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Table: 1, Figs : 9, Photograph: 1

Characterization of petal leachates of *Gliricidia sepium* (Jacq.) Kunth ex Walp for its herbicidal potential

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ABSTRACT

In petri plate, laboratory bioassays we studied the herbicidal potential of aqueous leachates of petals of *Gliricidia sepium* (Jacq.) Kunth ex Walp against the weed *Alternanthera tenella*. Colla. The petal leachates at all concentrations completely inhibited the seed germination of *A. tenella* Colla at 24 h. The reduction in root length and shoot length was concentration dependent. Seed germination and seedling growth of *A. tenella* was inhibited due to presence of phenolic compounds (Gallic acid, catechol and tannic acid) in the *G. sepium* petals. As compared to standard phenolic compounds, the petal leachates caused adverse effect on seed germination, root length and shoot length of *A. tenella* Colla in both petriplate and soil bioassays. In 20 % petal leachate, appreciable amount of total phenolics (24.12 mg per 100 ml) and phytochemicals Hydrocoumarin, 3-(2- Hydroxyphenyl) propionic acid, Diphenyl ether and Hydroxybiphenyl were detected and identified by GC-MS. The RP-HPLC technique quantified the final content of 28.91 mg/100ml for 3-(2-Hydroxyphenyl) propionic acid and 43.82 mg/100ml for Hydrocoumarin. Both 3-(2-Hydroxyphenyl) propionic acid and Hydrocoumarin at low concentrations (5, 10 and 15 ppm) adversely affected the seed germination and seedling growth of *A. tenella* Colla. However, except 10 ppm 3-(2-Hydroxyphenyl) propionic acid in soil bioassay slightly increased the root length and shoot length.

Key words: *Alternanthera tenella* Colla, GCMS, *Gliricidia sepium* (Jacq.) Kunth ex Walp, herbicidal potential, HPCL, laboratory bioassay, phenolic compounds, petal leachate, root length, RP HPLC, seed germination, seedling growth.

INTRODUCTION

In Asian countries weeding is done manually but now due to high wages of farm labours, manual weeding has been substituted by chemical weeding (1). But it has caused problems of environmental development of herbicidal resistance in weeds. Thus due to negative effects of herbicides, allelopathy, a module of integrated weed management using the allelochemicals has shown potential. *Gliricidia sepium* (Jacq.) Kunth ex Walp (family Leguminosae) is fast growing, due to its vital and repetitive re-sprout capacity after cutting (18). *Gliricidia* is propagated by cuttings or by seeds , it can establish itself in low fertile soil and also having drought tolerant competence (17). The chemical composition of its leaves, fruits, bark and heartwood is known (3,8,9,11,13,14). The leaf lopping of *G. sepium* are used against Coconut root grab (*Leucopholis coneophora* Bur.) and its leaf extract against insect pests in bitter gourd and brinjal (6, 7). Some workers have reported the allelopathic potential of *G. sepium* on seed germination and seedling growth of *Sorghum vulgare* L. (12), radicle elongation of *Lactuca sativa* L. (19), maize and cowpea seedlings (20) Kamara *et al*. (10) reported that *G. sepium* (Jacq.) Kunth ex Walp is popular tree in agroforestry and its mulch reduced the weeds density and biomass. The petals of

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Gliricidia are shed from December to May and at the time of shedding of petals, no vegetation was observed under its canopy. Up till now, not much work has been done on chemical composition of petals and its allelopathic potential. Hence this study aimed to do phytochemical screening of petal leachates and determine its herbicidal potential against an invasive weed *Alternanthera tenella* Colla.

Photograpgs *Gliricidia sepium* in flowering period and shedding of its petals

MATERIALS AND METHODS

The dried and dropped petals of *Gliricidia sepium* (Jacq.) Kunth ex Walp were collected from different trees in our city in December, 2015. Soil samples and seeds of *Alternanthera tenella* Colla were collected from village Kerle (Tehsil Karveer, District Kolhapur) from the agricultural fields in November, 2015 and 2016.

Petal leachate

To remove moisture content, petals were dried in shade for 8 days. To prepare the aqueous leachates and remove the impurities and dust etc. petals were washed 4-5 times with sterile distilled water. Then 5, 10 and 20 g petals were soaked in 100 ml of sterile distilled water for 24 h. After filtering through Whatman No. 1 filter paper, it was used for bioassays etc.

Total phenolics of petal leachates were estimated by mixing with Folin-Cioclateu reagent and 2% Sodium carbonate (16). The values were expressed as mg GA per 100ml.

To detect, identify and quantify the phytochemicals from petal leachates, 20% petal leachate was evaporated at 40^0C and residue was dissolved in methanol in 1: 5 proportions and analyzed by using GC-MS and HPLC techniques. Care was taken to avoid vapours and direct contact with petal leachates.

