Research Article

Phytotoxic Effects of Leaf Leachates of an Invasive Weed *Synedrella Nodiflora* and Characterization of its Allelochemical

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ABSTRACT

Synedrella nodiflora (L.) Gaertn. (Asteraceae) a dominant invasive weed from Ganeshkhind area has affected the phytodiversity of Pune University campus (M.S., India). Its leaf leachates inhibited seed germination and seedling growth in test crops like tomato and brinjal at all the concentrations indicating their phytotoxic nature. However maximum inhibition was recorded at higher concentrations (17.5 – 20% of original stock solution). The allelopathic leaf leachates had also adversely influenced the seedling physiology in both the vegetables. Hence to investigate its phytotoxic nature, isolation and characterization of allelochemicals in it, was undertaken. Leaf samples of *Synedrella* showed the presence of a ketone 3-(5-(1-(3-methylpentyloxy) propyl)-tetrahydro-2-oxofuran-3-yl)-dihydrofuran-2(3*H*)-one.

Keywords: *Synedrella nodiflora,* invasive weed, phytotoxicity, seed germination bioassay, seedling physiology.

INTRODUCTION

Invasive weeds continue to encroach on newer habitats and often replace native phytodiversity. These plants are often aggressive outside their natural range and become dominant in the communities due to their allelopathic potential[1-4]. Allelopathy is involved in both positive and negative interference between the plants and micro-organisms; these processes are difficult to understand without studying their ecophysiology and biochemistry. Allelochemicals released from such plants cause negative influence by prevention of seed germination and seedling growth by producing growth inhibiting allelochemicals[5]. This phytotoxicity may be due to high levels of inhibitory allelochemicals / ecochemicals that also affect the physiology of recipient plants[6-12].

The Pune University Campus with an area of about 164.8 hectares is rich in weed diversity. Through phytosociological studies,[13] it was indicated that invasive weeds like *Synedrella nodiflora* (L.) Gaertn. (Asteraceae), *S. vialis* (Less) A. Gray, *Cassia uniflora* Mill. Non Spreng. (Fabaceae), and *Alternanthera tenella* Colla. (Amaranthaceae) are becoming dominant on the campus and threatening native plants diversity. They further reported that *Synedrella nodiflora* (L.) Gaertn. was the next dominant species found on the campus after *Cassia*.

Frequent visits and ecological surveys conducted during the study period indicated that the plant species such as *Synedrella nodiflora* , *Cassia uniflora* and *Alternanthera tenella* were dominant compared to other plants on the campus of Pune University.

The phytotoxic nature of aqueous leaf leachates of *Synedrella nodiflora* on the growth of crop plants is well documented[13]. GC-MS studies of this weed indicated the presence of steam volatile components as caryophylene oxide, 2-pentadecanone, hexadecanoic acid, phytol and di-isooctyl phthalate as major compounds whereas nonpolar *n*-hexane extract showed the presence of 2-pentadecanone, neophytidiene and Hexadecanoic acid as major constituents[14]. From a review of current literature it revealed that little research has been done on isolation, identification and characterization of allelochemicals from different native and invasive weeds of this campus. In fact, little or no attention has been given to antimicrobial, insecticidal and herbicidal activities of novel allelochemicals existing in such weeds. The present investigation was conducted to explore the novel allelochemicals existing in *Synedrella nodiflora.*

MATERIALS AND METHODS

Leaf leachate preparation

Fresh leaf samples of *Synedrella nodiflora* were collected from the natural sites on the Campus at the flowering stage, brought to laboratory, cleaned with distilled water and spread on filter paper for shade drying. These dry leaves were powdered and the resulting powder (100g) was soaked in 1000 ml distilled water for 24 h at 25^0C. The leachate (20% - this is the original stock solution) was filtered through Büchner funnel using Whatman No. 1 filter paper and stored in a refrigerator in amber coloured bottles.

