2 and dried on in folds of ordinary filter paper. In each petri plate a Whatman No.1 filter paper was kept at bottom and there after 50 seeds each of test crop were arranged in circles on the top of the filter paper. Then 3 ml of the aqueous extract and ethanol extract or distilled water was added in each petri plate as per the treatments. Thereafter 2 ml solution of extract or distilled water was added uniformly as and when required till the end of the trial. As the weed seed endosperms were too little to support seedling growth in the petri dishes, the period of observation was limited to 7-10 days. The data on germination and seedling growth were recorded and the data were analysed statistically.

Nutsedge extract was prepared as per the procedure suggested by (Leela, 1995) and the allelochemicals present in the tubers were identified by High Performance Liquid Chromatography (HPLC). One hundred and seventy five gram dried tubers of nutsedge were finely ground in a blender and soaked in 500 ml of methanol for 30 minutes. Then it was filtered through a muslin cloth. The final volume was made to 1000 ml. The filtrate was concentrated on a vacuum flash evaporator. The residue (10 ml) was diluted in 50 ml water to which 2.5 g of NaCl is also added. This is extracted thrice with 25 ml ethyl acetate each time. The ethyl acetate extracts combined, concentrated and the residue was hydrolysed with 2N NaOH. Then the pH was adjusted to 2.0 using 2N HCl. This was again extracted with ethyl acetate three times and evaporated to dryness. The dried residue was treated with 0.1 N $NaHCO_3$ solution and the pH was adjusted to 2.0. This was re-extracted with ethyl acetate three times and washed with distilled water to remove last traces of HCl. This was evaporated to dryness. The residue was dissolved in 25 ml ethyl acetate and phenols present were analysed with High Performance Liquid Chromatography (HPLC).

RESULTS AND DISCUSSION

Of the four treatments tried, ethanol extract at 10 per cent concentration taken as control killed all the weed seeds completely, while aqueous extracts caused inhibition of growth of some of the weed seeds tested (Table-1, 2 and 3). Aqueous nutsedge extracts inhibited germination and growth of *Gomphrena decumbene* while it had no significant influence on germination of *Synedrella nodiflora* and *Chromolaena odorata*. In *Gomphrena decumbens*, an annual dicotyledenous upland weed, nutsedge extract taken after flowering caused greatest (68 per cent) reduction in seed germination while nutsedge extract taken before flowering caused a reduction percentage of 47. Suppression of plumule growth was observed in all the weed seeds tested and nutsedge extracts taken after flowering inhibited *Gomphrena decumbens* and *Chromolaena odorata* while extract taken before flowering inhibited *Synedrella nodiflora*. Significant reduction in radicle growth was caused by aqueous extract of nutsedge in all the three weed seeds. Presence of coumarins in *Cyperus* extract could interfere with root cell elongation, water relations and photosynthesis in plant (Lal and Oudhia, 1999).

The dry matter production is one of the deciding factors of plant vigour and it is a function of growth of both root and shoot. Dry weight of *Gomphrena decumbense* and *Chromolaena odorata* were significantly affected by nutsedge extracts. This reduction in dry weight is consequent to the earlier reduction in growth parameters like plumule and radicle growth. Vigour index was drastically reduced for all the weed plants. The reduction in vigour index was observed under both types of plant extracts. Inhibition of radicle growth, which in turn resulted in poor nutrient absorption and consequent poor biomass accumulation, could be the reason for such a drastic reduction in seedling vigour.

Table 1: Allelopathic influence of purple nutsedge extracts on germination and growth of *Chromolaena odorata*

Treatments	Germination percentage	Plumule length (cm)	Radicle length (cm)	Dry weight (mg $plant^{-1}$)	Vigour index
T_1	27.50 (31.53)	0.55	0.10	0.18	17.87 (4.20)
T_2	20.00 (26.18)	0.23	0.10	0.08	6.47 (2.51)
T_3	0.00 (0.00)	0.00	0.00	0.00	0.00 (0.00)
T_4	0.00 (0.00)	0.00	0.00	0.00	0.00 (0.00)
T_5	47.50 (44.28)	1.57	0.75	0.20	112.75 (10.25)
T_6	0.00 (0.00)	0.00	0.00	0.00	0.00 (0.00)
SEm(±)	**5.66**	**0.04**	**0.04**	**0.02**	**0.95**
LSD (0.05)	**NS**	**0.14**	**0.12**	**0.07**	**3.05**

*Figure in parenthesis indicate angular and square root transformed value*s

Table2: Allelopathic influence of purple nutsedge extracts on germination and growth of *Synedrella nodiflora*

Treatments	Germination (%)	Plumule length (cm)	Radicle length, (cm)	Dry weight, (mg plant^{-1})	Vigour index
T_1	52.50 (50.30)	1.05	0.13	0.18	58.87 (7.49)
T_2	47.50 (48.71)	1.25	0.16	0.14	68.50 (7.70)
T_3	0.00 (0.00)	0.00	0.00	0.00	0.00 (0.00)
T_4	0.00 (0.00)	0.00	0.00	0.00	0.00 (0.00)
T_5	80.00 (70.43)	3.93	0.31	0.20	344.42 (18.27)
T_6	0.00 (0.00)	0.00	0.00	0.00	0.00 (0.00)
SEm(±)	**13.54**	**0.16**	**2.21**	**2.73**	**1.57**
LSD (0.05)	**NS**	**0.52**	**7.08**	**NS**	**5.03**