CHEMICAL ANALYSIS

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

HPLC technique

Petal leachate analysis for 3-(2-Hydroxyphenyl) propionic acid was carried out in JASCO (Tokyo, Japan) High Performance Liquid Chromatography (HPLC) equipment with an Ultraviolet detector (UV) set at 280nm. All chromatograms were generated by ChromNav Software (JASCO, Japan). Method used a single HI-Bar C-18 reversed phase column (250mm X 4.6 mm, 5 μm). The injection volume of 20 μL and the flow-rate of 1 mL min-1 with Water : Acetonitrile : Acetic acid, 95:5:5 were used with isocratic elution for 60 min. The yield was calculated using the calibration curve of standard 3-(2-Hydroxyphenyl) propionic acid of the chromatogram.

Analysis of Hydrocoumarin was done by using same equipment with an ultraviolet detector (UV) set at 276nm. All chromatograms were generated by ChromNav Software (JASCO, Japan). Method used a single HI-Bar C-18 reserved phase column (250mm X 4.6 mm, 5 μm). The injection volume of 20 μL and the flow-rate of 1 mL min-1 with Water: Methanol, 30:70 were used with isocratic elution for 15 minutes. The yield was calculated by using the calibration curve of standard Hydrocoumarin of the chromatogram.

BIOASSAY

Bioassay I: We did bioassay in petriplates and with soil. Petriplates (9 cm dia) were first sterilised with 90% Ethanol and kept under UV light for 20 min. Healthy seeds were washed with sterile distilled water to remove surface impurities, then treated with 0.1% $HgCl_2$ for 5 min and rinsed with sterile distilled water for 4-5 times. Twenty seeds were sown in each petriplate. Each petriplate received 8 ml petal leachate as per treatment and sterilized distilled water served as control. The petriplates were kept under natural12 h light/12 h dark cycles and room temperature (24^0C-28^0C). Numbers of seeds germinated were recorded at 24, 48 and 72 h and root length and shoot length of seedling were recorded at 120 h.

Bioassay II: In plastic trays (22 cms x 17 cms x 4.2 cms), 750 g dry soil was used for bioassays (per tray). Thirty healthy, sterilized seeds were sown in each tray. Fifty ml petal leachate was added as per treatment and distilled water was used in control for 5 days (alternate days). After 10 days, root length and shoot length of seedlings was noted.

Bioassay III: Effects of identified phytochemicals were studied separately on seed germination and seedling growth of *A. tenella*. The 5, 10 and 15 ppm concentrations of 3-(2-Hydroxyphenyl) propionic acid (Sigma-Aldrich, molecular formula $C_9H_{10}O_3$) and Hydrocoumarin (Sigma-Aldrich, molecular formula $C_9H_8O_2$) and for positive control 20 ppm solutions of Gallic acid (Thomas Baker, $C_7H_6O_5H_2O$), Tannic acid (Qualigens, $C_{76}H_{52}O_{46}$) and Catechol (Thomas Baker, $C_6H_6O_2$) were applied in both wet paper petriplate and soil bioassays.

The inhibition or stimulation (%) was calculated as per the equation of Zhang *et al*. (23).

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). Means followed by different letters (a-j) are significantly different ($p< 0.05$) using Duncan's multiple range test by using SPSS software. LSD is calculated by using Microsoft Excel Software (MS office version 2010).

RESULTS AND DISCUSSION

SEED GERMINATION AND SEEDLING GROWTH

Petriplate bioassay :

Petal leachate : The petal leachates of *G. sepium* inhibited seeds germination of *A. tenella* at all concentrations at 24, 48 and 72 h (Figure. 1). The inhibitory response of petal leachate on seed germination was concentration dependent. The 5, 10 and 20 % petal leachate at 24 h showed 100 percent inhibition of the seeds germination. The petal leachates at all concentrations inhibited the seedling growth (root length and shoot length) (Figure. 2). The maximum inhibition in root length (-78.21%) and shoot length (-57.3%) was with 20% petal leachate.

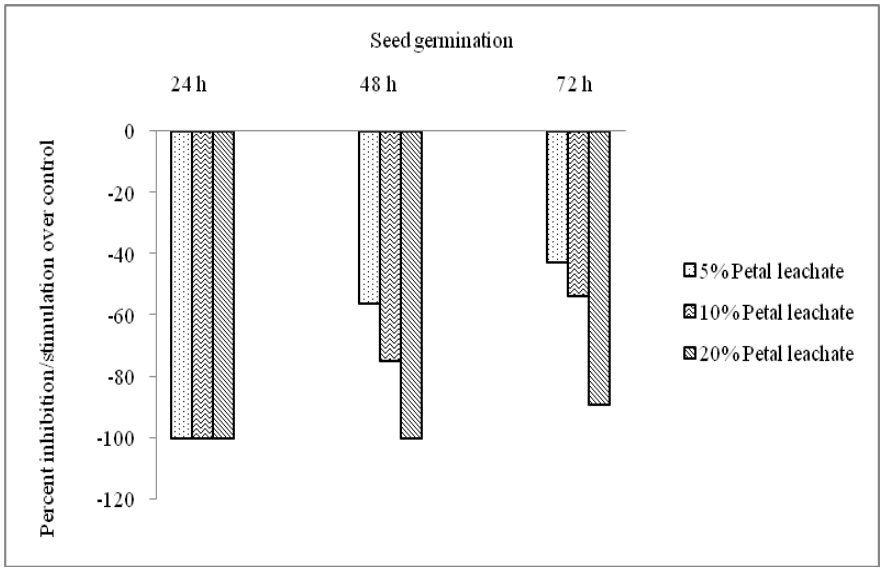

Figure 1. Effects of petal leachates of *G. sepium* on seed germination of *A. tenella* (Petriplate bioassay) Values are mean of three replicates