Seed Germination Bioassay

The seeds of tomato (*Lycopersicon esculentum* L. var.Vaishali) and brinjal (*Solanum melongena* L. var. Manjri gota) were obtained from the College of Agriculture, Pune. Healthy seeds were selected and used for bioassay study in a seed germination chamber using sterilized petriplates (9 cm diameter) lined with germination papers. The seeds of tomato and brinjal were surface sterilized with 0.02% aqueous $HgCl_2$ for two minutes. Then the seeds were thoroughly washed with distilled water. Seed germination papers were thoroughly moistened with respective concentrations of leaf leachates (5 ml.) of *Synedrella* (2.5% to 20%) that were prepared by dilutions with distilled water. Seeds placed on germination paper moistened only with distilled water were considered as a control.

Twenty seeds each of tomato and brinjal were uniformly placed on each petri-plate. Two ml leachates of each respective concentration were added to the appropriate petriplate on the third day. The petriplates were moistened with leachates only one additional time. All treatments were in triplicates. Seed germination (%), root length, shoots length and vigour index were recorded on the 7th day[15].

Biochemical analysis of seedlings

Randomly selected material of seedlings (control and leachates treated; 0.1g) was taken for analysis of starch[16], reducing sugars[17], total sugars[18], proteins[19] and phenols[20]. Absorbances were recorded on a UV-visible spectrophotometer (Shimadzu-1601) at respective wavelengths.

Extraction, Isolation and characterization of allelochemicals

Shade dried powdered plant material (25g) was refluxed with ethanol (100ml) for 18h. Solvent was removed under reduced pressure to get crude extract. TLC in a 20% ethyl acetate – toluene solvent system revealed 6 major spots of which one was UV active at 365nm. The crude ethanol extract was broad fractioned on silica gel (60-120, 5 g) using gradient polarity solvents. Each fraction of 100 ml was collected. Fractions were monitored by thin layer chromatography. Thus a total of four broad fractions (**A** to **D**) were collected. The details are given in Table 1.

Separation and purification of compound 'A' from *Synedrella*

The fraction **B** containing compound 'A' was concentrated and kept at room temperature for 18 h whereupon white crystals separated from the solvent. Compound A was further purified from this by preparative TLC and re-crystallized from ethanol. The pure compound (40 mg) produced single spot on TLC. The compound gets decomposed at 270°C.

Statistical analyses

The data were summarized as means of three replicates with standard deviation as the measure of variability. One way ANOVA was used to compare the mean values followed by Duncan's Multiple Range Test (DMRT) at p= 0.05 to compare the mean differences. SigmaStat 3.5 and Microsoft Excel 2007 were used for the data analyses.

RESULTS AND DISCUSSION

Seed germination bioassay of tomato and brinjal

Leaf leachates of *Synedrella nodiflora* at all concentrations tested inhibited seed germination and seedling growth of tomato and brinjal (Table 2 and 3). The germination percentage was found to decrease with an increase in concentrations. The IC_{50} value in both vegetables was 17.5% concentration. Reduction in root and shoot length, root**:**shoot ratio and vigour index was also recorded with increasing concentrations of leaf leachates. However, in tomato an opposite trend was noted for shoot length and vigour index at 2.5 and 5% concentrations. For all these parameters, brinjal was more sensitive than tomato to the higher concentrations of leaf leachates. The results were significant at p = 0.05. Leaf leachates of *Synedrella* were also tested for antimicrobial activity and the results obtained were positive. Therefore further investigation and characterization of pure compound was continued.

Changes in germination usually result from differences in membrane permeability, DNA transcription, protein translation, function of the secondary messengers, respiration, confirmation of enzymes and receptors in the membranes or joint action of these changes. Phytotoxic effects are generally recorded on the seed germination ability which indicates negative influence of allelochemicals[21, 22]. Inhibition in seed germination may have adverse effect on seedling establishment, because this inhibition can affect the ability of the seedlings to establish in natural conditions[23, 24].

