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Table: 2, Figs : 6, Photograph : 1

Allelopathic potential of petal leachates of *Cassia fistula* L. against an invasive weed *Alternanthera tenella* L.

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ABSTRACT

In Laboratory bioassays, the allelopathic potential of aqueous petal leachates of *Cassia fistula* L. were studied against *A. tenella*. Petal leachates of *C. fistula* at all concentrations inhibited the seed germination and seedling growth *A. tenella* in both petriplate and soil bioassays. Seed germination and seedling growth was inhibited due to the phenolic compounds (Gallic acid, Catechol and Tannic acid) except Gallic acid and Catechol in soil bioassay. Petal leachates caused adverse effects than standard phenolic compounds. The 20 % petal leachate of *C. fistula* contained (42.67 mg/100 ml) total phenolics using the GC-MS, we detected and identified following phytochemicals : 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one; 2,4,5-Trimethyl-1,3-dioxolane; Hexahydro-1,2,4,5 tetramethyl-1,2,4,5-tetrazine; Diphenyl ether; 1,1-Dimethyl-4,4-diethyl-.DELTA.[2]-tetrazene; o-Hydroxybiphenyl in petal leachates of *Cassia fistula*. Spectrophotometric analysis quantified 35.78 mg/100ml o-Hydroxybiphenyl. In petri plate and soil bioassays the o-Hydroxybiphenyl at 5, 10 and 15 ppm concentrations of *C. fistula* inhibited the seed germination and seedling growth of *A. tenella*.

Key words: *Alternanthera tenella*, *Cassia fistula*, GCMS, laboratory bioassay, petal leachate, phenolic compounds, phytochemical screening, phytosociology, seed germination, seedling growth

INTRODUCTION

The agricultural lands, disturbed habitats, metropolitan ecosystems and ignored habitats are infested by invasive weeds due to their wider adaptations, ecological amplitude and allelopathic characters (3). Once the invasive weeds get established and developed their own monocultures impeded the growth of native plants (22). Mack *et al.* (31) have stated about the destruction of biodiversity, natural system functioning and aesthetic value of habitats. Continued use of synthetic herbicides to control weeds and to increase crop yields have led to many problems (environmental pollution, increase number of resistant weeds and contamination of water resources). Tanveer *et al*. (46) reported that in India, *Alternanthera* genus has highest number of alien invasive species [*A. paronychioides* St. Hill (Smooth joyweed), *A. philoxeroides* (Mart) Griseb, *A. pungens* Humb. Bonpl & Kunth (Khaki weed), *A. sessilis* (L.) DC and *A. tenella* Colla (Joyweed)]. The *Alternanthera* species drastically reduced the seeds germination, seedling growth and yields of crops. *A. tenella* (native to Tropical America) has widely spread due to its immense branching and vegetative propagation (42). *Cassia fistula* L., native to India and Sri Lanka is used as ornamental plant in Mauritius, India, South Africa, Mexico, China, West Indies, East Africa and Brazil (36). *Cassia fistula* flowers from March to August (1,18,20,21,40,50). Phytochemical screening of its different parts showed that it has antibacterial, antifungal, insecticidal and allelopathic properties.

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C. fistula is widely cultivated as an ornamental plant for its bright yellow flowers. The flowers were examined chemically by many workers (11,25,29,38,43,48). Luximon-Ramma *et al*. (29) have implied higher total phenolic, proanthocyanidin and flavonoid contents in flowers of *C. fistula*. Siddhurajua *et al*. (43) have indicated low antioxidant activity of methanol extract of flowers due to presence of proantioxidant chrysophanol and reducing sugars. (E)- nerolidol and 2-hexadecanone main components of flower oil reported by Tzakou *et al*. (48). Duraipandiyan and Ignacimuthu (11) have tested hexane, chloroform, ethyl acetate, methanol and water extracts of flowers for its antibacterial and antifungal activity and noticed antibacterial activity of all the extracts against *Staphylococcus aureus, S. epidermidis, Bacillus subtilis, Enterococcus faecalis* and *Pseudomonas aeruginosa*. They have isolated and confirmed 4-hydroxy benzoic acid hydrate by chromatography and X-ray crystallography techniques which exhibited antifungal activity against *Trichophyton mentagrophytes* and *Epidermophyton floccosum*. Larvicidal activity of crude extracts of flower against *Culex tritaeniorhynchus, Aedes albopictus* and *Anopheles subpictus* has been shown by Govindarajan (14).