Soil bioassay : The petal leachates at 5%, 10% and 20% concentrations inhibited the root length and shoot length of 10 days old seedlings than control (Figure. 2). The inhibition pattern of seedling growth was not dependent on concentrations of petal leachate concentrations. In case of root length 20% petal leachate was more inhibitory with -46.47% and in case of shoot length 10% was more inhibitory with -22.43%.

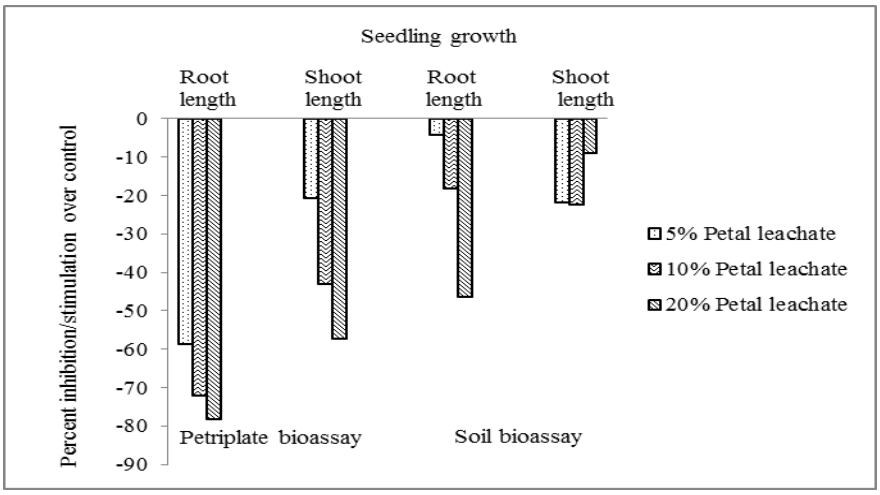

Figure 2. Effects of petal leachates of *G. sepium* on growth of 5 days old seedlings (Petri plate bioassay) and 10- days old seedlings (soil bioassay) of *A. tenella*

Gallic acid, Catechol and Tannic acid

Petriplate bioassay

All the standard phenolic compounds inhibited the seed germination of *A. tenella* Colla at 24, 48 and 72 h stages (Figure 3). Gallic acid, catechol and tannic acid reduced seedling growth relating to root length and shoot length, with 20 ppm tannic acid causing more decline in both root (-22.01%) and shoot length (-10.51%) (Figure 4).

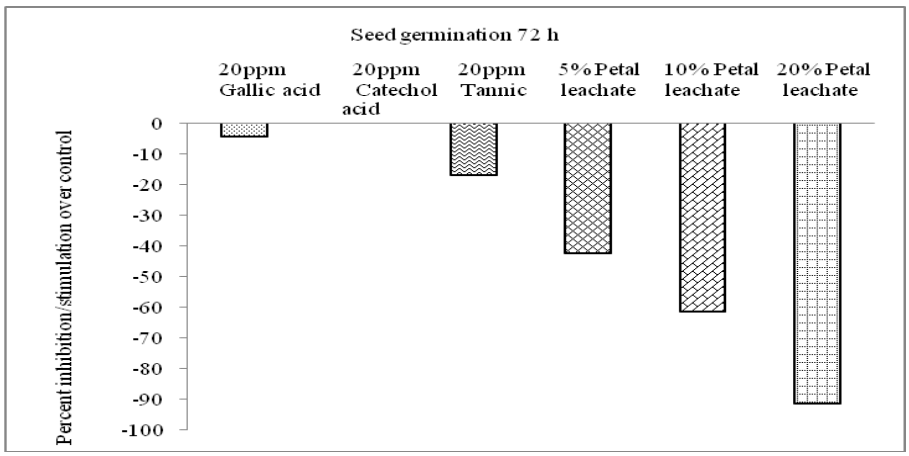

Figure 3. Effects of standard phenolic compounds and petal leachate of *G. sepium* on seed germination of *A. tenella* (Petriplate bioassay)

Soil bioassay: The gallic acid, catechol and tannic acid suppressed the root length and shoot length also in soil bioassay (Figure 4).

