Allelopathic perspectives of some plants for biocontrol of invasive alien weed: *Hyptis suaveolens* L. (Poit.)

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ABSTRACT

Vitex negundo L. (verbenaceae) and *Ricinus communis* L. (Euphorbiaceae), are especially well known for their industrial, pharmacological, and toxicological properties, but to date very little is known about their allelopathic potential. Hence the present study was conducted to determine the allelopathic effect of both the plants on seed germination, seedling growth; fresh and dry weight of *Hyptis suaveolens* (L.) Poit. which is an emerging alien invader. Various concentrations (2.5%, 5%, 7.5%, and 10%) of leaf leachates of selected plants were used to test their effect on test species. Results of present study indicated that the seed germination and early stages of growth were significantly inhibited in *Hyptis* at all concentration of aqueous leaf leachates of selected plants. The effect of *Ricinus* leaf leachates was more pronounced than that of *Vitex*. Since *Ricinus* leaf leachates had greater activity than *Vitex* against major emerging invader, this plant could be best candidate for isolation and identification of allelochemicals, which might promote the discovery of new biocontrol for invasive weeds.

Key words: Allelopathic, alien invader, *Hyptis suaveolens* L., *Vitex negundo* L., and *Ricinus communis* L.

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INTRODUCTION

Throughout the world weeds have been, are, and will continue to be a major constraint to agriculture production (National Research Council, 1996). For controlling obnoxious weeds synthetic weedicide were an effective tool, but its excessive use led to a reduction in yield, environmental pollution and increase in herbicide resistant weeds. Hence to minimize the dependency on synthetic herbicides for controlling obnoxious weeds there is a need to find natural ways (Bhadoria, 2011). For sustainable weed management the use of allelopathic behaviour is one of the new options. Allelopathy is defined as the direct or indirect detrimental or profitable effects of one plant or another through the production of biochemical compounds that escape into the environment (Rice, 1984). Phytodiversity and native species is greatly influenced by invasive exotic species (Lee and Klasing, 2004). Creating competition between native and alien species would be a good control method and it has gained momentum recently. Efforts have been made to use allelopathic potential of numerous plants in weed control (Knox, 2008). Almost all plants possess allelochemicals and present in many tissues, like leaves, stems, flowers, fruits, seeds and roots (Putnam, 1988). Allelochemicals are released into the environment through root exudation, leaching and decomposition of plant residues as they are often water soluble substances (Aminidehaghi *et al.*, 2006). According to Rice (1984), seed germination and plant growth is modified by allelopathy, and hence germination is one of the important tools for the study of allelopathy. Allelopathy plays a significant role in agro-ecosystem, and affects the growth and quantity of the products by the interactions among crops, weeds and trees. Allelochemicals produced by alien plants significantly affect the native plants irrespective of whether a native species produces allelochemicals or not (Msafiri *et al*., 2013).

The extent to which allelopathy of one plant affect the other plant has been worked out by several researchers using one of the other indices like germination %, inhibition %, germination capacity%, germination rate, radicle and plumule length, relative elongation ratio, fresh and dry weight of seedling, root-shoot ratio, vigour index (Netsere and Mendesil 2011; Khan *et al*.,2011; Chatiyanon *et a*l., 2012; Kavitha *et al*.,2012; Islam and Kato-Noguchi 2013; Alagesaboopathi , 2014; Desalegn T, 2014; Gulzar *et al*., 2014; Ghayal *et al*., 2014; Joshi *et al*., 2015; Pathare *et al*.,2014; Nekonam *et al*., 2014; Naz and Bano 2014; Vijayan, 2015). To investigate probable involvement of allelopathy laboratory bioassay is the first step (Foy, 1999).

For present study an alien invader *Hyptis suaveolens* L. (Poit.) was selected which is commonly known as horehound weed or mint weed, is native to tropical America, and is now a pantropic weed (Hutchinson and Dalziel, 1963; Hickey and King, 1988). The weed extends from Southern United States through Caribbean region and Central America, South to Argentina in the warmer parts of the old world. Because of *Hyptis suaveolens* regions in western and southeast Africa, southwest Asia, and north Australia might face high invasion risk (Padalia *et al*., 2015).