Inflorescence of *C. fistula* Flowering Dropped petals

Photograph 1. *Cassia fistula* L. at flowering and shedding of petals stages.

Alternanthera tenella is perennial herb (45), grows erect or decumbent with persistent bracteoles. It germinates and grows in Monsoon season (May- June) and flowers and fruit from August to November (9,39). It has green leaves, flowers are in axillary and terminal dense cluster of 2-5 spikes. The sepals are 5 in number and white or pale yellow in colour and with 3- nerves, alternating with as much as long, pseudo-staminodes 3-5-toothed or lobed at apex. Perianth stamens of *A. tenella* are with 3 nerves and 5 stamens. Its bract and perianth lobes are not spinoscent. Its flowering and fruiting period is from August to November and fruits are acute or pointed at apex, brown in colour (50). It is vegetatively propagated, hence, wide spread, hence is very hard to control (42). It leaves extracts are allelopathic to rice (47) and *Cicer arietinum* (13).

Muhammed *et al.* (35) examined the allelopathic potential of *C. fistula* aqueous extracts of leaves, bark and fruits against *Lactuca sativa, Setaria italica* and *Pennisetum americanum.* They reported that *Cassia fistula* allelopathic potential may be used for weed control (35).

This study aimed to determine the effects of petal leachates of *C. fistula* on growth of *A. tenella* weed.

MATERIALS AND METHODS

Study area and plant material collection

The seeds of *A. tenella* and soil samples were collected in November, 2015 and 2016 from 5 sites from the agricultural fields in village Kerle (Taluka Karveer, District Kolhapur, Maharashtra State, India). The dropped petals of *Cassia fistula* L. were collected from the different localities of Kolhapur city from April to June, 2015 and 2016.

Table 1. GPS Co-ordinates and Elevations of Sampling areas

GPS Co-ordinates	Elevation (m)
N-16^0 45. 071’, E-$074^0$10. 635’	543
N- 16^0 45. 778’, E- 074^0 10. 038’	553
N- 16^0 46. 250’, E- 074^0 09. 701’	574
N- 16^0 46. 886’, E- 074^0 09. 404’	584
N- 16^0 47. 215’, E- 074^0 09. 357’	603

Petal leachates

Collected petals were washed with sterile distilled water to remove the dust and impurities and shade dried for 8 days at room temperature about 28 ^{0}C. Then 5, 10 and 20 g petals were soaked in 100 ml sterile distilled water for 24 h. These leachates were filtered through Whatman No.1 filter paper and used for chemical analysis and bioassays.

Total phenolics of petal leachates at 5, 10 and 20 % were quantified by treating the petal leachates with 1ml Folin-Cioclateu reagent and diluted in 1:5 proportion with distilled water. After 10 min reaction mixture was treated with 2 % NaCO3. After 2 h absorbance was recorded at 760 nm and values were expressed as mg per 100 ml (44).

To detect and identify the phytochemicals, 100 ml of 20 % petal leachate was evaporated at 40 ^{0}C on water bath and the residue was dissolved in methanol in 1:5 proportion for the analysis by GCMS.