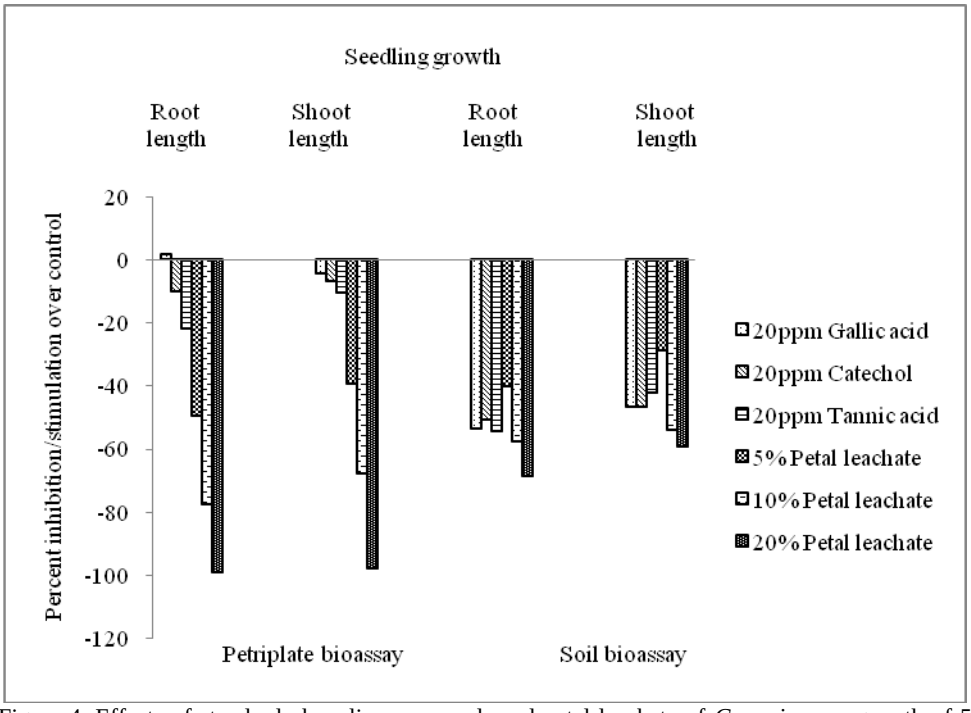

Figure 4. Effects of standard phenolic compounds and petal leachate of *G. sepium* on growth of 5 days old seedlings (Petri plate bioassay) and 10- days old seedlings (soil bioassay) of *A. tenella*

3-(2- Hydroxyphenyl) Propionic acid and Hydrocoumarin

The effects of phytochemicals identified and quantified from petal leachates of *G. sepium* on seed germination and seedling growth were studied separately. Different concentrations (5, 10 and 15 ppm) of 3-(2-Hydroxyphenyl) propionic acid inhibited the seed germination (at 24, 48 and 72 h) of *A. tenella* with more adverse effect due to 15 ppm with -50% and 29.79% at 24 h and 48 h and 72 h respectively. (Figure 5).

In petriplate bioassay 3-(2-Hydroxyphenyl) propionic acid at 15 ppm concentration affected root growth (-38.23%) adversely than shoot growth (-26.67%). Reduction in root length and shoot length was concentration dependent (Figure 6). The treatment of 3-(2-Hydroxyphenyl) propionic acid caused decrease in root length and shoot length except 10 ppm concentration which brought about the increase in root length with 4.28% and shoot length with 1.44% in soil bioassay (Figure 6).

Hydrocoumarin at 5, 10 and 15 ppm concentrations inhibited the seed germination at 24 and 48 h and at 72 h stage in petriplate bioassay (Figure 7). The Hydrocoumarin at 10 ppm concentration did not have consistent effect as compared to 5 ppm and 15 ppm concentrations (Figure 8). Hydrocoumarin at all concentrations reduced the root length

and shoot length with more inhibition, -28.83% in root length and -22.27% in shoot length at 15 ppm concentration in soil bioassay (Figure 8).

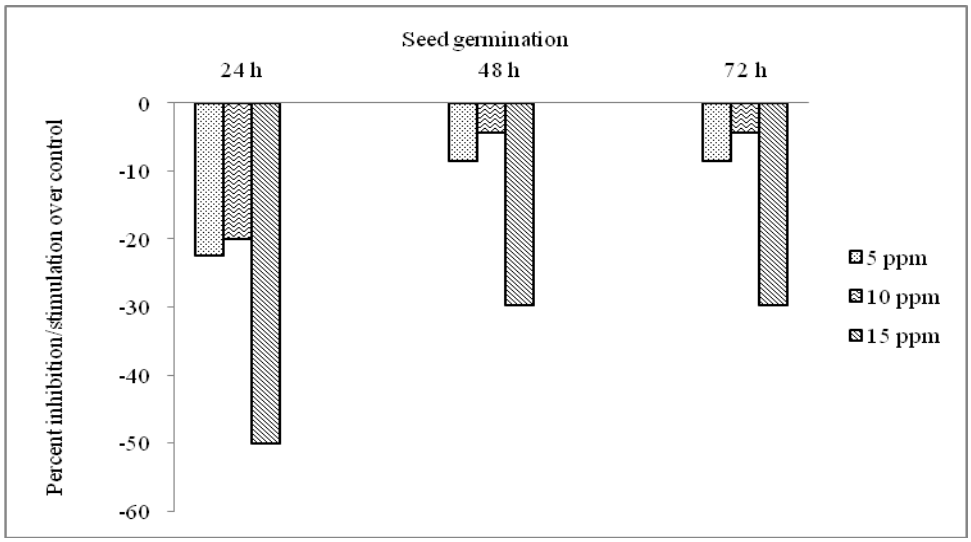

Figure 5. Effects of 3-(2-Hydroxyphenyl) propionic acid on seed germination of *A. tenella* (Petriplate bioassay)
Values are mean of three replicates