The results of the present investigation as well as in root length, shoot length, root:shoot ratioand vigour index are in agreement with those of other researchers[25,26], who worked with rice, cowpea, green gram and pigeon pea[27,31].

Table 1: Broad fractionation of ethanol extract of *Synedrella nodiflora* leaves

Fraction	Solvent	Vol. ml	Wt. of Fraction g	Approximate composition
A	Acetone	4 X 100	0.640	Mixture of unidentified compounds
B	**5% EtOH- Acetone**	10 X 100	0.513	Mixture of unidentified compounds + **Compound 'A'**
C	100% EtOH	9 X 100	0.930	Mixture of unidentified compounds
D	100% MeOH	4 X 100	0.200	Mixture of unidentified compounds

Table 2: Effects of *Synedrella nodiflora* Leaf Leachates on Seed Germination of Tomato

Conc. %	% Germination	Root length cm Mean ± SD	Shoot length Mean ± SD	R : S Ratio Mean ± SD	Vigour index Mean ± SD
Control	100	6.93 a ± 2.40	11.37 a ± 0.35	0.61 ± 0.19	550 b ± 26.46
2.50	100	5.17 abc ± 0.85	11.4 a ± 1.44	0.45 ± 0.03	650 a ± 72.11
5	95	5.73 ab ± 0.80	12.5 a ± 0.17	0.46 ± 0.07	617.5 a ± 68.51
7.50	95	5.5 ab ± 0.7	10.87 a ± 3.13	0.53 ± 0.15	546 b ± 85.06
10	90	5.07 abc ± 0.67	10.4 a ± 0.26	0.49 ± 0.07	413.67 c ± 85.14
12.50	75	4.57 bcd ± 1.16	9.63 ab ± 1.86	0.47 ± 0.03	392c ± 28.84
15	65	3.77 bcd ± 0.67	10.47 a ± 0.96	0.37 ± 0.10	485.33 bc ± 54.37
17.50	55	3.33 cd ± 0.42	7.07 bc ± 0.67	0.48 ± 0.11	256.67 d ± 45.37
20	35	2.67 d ± 0.76	4.9 c ± 1.9	0.67 ± 0.51	94.67 e ± 20.53
p-value		**0.004***	**<0.001***	**0.733 - NS**	**<0.001***

*Values followed by different letters differ significantly by Duncan's multiple range test at p = 0.05

Table 3: Effects of *Synedrella nodiflora* Leaf Leachates on Seed Germination of Brinjal

Conc. %	% Germination	Root length cm Mean ± SD	Shoot length cm Mean ± SD	R : S Ratio Mean ± SD	Vigour index Mean ± SD
Control	100	3.87 abc ± 0.42	5.5 bc ± 0.26	0.70 ± 0.05	550.0 b ± 26.46
2.50	100	4.27 ab ± 0.32	6.5 ab ± 0.72	0.66 ± 0.11	650.0 a ± 72.11
5	95	3.97 abc ± 0.15	6.5 ab ± 0.72	0.62 ± 0.09	617.5 a ± 68.51
7.50	90	3.97 abc ± 0.15	6.07 abc ± 0.95	0.67 ± 0.14	546.0 b ± 85.06
10	85	3.63 bc ± 0.55	4.87 c ± 1.00	0.77 ± 0.22	413.6 c ± 85.14
12.50	80	3.67 bc ± 0.32	4.9 c ± 0.36	0.75 ± 0.01	392.0 c ± 28.84
15	70	4.47 a ± 0.31	6.93 a ± 0.78	0.65 ± 0.10	485.3 bc ± 54.3
17.50	50	3.53 c ± 0.42	5.13 bc ± 0.91	0.69 ± 0.05	256.6 d ± 45.37
20	40	2.27 d ± 0.25	2.37 d ± 0.51	1.01 ± 0.35	94.67 e ± 20.53
p-value		**<0.001 ***	**<0.001 ***	**0.181**	**<0.001 ***

*Values followed by different letters differ significantly by Duncan's multiple range test at p = 0.05

The phytotoxic effects of various weeds on seed germination and growth of different crops also were reported[32 - 35]. The allelopathic impact of leachates was more harmful to radical[36] as it was coming in close contact with the leachates/extracts. The favourable or adverse impact of allelochemicals on processes as seed germination is concentration/dose dependent which is explained[37] as "hormesis"; or stimulation of growth at lower concentration and inhibition at higher concentrations of leachates/extracts.