CHEMICAL ANALYSIS

GC-MS chemical analysis was done on SHIMADZU GC-2010, GC-MS QP-2010. The column used was Restek Rtx-5 MS measuring 60 m X 0.25 mm ID thickness of 0.25 µm composed of 95 % Dimethyl polysiloxane. The carrier gas used was Helium at a flow rate of 1ml/minute. One µl sample injection volume was utilized. The oven temperature was programmed initially at 60 ^{0}C for 5 minutes then an increased to 240 ^{0}C for 5 min. Then programmed to increase up to 280 ^{0}C for 2 min @ 10 ^{0}C per min ending with 5 min. The spectrums of components were compared with NIST library. Measurement of peak areas and data processing were done by Read Time analysis software.

Quantification of o-Hydroxybiphenyl

To quantify the o-Hydroxybiphenyl, 20 % petal leachate was evaporated at 40 ^{0}C on waterbath and the residue was dissolved in methanol in 1:5 proportion. The sample was treated with the reagent Titanium Sulphate and incubated for 1 h. The absorbance was recorded at 450 nm and the value was expressed as mg per 100 ml by using calibration curve of 2-Phenylphenol (6).

BIOASSAYS

The experimental treatments consisted of 3 factors: (i). *C. fistula* petal leachates concentrations : 4 (0,5,10,20 %), (ii) Phenolic compounds concentrations: 2 (0, 20 ppm) and (iii). 2-Phenylphenol concentrations : 4 (0, 5, 10, 15 ppm).

(i). Laboratory bioassay : Petriplates (9 cms dia) were first sterilized with 90 % Ethanol. The petriplates and filter papers were sterilized by exposing to UV light for 20 min. Healthy seeds of *A. tenella* were cleaned by distilled water to remove surface dust and sterilized with 0.1% $HgCl_2$ for 5 min and rinsed 4-5 times with distilled water. Twenty sterilized seeds were sown equidistant in each petriplate lined with filter paper. As per treatment, each petri plate received 8 ml petal leachates or 8 ml sterile distilled water (control). The petri plates were kept in Laboratory at room temperature (24-28 ^{0}C) under natural light and dark cycles. Numbers of seeds germinated were recorded at 24, 48 and 72 h after sowing. Root and shoot lengths were recorded at 120 h after sowing.

(ii). Soil bioassay : The soil bioassays were done in plastic trays (22 cm x 17 cm x 4.2 cm) filled with 750 g dry soil. First soil was moistened with 100 ml of distilled water. Thirty sterilized seeds of *A. tenella* were sown at 2-3 cms depth in soil in equidistant rows. As per treatment, 50- ml petal leachates (5, 10, 20 %) or 50 ml sterile distilled water for control were applied to each tray on alternate days for 5 days. Root length and shoot length were recorded 10 days after sowing.

(iii). Chemical compounds bioassay : The effects of phenolic compounds and the identified compounds were studied separately on seed germination and seedling growth of *A. tenella*. For positive control assay, 20 ppm solutions of Gallic acid (Thomas Baker, $C_7H_6O_5H_2O$ and M.W. 188.14), Catechol (Thomas Baker, $C_6H_6O_2$ and M.W. 110.1) and Tannic acid (Qualigens, $C_{76}H_{52}O_{46}$ and M.W.1701.206) along with 5, 10 and 20 % petal leachates were applied in both petriplate and soil bioassays.

(a) **Petriplate bioassay:** In Petriplates (9 cms dia), 20 healthy and sterilized seeds (with 0.1% $HgCl_2$) were sown in sterilized petriplates lined with sterilized filter paper. Eight ml Gallic acid, Catechol and Tannic acid of 20 ppm concentrations and 8 ml petal leachates (5,10 and 20%) were applied per petri plate and in control 8 ml sterile distilled water was used.

(b) **Soil bioassay**: In plastic trays (22 cm x 17 cm x 4.2 cm), 30-sterilized seeds were sown at 2-3 cms depth in equidistanct rows. Fifty ml of 20 ppm Gallic acid, Catechol and Tannic acid and 50 ml of 5,10 and 20 % petal leachates were applied as per treatments and 50 ml of sterile distilled water was used for control.