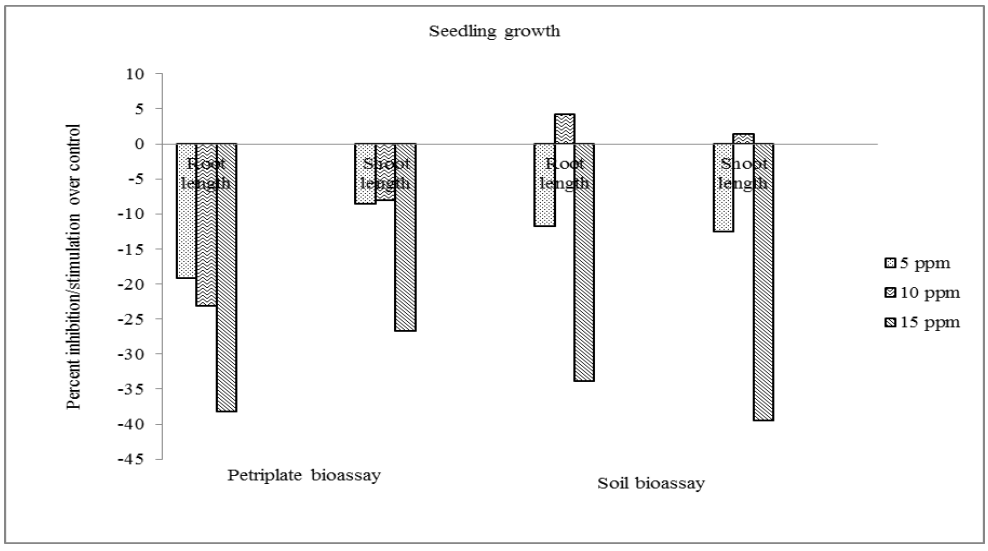

Figure 6. Effects of 3-(2-Hydroxyphenyl) propionic acid on seedling growth of 5 days old seedlings (Petri plate bioassay) and 10- days old seedlings (soil bioassay) of *A. tenella*

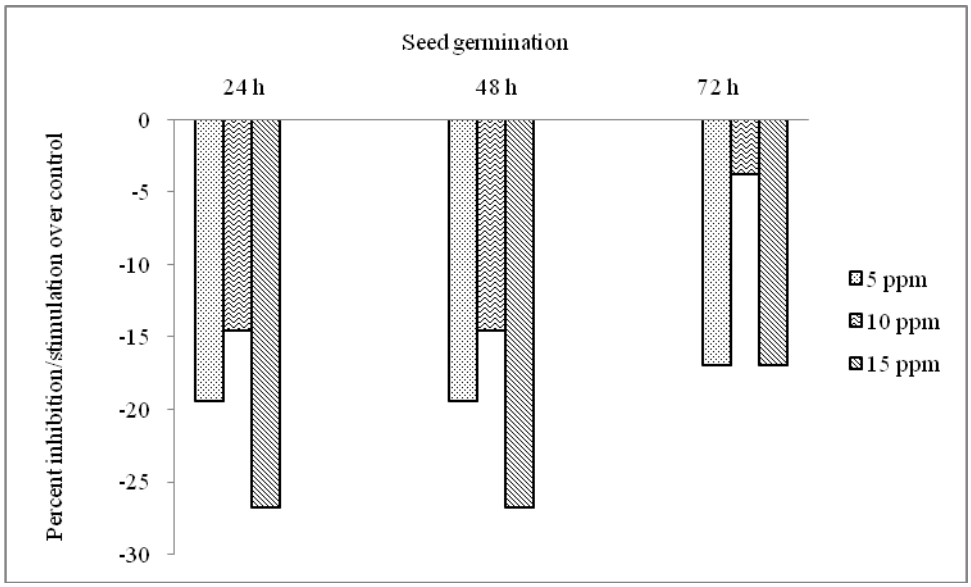

Figure 7. Effects of Hydrocoumarin on seed germination of *A. tenella* (Petriplate bioassay)
Values are mean of three replicates