A negative influence of higher concentrations of leachates and extracts of different allelopathic plant species including weeds on seed germination has been reported by many workers performing seed germination bioassays and testing allelopathic influence[38 - 51]. The stimulatory as well as inhibitory effects were concentration dependant. The results of the present investigation were in agreement with the above studies.

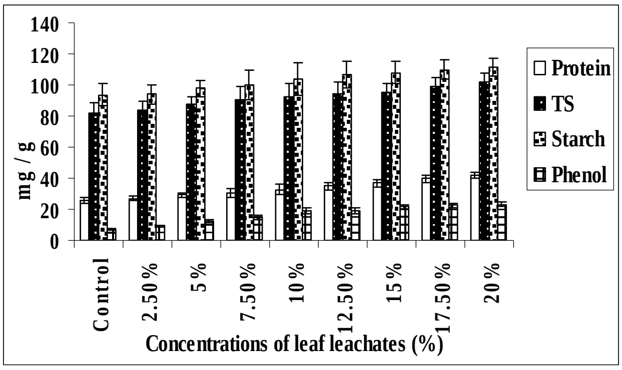
Allelochemicals present in the leachates may be responsible for changes in water relations, membrane permeability and enzymatic activities of protein and carbohydrate degradation during the seed germination process, which determined the success of seed germination[52]. Similar effects may explain the changes in germination of tomato and brinjal produced by leachates of the invasive weed *Synedrella nodiflora*.

Seedling physiology

Physiological analysis of seedlings illustrates their metabolic status and capability to mobilize and effectively utilize the reserve food materials, resulting into successful seedling development, growth and establishment. Hence investigation of the physiology and biochemistry of germinating seeds was under taken.

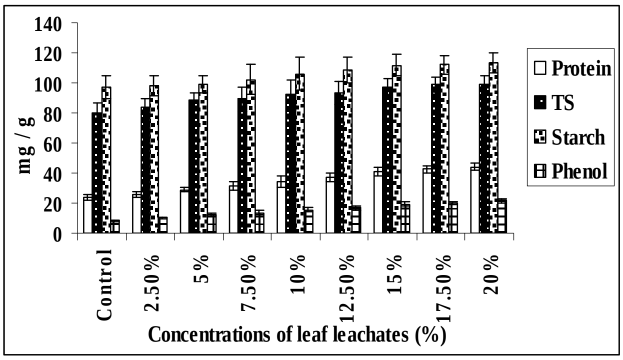
The results represented in Figures 1 and 2 indicated that lower concentrations of *Synedrella nodiflora* leaf leachates exhibited significantly less reduction in total sugar contents in tomato and brinjal seedlings, as compared to controls, which increased along with the concentrations.

Figure 1: Effects of *Synedrella nodiflora* Leaf Leachates on Seedling physiology of Tomato



TS = Total sugars; #Data columns are the pooled means of three replicates with standard deviation as error bars.

Figure 2: Effects of *Synedrella nodiflora* Leaf Leachates on Seedling physiology of Brinjal



TS = Total sugars; #Data columns are the pooled means of three replicates with standard deviation as error bars.

At lower concentration treatments with *Synedrella* leaf leachates there was significantly less reduction in starch content of seedlings of both vegetables in comparison to controls. However, starch content increased with higher concentrations of *Synedrella* leaf leachates. Similar observations were recorded for protein and phenolic contents of the seedlings of both test crops. This increase may be ascribed to inhibition of seed germination and non-utilization of reserved food during process[53-58]. However the increased phenolics may be due to stress created by the allelochemicals present in leaf leachates. This role of phenolic compounds as antioxidants is well explained by many workers (Figures 1 and 2).