(iv) o-Hydroxybiphenyl : The effects of o-Hydroxybiphenyl (2-Phenylphenol, Sigma-Aldrich, $C_{12}H_{10}O$ and M.W. 170.21) at 5, 10 and 15 ppm concentrations were tested separately on germination and seedling growth of *A. tenella* using both petriplate and soil bioassays.

(a) **Petriplate bioassay :** In sterilized petriplates (9 cms dia) with sterilized filter paper 20 healthy and sterilized seeds were placed equidistant and asper treatments 8 ml of 5, 10 and 15 ppm of o-Hydroxybiphenyl and 8ml of sterilized distilled water for control were applied.

(b) **Soil bioassay**: Thirty sterilized seeds of *A. tenella* were sown in plastic trays (22 cm x 17 cm x 4.2 cm) at equidistant rows at 2-3 cms depth. Fifty ml of 5, 10 and 15ppm of o-Hydroxybiphenyl for treatment and 50ml of sterilized distilled water for control were applied.

The inhibition or stimulation (%) was calculated as per Zhang *et al*. (51).

Inhibition/Stimulation (%) = (Treatment data - Control data)/ Control data X 100.

Statistical Analysis

The mean value, Standard deviation and Standard error were derived using Microsoft Excel Software (MS office version 2010). Mean values followed by different letters (a-e) are significantly different ($p < 0.05$) using Duncan's multiple range tests by using professional SPSS software.

RESULTS AND DISCUSSION

PETAL LEACHATE

(i) Seed Germination : The petal leachate of *Cassia fistula* at 5, 10 and 20 % concentrations inhibited the seed germination of *Alternanthera tenella* (Figure 1). The inhibitory effects of petal leachates were concentration dependent. The 20 % leachates caused maximum inhibition (-84.31 %) in seed germination at 72 h.

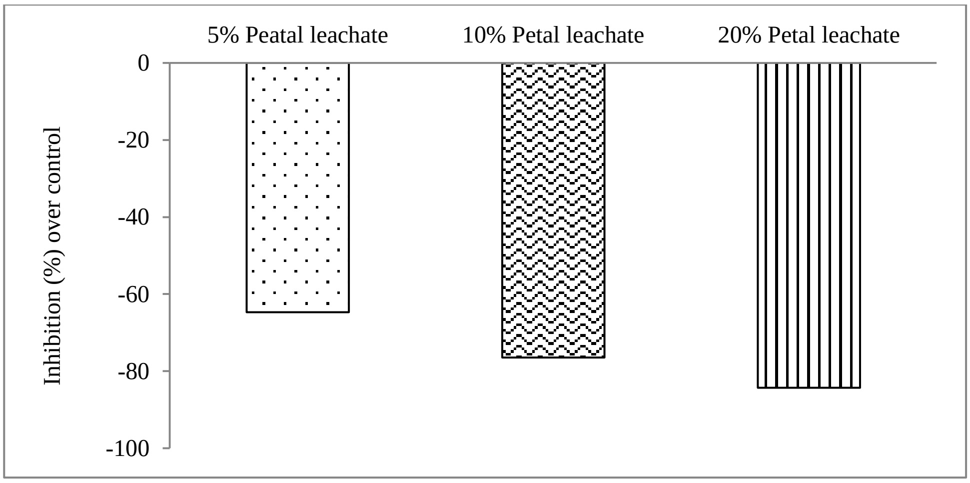

Figure 1. Effects of petal leachates of *Cassia fistula* on seed germination of *Alternanthera tenella* (Petriplate bioassay) at 72 h.

(ii) Seedling growth :

(a) Petri-plate bioassay : Petal leachates of 5,10 and 20% concentrations decreased the root length at 5 days after sowing (DAS) by 75 %, 87.5 %, 93.75 % and shoot length 5 DAS by 77.77 %, 87.40 %, 91.85 % (Figure 2).

(b) Soil bioassay : Application of petal leachates (5,10 and 20%) reduced the root length by 32.81 %, 48.43 %, 46.09 % and shoot length by 37.30 %, 46.82 %, 52.38 % at 10 days after sowing (Figure 2).