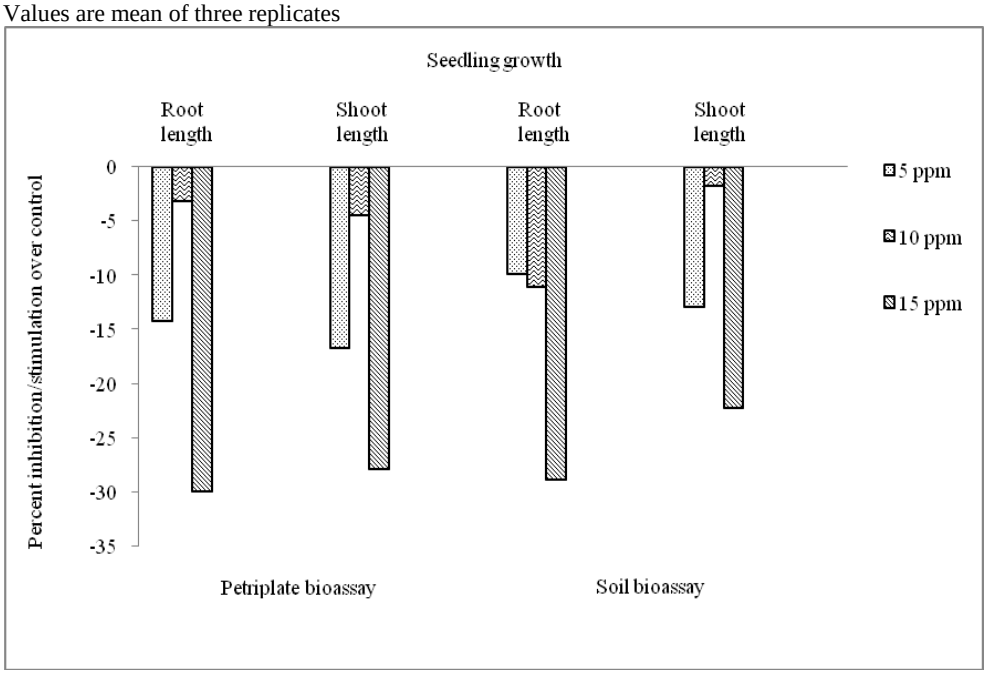

Figure 8. Effects of Hydrocoumarin on seedling growth of 5 days old seedlings (Petri plate bioassay) and 10- days old seedlings (soil bioassay) of *A. tenella*

The allelochemicals inhibits the seed germination and reduced the shoot and root growth (5,22). In an ecosystem detrimental effects of allelochemicals decreased and delayed the seed germination and caused seedling mortality (4). As seed germination and seedling growth are frequently evaluated by the morphological parameters to study the allelopathic potential, in present study we also tried to assess seed germination and growth performance under the influence of petal leachates and phytochemicals. Present findings are in accordance with the reports, where seed germination and seedling growth are the evaluated parameters under the influence of extracts and leachates of leaves and mulches of *G. sepium*, Tian and Kang (20) have reported significant decrease in growth of maize seedlings due to leachates of *Gliricidia* prunings. Takemura *et al*. (19) observed inhibition of growth of radicle of seedlings of lettuce due to methanolic extracts of leaves of *G. sepium* and crude extract containing 9.7 µmol/L coumarin inhibited radicle growth by 50%. Ramamoorthy and Paliwal (12) have tested different fractions of crude extract of leaves of *Gliricidia sepium leaves* against the *Sorghum vulgare* and found that germination and root elongation of test crop was inhibited by all fractions. They have also reported that application of 800 g of *Gliricidia* leaf/ m^2 showed better crop yield among 400, 800 and 1200 g/m^2 doses. Silva *et al*. (15) reported intercropping with *Gliricidia* reduced weed population without affecting corn and bean growth.

Topala *et al*. (21) have studied herbicidal potential of Catechol against weed species Poppy (*Papaver rhoeas*), creeping thistle (*Circium arvense*), Henbit (*Lamium amplexicaule*) and wild mustard (*Sinapis arvensis*) along with 2,4-D and found rhat the 13.64mM of Catechol was as effective as 2,4-D to kill field poppy and also having suppressive herbicidal effect on remaining weeds by inhibiting their growth. Hegab *et al*. (2) have noticed the herbicidal potential of Gallic acid on *Amaranths hybridus, A. lividus, Avena sterilis, A. fatua, Cichorium endivia, Convolvulus arvensis, Corchorus olitorius, Cynodon dactylon, Cyperus rotundus, Echinichloa colona, Euphorbia helioscopia, Euphorbia prostate, Melilotus indicus, Hibiscus trionum, Panicum repens, Paspalum distichum, Phalaris paradoxa* and *Portulaca oleracea.*

Petal leachate analysis

The petal leachates of all concentrations (5, 10 and 20 %) were analyzed to determine their total phenolics. The higher content of phenolics was found in 20 % petal leachate (24.12 mg/100 ml) than 10 % (18.48 mg/100 ml) and 5 % (13.36 mg/100 ml) (Table 1). The GC-MS analysis of petal leachate of *G. sepium* was detected and identified total 4 phytochemicals [Hydrocoumarin, 3-(2-Hydroxyphenyl) propionic acid, Diphenyl ether and Hydroxybiphenyl] in petal leachate (Table 1). Among these 4 phytochemicals, quantitative determination of 3-(2-Hydroxyphenyl) propionic acid and Hydrocoumarin was done using RP-HPLC method and results were expressed as mg per 100 ml with Criterion of Correlation (R2) value equal to 0.9998 for 3-(2-Hydroxyphenyl) propionic acid and Hydrocoumarin. Final output of 3-(2-Hydroxyphenyl) propionic acid was 28.91 mg/100 ml and 43.82 mg/100 ml for hydrocoumarin (Table 1).