Figure in parenthesis indicate angular and square root transformed values NS- Non significant

The study revealed greater inhibition rate for extracts collected after flowering, which indicated that allelochemical production is more after flowering. Differential inhibition by nutsedge extracts taken at different stages of growth can be explained by the fact that production of allelochemicals is regulated by the stage of the plant and is modified by environmental stresses like temperature extreme, nutrient moisture variables, insects and diseases and radiation (Einhellig, 1995). Inhibition obtained at post flowering stage may be due to the higher level of allelochemical production after flowering in nutsedge (Jha and Sen, 1982). Presence of p-hydroxy benzoic acid, caffeic acid, o-coumaric acid and ferulic acid in *Cyperus rotundus* reported earlier by Leela (1995). Allelopathy even though considered as an undesirable property these characters can be profitably exploited. Collectively, these results showed differential toxicity of the allelopathic chemicals among the sp depending upon the stage and method of application. The use of water extracts of allelopathic crops particularly nutsedge alone or in combination with other water extracts will provide an economical, environmentally safe and effective weed control technique as an alternative for herbicides. Hence

Table 3: **Allelopathic influence of purple nutsedge extracts on germination and growth of *Gomphrena decumbense***

Treatments	Germination percentage 7 DAS	Plumule length, (cm)	Radicle length, (cm)	Dry weight, (mg plant^{-1})	Vigour index
T_1	45.00 (42.03)	1.37	0.23(0.47)	0.25	72.75 (8.43)
T_2	27.50 (31.01)	1.15	0.28(0.51)	0.49	39.75 (6.12)
T_3	0.00 (0.00)	0.00	0.00(0.00)	0.00	0.00 (0.00)
T_4	0.00 (0.00)	0.00	0.00(0.00)	0.00	0.00 (0.00)
T_5	85.00 (70.05)	2.35	1.03(1.00)	0.54	289.00(16.93)
T_6	0.00 (0.00)	0.00	0.00(0.00)	0.00	0.00 (0.00)
SEm(±)	**5.09**	**0.07**	**0.06**	**0.04**	**0.83**
LSD(0.05)	**16.31**	**0.22**	**0.20**	**0.13**	**2.65**

Figure in parenthesis indicates angular or square root transformed values

Table 4: Identification of allelochemicals in the tuber extract of purple nutsedge (*Cyperus rotundus* L.)

Sl. No.	Reference compounds	Retention time(min.)	Retention of peaks in sample (min)
1	Gallic acid	1.98	
2.	3,4-dihydoxy phenyl acetic acid	3.08	
3.	p-hydroxy benzoic acid	4.40	4.40
4.	Caffeic acid	4.84	
5.	Vanillic acid	5.28	
6.	Gentisic acid	5.73	5.88,6.07,6.79,7.88
7.	p-coumaric acid	8.58	8.71
8.	Ferulic acid	11.45	
9	m-coumaric acid	13.43	13.31,14.61
10.	o-coumaric acid	20.69	
11.	Salicylic acid	27.96	

it is probable that these chemicals would be useful as applied herbicides and can be effectively utilized for the management of other weeds.

In the present study, the tuber extracts of nutsedge as identified by HPLC technique revealed the presence of phenolic compounds *viz.* p-hydroxybenzoic acid and p-coumaric acid (Table 4). Elrokiek (2010) have reported that *C. rotundus* contains phenolic compounds such as cyperene and culmorin. Phenolic compounds with high solubility in water have reported to have low allelophaty activities (Seigler, 1996). The readily visible effects of these allelochemicals include inhibition or retardation of germination, reduced radicle or coleoptile extension, curling of the root axis, discolouration, increased number of seminal roots, reduced dry weight accumulation, and lowered reproductive capacity (Turk *et al.*, 2002; Weir *et al.*, 2004). These gross morphological effects may be secondary manifestations of primary events caused by a variety of more specific effects acting at the cellular or molecular levels in receiver plants (Rice, 1970). Presence of p-hydroxy benzoic acid, caffeic acid, o-coumaric acid and ferulic acid in tuber extracts of *Cyperus rotundus* is reported earlier by Leela (1995) and reduction in germination and growth of weed seeds observed in present study corroborate her findings.

Allelochemicals are present in virtually all plant tissues including leaves, flowers, fruits, stems, roots, rhizomes, seeds and pollen. The inhibition in germination and growth of test crops observed in bioassay was marginal. Considering the uncontrolled growth in the field situation and a relatively high proportion of live and dormant tuber remaining in the soil coupled with the unfavourable stress conditions to which the soil will be exposed the growth of adjacent crops can expected to be more pronounced in the field situation than the conditions prevailing in the present study. However, phenolic compounds with high solubility in water have reported to have low allelophathic activities (Seigler 1996). Therefore, for its application in the field as bioherbicide, further studies are needed. .

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