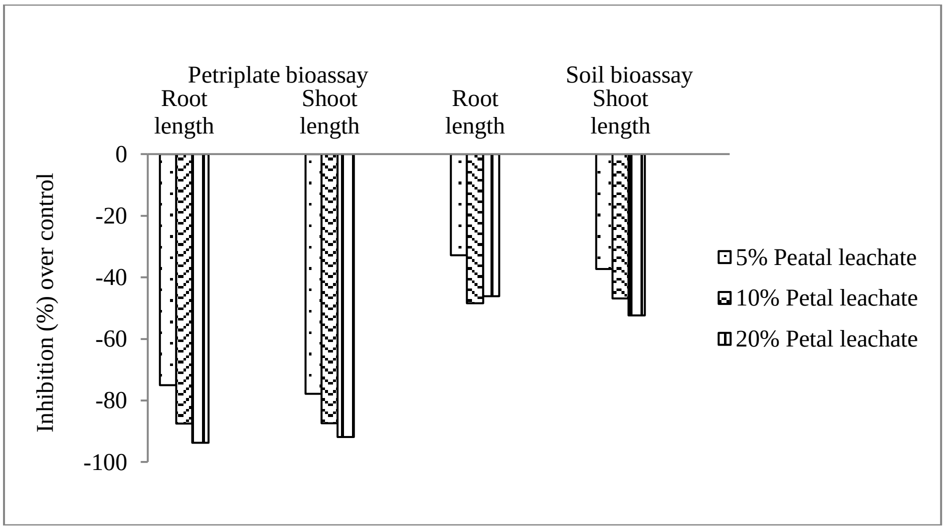

Figure 2. Effects of petal leachates of *Cassia fistula* on growth of 5 days old seedlings (Petriplate bioassay) and 10 days old seedlings (Soil bioassay) of *Alternanthera tenella*.

The use of synthetic herbicides leads to contamination of agricultural products, environmental pollution and development of herbicide resistant weeds. Under such conditions, the use of allelopathic potential of plants may provide an alternative for weed control in crops (2,10,24). Laboratory bioassays are most convenient to study the allelopathic potential of plant extracts or leachates or a compound on the seed germination, seedling growth and the morphological parameters (26,49). In present study, we determined the effects of *C. fistula* petal leachates (and its phytochemicals) on the seed germination and seedling growth of *A. tenella*. The present findings are supported by the results of workers (18,35,47). Hong *et al*. (18) the inhibitory effects of 5 % extracts of dry material of root, stem and leaves of *C. fistula* on germination, seedling growth and root length of radish. Thomas and Rizvi (47) studied the effects of 05 %, 10 %, 15 % and 20 % w/v concentrations of green leaves on extracts seed germination Percentage (SGP) and seedling vigor index (SVI)} of weedy rice variety and 4-cultivated rice varieties [AT 362 (Rosa Kekulu), BG 39/16, Samba 365/3 and Sudu 400/2]. They reported that 15 % and 20 % *C. fistula* leaf extracts, inhibited the seed germination Percentage (SGP) and seedling vigor index (SVI) of all tested varieties.

GALLIC ACID, CATECHOL AND TANNIC ACID

(i) Seed Germination : The phenolic compounds, gallic acid, catechol and tannic acid inhibited the seed germination at 72 h stages (26.08 %, 23.91 % and 34.77 %), respectively. Tannic acid was most inhibitory to seed germination at 72 h than gallic acid and catechol (Figure 3).