Table 1. Chemical analysis of petal leachates of *G. sepium*

Petal leachates	Total Phenolics (mg/100ml)	Phytochemicals detected by GC-MS (20% aqueous petal leachate)	Phytochemicals quantified by HPLC (20% aqueous petal leachate)
5% Petal leachate	13.36± 0.204*	Hydrocoumarin Retention time in minute-17.600 Area%- 13.44 3-(2-Hydroxyphenyl) propionic acid Retention time in minute-17.600 Area%- 13.44 Diphenyl ether Retention time in minute-17.742 Area%-5.78 Hydroxybiphenyl Retention time in minute-17.742 Area%-5.78	Hydrocoumarin Retention time in minute-5.850 Area%- 0.294 43.82 mg/100ml 3-(2-Hydroxyphenyl) propionic acid Retention time in minute-42.775 Area%- 17.499 28.91 mg/100ml
10% Petal leachate	18.48± 0.126*		
20% Petal leachate	24.123± 1.012*		

*All values are means of three replicates ± Standard error

The chemical composition of various parts of *G. sepium* has been reported by many workers and various compounds have been identified and quantified using the GC-FID, GC-MS, HPLC. The compounds,12a-Hydroxyrotenoids like gliricidol, 2-methoxyglirici dol and gliricidin were isolated and identified from bark (14). Two saponins gliricidoside A and B from roots as well as pterocarpon, 2 isoflavans and flavan glycosides from leaves were isolated by Rastrelli *et al*. (14). Three hederagenin- based saponins, hederagenin-3-O-(4-O-acetyl-fi-D-xylopyranosyl)-(1~3)-:~-e-rhamnopyranosyl-(l~2)-~-L-arabinopyrano-side, hederagenin-3-O-(3,4-di-O-acetyl-fl-D-xylopyranosyl)-(1--*3)-c~-t-rhamnopyrano syl-(1---,2)-e-t-arabinopyranoside and hederagenin-3-O-(3,4-di-O-acetyl-~-L-arabinop-yranosyl)-(l ~ 3)-c~-L-rhamnopyranosyl-(l---,2)-~-L-arabinopyranoside were isolated from fruits of *G.sepium* (11). A phenolic Iso-Flavan-3- ene and two new isoflavenes, viz. 2',3',7-trihydroxy-4'-methoxyisoflav-3-ene(sepiol)and 3',7-dihydroxy-2',4'-dimethoxyiso-flav-3-ene (2'-0-methylsepiol), and a new phenolic isoflavan, robinetin, and 7,3',4'-trihydroxyflavanone have been reported from extract of heartwood of *G. sepium* (8,9). Our results corroborate the findings of Ramamoorthy and Paliwal (12) who quantified and identified 15 compounds (gallic acid, protocatechuic acid, p-hydroxybenzoic acid, gentisic acid, β-resorcyclic acid, vanillic acid, syringic acid, p-coumaric acid, m-coumaric acid, o-coumaric). In bioassays against *Sorghum vulgare*, the compounds in the extract decreased the seeds germination and root elongation. Takemura *et al*. (19) identified the coumarin from methanolic extract of *G. sepium* leaves, it was very inhibitory to lettuce radicle growth. In our study also, the hydrocoumarin showed high inhibitory activity than 3-(2-

Hydroxyphenyl) propionic acid. In bioassays against *Sorghum vulgare*, the compounds in the extract decreased the seeds germination and root elongation. Takemura *et al*. (19) identified the coumarin from methanolic extract of leaves of *G. sepium*, it was very inhibitory to lettuce radicle growth. In our study also, the hydrocoumarin showed high inhibitory activity than 3-(2-Hydroxyphenyl) propionic acid.

CONCLUSIONS

The petal leachates of *G. sepium* inhibited the seed germination and seedling growth of *A. tenlla* due to the presence of different phytochemicals. The petal leachates were more inhibitory to germination, root length and shoot length than standard phenolic compounds. The adverse effects of 3-(2- Hydroxyphenyl), propionic acid and hydrocoumarin present in the *G. sepium* leachate on *A. tenella* showed the herbicidal potential of *G. sepium* leachate. Application of bioleachates of *G. sepium* may help to control the invasive weed *A. tenella*.