The results of seedling physiology studies indicated the greater sensitivity of brinjal to leaf leachates of *Synedrella* than tomato. This corroborates with the results of seed germination bioassays.

The importance of physiological studies on seed germination as affected by allelopathy has been previously emphasized[53]. The results of studies on seedling physiology also indicated decreased mobilization efficiency of the reserve food materials during seed germination of seeds treated with leaf leachates.

Alterations in protein metabolism play an important role in seed germination and seedling growth, which are influenced by treatments with leachates, extracts and residues from different allelopathic plant species. Germinating seeds of tomato and brinjal exhibited an increase in protein content, which may be due to non-mobilization and non-utilization of reserve foods. The positive or negative impact of allelobiogenetic stress induced by treatments with leachates and extracts of different ferns, *Eucalyptus, Dalbergia, Parthenium* and *Andrographis* on protein content in germinating seeds of mung bean and rice is well documented[59-61]. The different allelochemicals present in the leaf leachates may act synergistically on the processes of seed germination, seedling growth and physiology of these events. Similar inhibition of growth and oxidative damage in tomato due to the effect of certain allelochemicals has been reported[62]. In order to understand the allelopathic influence of phenolics present in the leaf leachates of *Synedrella* detailed investigations were carried out with tomato and brinjal seeds from the seed germination process up to seedling growth. Phenols as secondary metabolites have a predominant role in ecophysiological investigations and often cause adverse effects on seed germination, growth, development and metabolic functioning of plants. Increased phenolic contents in leachate treated mung bean, black gram, corn, sorghum, radish, mustard, maize and rice at higher concentrations of *Terminalia, Parthenium* and *Eucalyptus* have previously been noted[63, 64].

Identification and Characterization of allelochemicals

The stimulation or inhibition of seed germination for the test crops due to allelopathic influence at either lower or higher concentrations of leachates can be ascribed to the different types of allelochemicals present in leaf leachates of *Synedrella nodiflora*. The isolation, identification and characterization of such allelochemicals revealed following results.

IR, ^{1}HNMR, ^{13}CNMR, MASS and DEPT spectral analysis were important for the determination of the molecular formula, structure and physical characters of compound **A** (Table 3) from the leaves of *Synedrella nodiflora.*

Compound **'A',** obtained as a white, crystalline solid, was purified by crystallization using ethanol as a solvent. This substance decomposed at 270ºC. The molecular formula, $C_{17}H_{28}O_5$, was determined by mass spectrometry (*m/z* = 312). The IR spectrum had characteristic bands at 1763 and 1751 (cyclic lactones) cm^{-1}. The ^{1}HNMR spectrum (Tables 4 and 5) showed the presence of downfield oxymethylene protons of a lactone ring at δ 4.36 (*t*, *J* = 8Hz, 2H), methine protons at δ 3.44 (*m*, 2H) for H-C (3' and 3"), and an upfield methine proton at δ 2.68 (broad singlet, 1H) for H-C (1a). The spectrum exhibited a doublet at δ 0.94 (*J* = 8Hz) corresponding to methyl protons at the C_3 position. Two triplets were assigned at δ 1.06 and δ 0.85 (*J* = 8Hz) for C-3a and C-5 methyl protons.

The ^{13}CNMR spectrum (Tables 4 and 5) of compound 'A' had 17 carbon atoms. Multiplicities of carbon signals were determined by a DEPT plus sequence. Compound 'A' had 5-CH, 7-CH_2, 3-CH_3 and (by difference) two quaternary carbons. The probable structure of the compound which was: 3-(5-(1-(3-methylpentyloxy) propyl)-tetrahydro-2-oxofuran-3-yl)-dihydrofuran-2(3*H*)-one. This compound has been isolated for the first time from *Synedrella* plant.