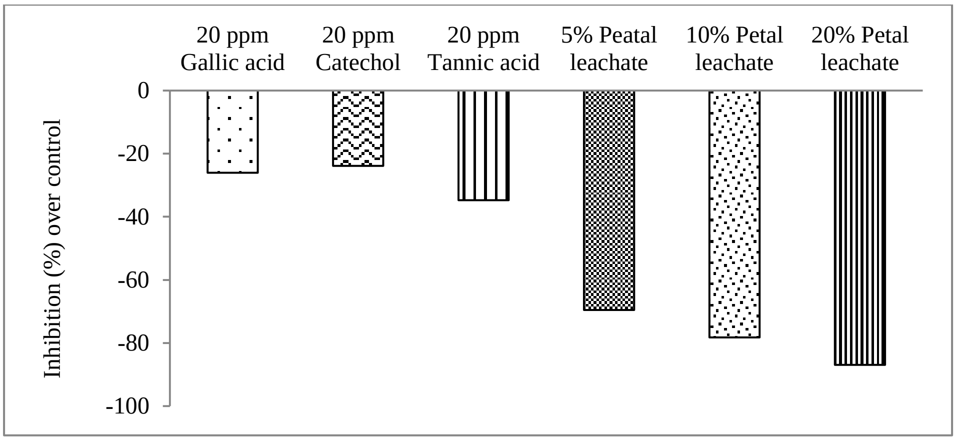

Figure 3. Effects of phenolic compounds and petal leachates of *Cassia fistula* on seed germination of *Alternanthera tenella* (Petriplate bioassay) at 72 h.

(ii) Seedling growth :

(a) Petri-plate bioassay : Gallic acid, catechol and tannic acid reduced the root length by 23.07 %, 17.30 % and 28.84 % and shoot length by 25.75 %, 17.42 % and 28.3 % in petri-plate bioassay (Figure 4).

(b) Soil bioassay: Only tannic acid suppressed the root length (12.38 %) and shoot length (11.90 %) in soil bioassay (Figure 4).

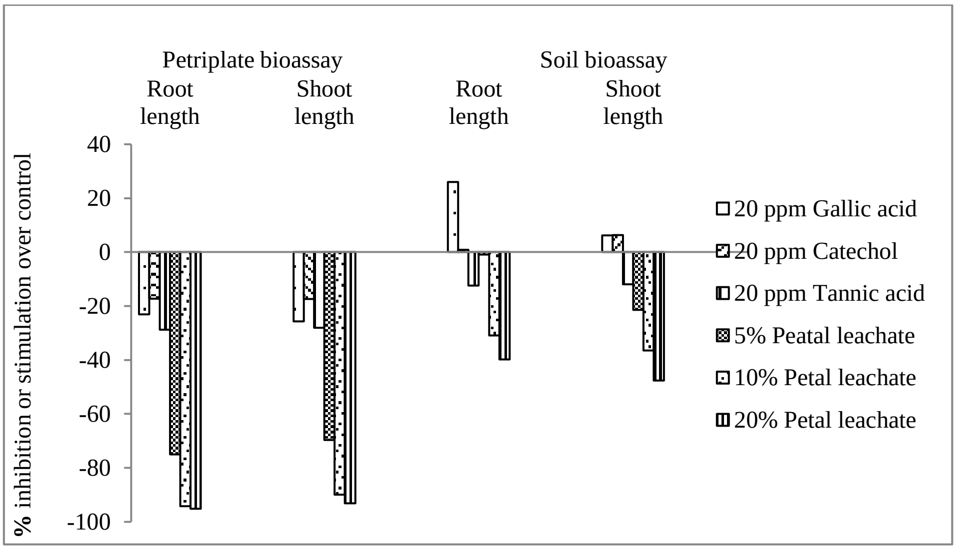

Figure 4. Effects of phenolic compounds and petal leachates of *Cassia fistula* on growth of 5 days old seedlings (Petriplate bioassay) and 10 days old seedlings (Soil bioassay) of *Alternanthera tenella*.