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REFERENCES

1. Chauhan, B.S., Ahmed, S. and Awan, T.H. **(**2015). Performance of sequential herbicides in dry-seeded rice in the Philippines. *Crop Protection* **74:** 124-130.
2. Hegab, M.M., Erdei L. and Abdelgawad, H. (2016). Isolation and phytotoxicity of an active fraction and its pure compound (gallic acid) from sun spurge (*Euphorbia helioscopia* L.) against harmful weeds. *Acta Biologica Szegediensis* **60**(1): 17-25.
3. Herath, H.M.T.B., Dassanayake, R.S., Priyadarshani, A.M.A., De Silva, S., Wannigama, G.P. and Jamie, J. (1998). Isoflavonoids and a pterocarpan from *Glyricidia sepium*. *Phytochemistry* **47**: 117-119.
4. Herro, J.L. and Callaway, R.M. (2003). Allelopathy and exotic plant invasion. *Plant and soil* **256**: 29-39.
5. Jabran, K., Mahajan, G., Sardana, V. and Chauhan, B.S. (2015). Allelopathy for weed control in agricultural systems. *Crop Protection* **72**: 57-65.
6. John, J., Sreekumar, K.M., Rekha, P. and Abhilash, E.K. (2004). Allelopathic effects of leaf loppings of multipurpose trees and cashewnut shell liquid to control coconut root grub (*Leucopholis coneophora* Bur.). *Allelopathy Journal* **14**: 221-226.
7. John, J., Leela, N.K., Sreekumar, K.M., Anesh, R.Y. and Hema, M. (2007). Phytotoxicity of leaf extracts of multipurpose trees against insect pests in bitter gourd (*Momordica charantia*) and brinjal (*Solanum melongena*). *Allelopathy Journal* **20**: 411-417.
8. Jurd, L. (1976). A phenolic isoflav-3-ene from *Glyricidia sepium*. *Tetrahedron Letters* **21**: 1741-1744.
9. Jurd, L. and Manners, G.D. (1977). Isoflavene, isoflavane and flavonoid constituents of *Glyricidia sepium*. *Journal of Agricultural and Food Chemistry* **25**: 723-726.
10. Kamara, A.Y., Akobundu, I.O., Chikoye, D. and Jutzi, S.C. (2000). Selective control of weeds in an arable crop by mulches from some multipurpose trees in Southwestern Nigeria. *Agroforestry Systems* **50**: 17-26.
11. Kojima, K., Zhu, X.B. and Ogihara, Y. (1998). Saponins from *Glyricidia sepium. Phytochemistry* **48**: 885-888.
12. Ramamoorthy, M. and Paliwal, K. (1993). Allelopathic compounds in leaves of *Glyricidia sepium* (Jacq.) Kunth ex Walp. and its effect on *Sorghum vulgare* L. *Journal of Chemical Ecology* **19**: 1691-701.
13. Rastrelli L., Berger I., Kubelka W., Caceres A., De Tommasi N. and De Simone F. (1999). New 12a-Hydroxyrotenoids from *Gliricidia sepium* Bark. *Journal of Natural Product* **62**: 188-190.

14. Rastrelli, L., Caceres A., De Simone, F. and Aquino, R. (1999). Studies on the constituents of *Glyricidia sepium* (Leguminosae) leaves and roots: Isolation and structure elucidation of new triterpenoid saponins and aromatic compounds. *Journal of Agricultural and Food Chemistry* **47**: 1537-40.
15. Silva, P.S.L. Cunha, T.M.S., Oliveira, R.C., Silva, K.M.B. and Oliveira, O.F. (2009). Weed control via intercropping with glyricidia. II. corn crop. *Planta daninha* **27**: 105-112.
16. Slinkard, K. and Singleton, V.L. (1977). Total phenol analysis: Automation and comparison with manual method. s*Journal of Enology and Viticulture* **28**: 49-55.
17. Solangi, A.H., Mal, B., Kazmi, A.R. and Iqbal, M.Z. (2010). Preliminary studies on the major characteristic, agronomic feature and nutrient value of *Gliricidia sepium* in coconut plantations of Pakistan. *Pakistan Journal of Botany* **42**: 825-832
18. Stewart, J.L., Dunsdona, A.J., Kassb, M., Lopez Ortizb, S., Ortizb, A., Larbic, A., Premaratned, S., Tangendjajae, B., Winae, E. and Vargasf, J.E. (1998). Genetic variation in the nutritive value of *Glricidia sepium*:1. Acceptability, intake, digestibility and live weight gain in small ruminants. *Animal Feed Science and Technology* **75**: 111-124.
19. Takemura, T., Kamo, T., Sakuno, E., Hiradate, S. and Fujii, Y. (2013). Discovery of coumarin as the predominant allelochemical in *Glyricidia sepium*. *Journal of Tropical Forest Science* **25**: 268-272.
20. Tian, G. and Kang, B.T. (1994). Evaluation of phytotoxic effects of *Glyricidia sepium* (Jacq.) Walp prunings on maize and cowpea seedlings. *Agroforestry Systems* **26**: 249-254.
21. Topala, S., Kocacalıskana, I. and Arslanb, O. (2006). Herbicidal potential of catechol as an allelochemical. *Zeitschrift fur Naturforsch*ung **61C:** 69-73.
22. Weston, L.A. and Duke, S.O. (2003). Weed and crop allelopathy. *Critical Reviews in Plant Sciences* **22**: 367-389.
23. Zhang, E., Zhang, S. and Li, L. (2015). Effects of tomato (*Solanum lycopersicum* L.) plant part extracts, root exudate and tomato grown soil extract on seed germination and seedling growth of tomato. *Allelopathy Journal* **35**: 1-10.