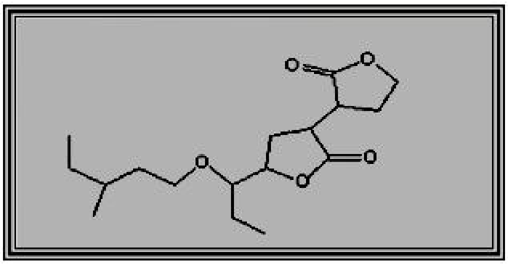
Table 4: ^{1}H-NMR spectral data of compound 'A' from *Synedrella nodiflora* leaves (500 MHz, DMSO)

Atom No.	δ ppm
H-C (5") (*t*, *J* = 8Hz, 2H)	4.36
1H (3') & 1H (3"), *m*	3.44
H-C (1a) (br *s*)	2.68
methyl protons- C3a, (*t*, *J* = 8Hz, 3H)	1.06
methyl protons- C3(*d*, J = 8Hz, 3H)	0.94
methyl protons – C5, (*t*, *J* = 8Hz, 3H)	0.85

Table 5: ^{13}C-NMR spectral data of compound 'A' from *Synedrella* leaves (125 MHz, DMSO)

Atom no.	δ ppm
CH_3-C3a	9.59, *q*
C-5	16.44, *q*
CH_3-C3	22.21, *q*
C-2a	26.70, *t*
C-4'	26.70, *t*
C-4"	26.70, *t*
C-4	32.56, *t*
C-2	35.48, *t*
C-3	37.24, *d*
C-3'	42.28, *d*
C-3"	42.28, *d*
C-5"	67.68, *t*
C-1	75.25, *t*
C-1a	81.98, *d*
C-5'	88.44, *d*
C-2'	181.39, *s*
C-2"	181.39, *s*

Structure of the compound isolated and characterised from *Synedrella* leaf



3-(5-(1-(3-methylpentyloxy)propyl)-tetrahydro-2-oxofuran-3-yl)-dihydrofuran-2(3*H*)-one

Allelochemicals with inhibitory effect on recipient plants may be leached from the invasive weed *S. nodiflora*. This allelopathic potential was confirmed by seed germination bioassay. The structure of allelochemicals in leaves was confirmed with the help of NMR, CMR, MASS, IR, DEPT [DEPT = Distorsionless Enhancement by Polarization Transfer]. Amongst all allelochemicals the detected and elucidated compound was in highest quantity as compared to other fractions. As this was identified and characterized for the first time in this plant, it has not been assigned any common name. Identification and characterization of other fractions is also in process.

Allelochemicals from the allelopathic plants belonging to the family Asteraceae such as *Mikania micrantha*[65], *Echinops echinatus*[66], *Mentha piperata*[67], *Ageratina adenophora*[68] were confirmed by using above mentioned techniques. *Synedrella* belonging to same family supports the above work[69]. The allelochemicals from different allelopathic plants other than family Asteraceae such as Euphorbiaceae (*Croton*)[70], Poaceae (*Arundo donax)*[71], Moraceae (*Ficus benghalensis*)[72], Convolvulaceae (*Ipomoea cairica*)[73], Asparagaceae (*Polygonatum odoratum*)[74].

All above allelopathy workers have also studied the allelopathic influence of plants containing allelochemicals on metabolic processes such as seed germination, nutrient uptake and assimilation, flowering, fruiting etc. They also investigated biochemical and enzymological activities during growth and development of recipient plant species[75, 76].

The present investigation was attempted to focus on seed germination process, seedling physiology of test crops tomato and brinjal when treated with leaf leachates of invasive weed *Synedrella nodiflora.* To know the allelochemical responsible for negative influence on above processes its isolation and characterization was also attempted.

CONCLUSION

The inhibitory effect on seed germination, seedling growth, vigour index and seedling physiology have confirmed the allelopathic nature of *Synedrella nodiflora* which was assigned to the complex mixture of different compounds in leaf leachates. The suppression and inhibition of different plant species naturally growing in association with *Synedrella* might be due to the same reason.

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