We have used the gallic acid, catechol and tannic acid as positive control against *Alternanthera tenella* and compared with petal leachates of *C. fistula*. Allelopathic potential of gallic acid, catechol and tannic acid has been reported (5,8,12,15,16,17,23,27,28,32,33,34,38). Muzaffar *et al*. (37) studied the phytotoxicity of gallic acid and catechol against *Cucumis sativus* L. in petriplate bioassay and reported inhibition of seed germination and growth of radicle and hypocotyl by catechol and gallic acid than control (distilled water). Chaudhuri and Ray (7) have used tannic acid (0.05, 0.2, 0.4, 0.6, 1 mg/ml concentrations) as positive control to examine growth retardation pattern of wheat seedlings with successive solvents (petroleum ether, chloroform, ethyl acetate, methanolic, aqueous) extract fractions of leaves of *Ampelocissus latifolia* (Roxb.) Planch. Tannic acid caused drastic reduction (93.76 %) of root growth of wheat at 1 mg/ml after 48 h in a dose dependent manner with inhibition. Allelochemicals can be applied as an alternative to synthetic herbicides, as they are not toxic or has no residual effects like herbicides (4). Sustainable development of agriculture include use of allelopathic effects in agricultural production and reduce the use of chemical pesticides (19,30). It is crucial to imply synthetic source of allelochemical to study its mode of action and to set up a basic structure- activity profile for its activity (48).

O-HYDROXYBIPHENYL

(i) Seed Germination : At 72h, the o-Hydroxybiphenyl at 5,10 and 15 ppm concentrations suppressed the seed germination at 72 h (28.57 %, 25.71 % and 39.99 %, respectively) (Figure 5).

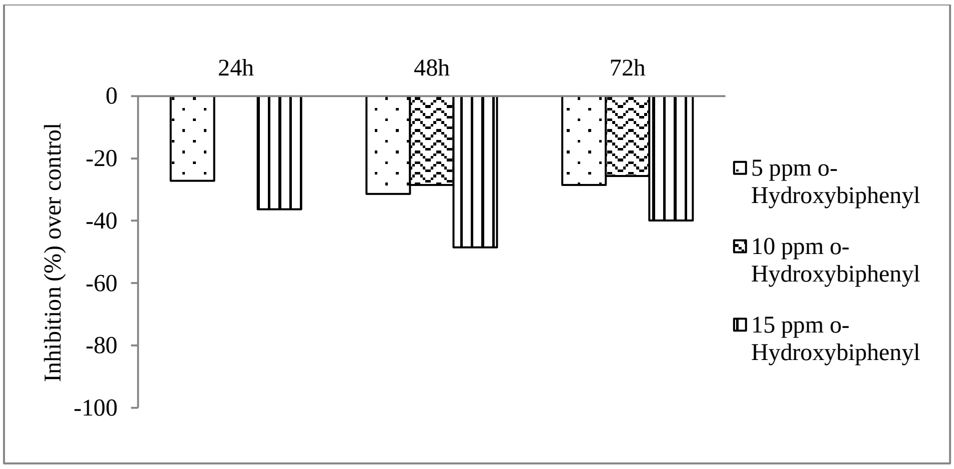

Figure 5. Effects of o- Hydroxybiphenyl on seed germination of *Alternanthera tenella* (Petriplate bioassay) at 72 h.

(ii) Seedling growth :

(a) Petri-plate bioassay : O-Hydroxybiphenyl at 5,10 and 15 ppm concentrations inhibited the seedling growth of *A. tenella*. At 15 ppm, the inhibition in root length was 32.97 % and shoot length was 58.82 % (Figure 6).

(b) Soil bioassay : The O-Hydroxybiphenyl at 5,10,15 concentrations reduced the root length (28.77 %, 33.09 % and 29.49 %) and shoot length (26.57 %, 25.87 % and 34.26 %) (Figure 6).

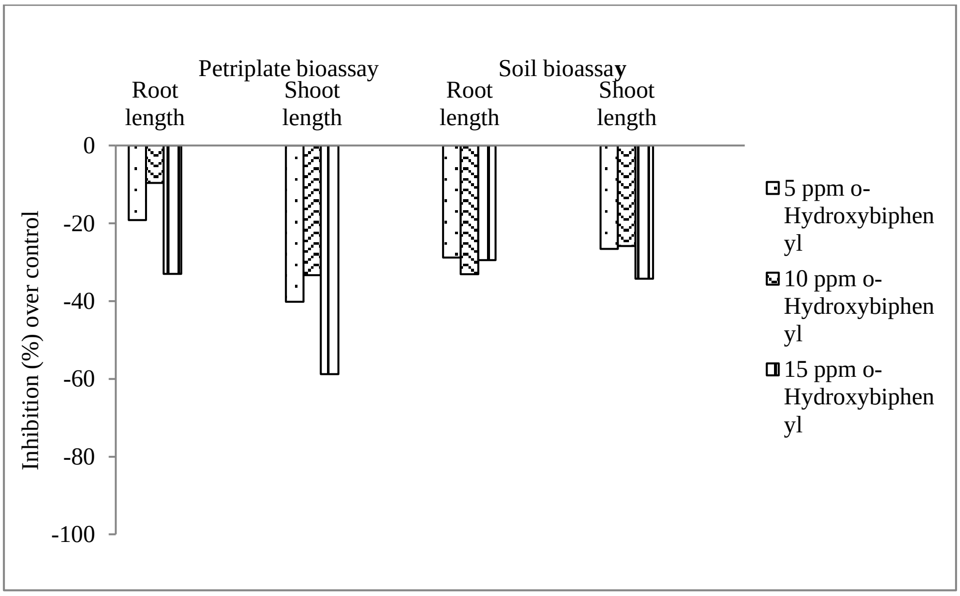

Figure 6. Effects of o- Hydroxybiphenyl on growth of 5 days old seedlings (Petriplate bioassay) and 10 days old seedlings (Soil bioassay) of *Alternanthera tenella*.

The o-Hydroxybiphenyl (o-phenylphenol) was detected in petal leachate (20%) of *C. fistula* by GC-MS. It is antifungal agent for fruits and vegetables during the post-harvest period to control their rotting during the transportation and storage (41). Hence to find herbicide potential its effect was studied on seed germination and seedling growth of *A. tenella* and was found inhibitory.

Table 2. Phytochemicals detected by GC-MS in 20 % petal leachates of *Cassia fistula*

Phytochemicals detected by GC-MS	o-Hydroxybiphenyl (mg/100ml)
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, 2,4,5-Trimethyl-1,3-dioxolane, Hexahydro-1,2,4,5-tetramethyl-1,2,4,5-tetrazine, Diphenyl ether, 1,1-Dimethyl-4,4-diethyl-.DELTA.[2]-tetrazene, o-Hydroxybiphenyl	35.78±0.85

Significant differences in Total phenolics of petal leachate s were observed.
The Total phenolics content was 18.87, 37.36 and 42.67 mg/100 ml in 5,10, 20 % petal leachates, respectively.

Petal leachate analysis

Total phenolic content was higher in 20% petal leachates (42.67 mg/100 ml) than 10 % (37.36 mg/100 ml) and 5% (18.87 mg/100 ml). Analysis of the petal leachate (20 %) by GC-MS detected and identified six compounds viz., 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one; 2,4,5-Trimethyl-1,3-dioxolane; Hexahydro-1,2,4,5 tetramethyl-1,2,4,5- tetrazine; Diphenyl ether; 1,1-Dimethyl-4,4-diethyl-.DELTA.[2]-tetrazene; o-Hydroxybiphenyl. Petal leachate (20 %) contained 35.78 mg/100 ml o-Hydroxybiphenyl by spectrophotometer quantification (Table 2).

CONCLUSIONS

Petal leachates of *C. fistula* adversely affected the seed germination and seedling growth of *A. tenella* due to presence of phytochemicals. Petal leachates were more inhibitory to seeds germination and seedlings growth of *A. tenella* weed than standard phenolic compounds. o- Hydroxybiphenyl even at low concentration inhibited the seed germination and seedling growth of *A. tenella*. Application of petal leachates after more research may provide an alternative to eradicate the invasive weed *A. tenella*.

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