In India also it is invading at an alarming rate occupying almost major parts of the country. *Hyptis suaveolens* may be considered as one of the most serious invaders in the Vindhyan dry deciduous forest of India after the invasion of *Lantana camara* (Sharma *et al*. 2005; 2007; Sharma and Raghubanshi, 2006).

In recent times to control weeds either directly or as natural herbicides developed from allelochemicals isolated from allelopathic plants particularly those with medicinal properties have been gaining interest (Sodaeizadeh *et al*. 2009). It is easier to screen allelopathic plant from medicinal plants species than other plants (Fujii *et al*., 2003). But again we couldn't overlook the possibility that medicinal plants may contain more bioactive compounds than other plants. Therefore, to combat the hazardous effects of synthetic herbicides on environment allelopathic medicinal plants might be helpful to discover new natural herbicides for sustainable agriculture (Khanh *et al*., 2007).

On the basis of hypothesis that opponents who are an equal match in characters can be used as a check, few wild and weedy plants were selected to evaluate as a biocontrol or bioherbicides against *H. suaveolens*.

Ricinus communis L. is a plant belonging to Euphorbiaceae, commonly found in the tropical and temperate climates of the world (Lakshmamma and Prayaga, 2006; Raoof and Yasmeen, 2006), which is well known for many of its medicinal and industrial uses (Ogunniyi, 2006; Islam *et al*., 2011; Jena and Gupta, 2012). *Vitex negundo* L. an aromatic shrub belonging to verbenaceae widely known for its use as green manure, medicine in ayurvedic, unani systems of medicine and as a mosquito repellent.

This study was conducted to investigate the bioherbicidal potential of different concentrations of aqueous leaf leachates of *V. negundo* and *R. communis* on the seed germination and seedling growth of alien invader *H. suaveolens* and an attempt has been made to find out alternate ecofriendly approach for weed management.

MATERIALS AND METHODS

Experimental site

Experiments were conducted at the Post Graduate Teaching Department of Botany; RTM Nagpur University, Nagpur, India.

Seeds collection of test plant

Mature seeds of *Hyptis suaveolens* L. (Poit.) were collected from the University campus; RTM Nagpur University, Nagpur, India.

Collection and extraction of plant materials

The leaves of both *V. negundo* and *R. communis* were collected from Nagpur during the full stage and washed with tap water followed by shade drying. The dried leaves were grinded to powder using laboratory blender. 10g powdered leaves were soaked in 100ml distilled water for 24 h at 25^0C and the leachate was first filtered through muslin cloth then through Whatman filter paper No.1. The obtained leachates (10%) of *V. negundo* and *R. communis* were used as stock solutions and stored in amber coloured bottle. Different concentrations (2.5%, 5%, 7.5 %,) were prepared from these stock solutions during the bioassay experiment.

Seed germination bioassay

Healthy seeds were used for bioassay experiment. The seeds of *H. suaveolens* were surface sterilized with 5% sodium hypochlorite for 10 min. The seeds were then thoroughly rinsed three times with distilled water. Sets of autoclaved petri plates (9 cm diameter) lined with single layered Whatman filter paper No.1 and moistened with 5ml of leaf leachates of each plant for each concentration (2.5%=T1, 5%=T2, 7.5 %=T3, and 10%=T4). The petri plates treated with distilled water were taken as a control and considered to be set 0=T0. 10 seeds of *H. suaveolens* were uniformly kept in each petri plates. Moisture in petri plates was maintained by adding 2ml of leaf leachates of each plant of respective concentrations or distilled water only on fourth day. The treatments were replicated thrice. The experiments were repeated thrice. The experiment was carried out for ten days. The seeds were considered germinated upon the emergence of the radicle. Germinated seeds were counted daily. The seedlings were sampled on 11^{Th} day after sowing to record various observations.

Data analysis

The results were quantified as germination percentage, inhibition percentage of seed germination, radicle and plumule length, relative elongation of root and shoot, seedling vigour index, fresh and dry weight of seedlings and relative biomass ratio.

The radicle and plumule length (cm) of the respective treatments were measured by using graph sheet method by taking ten seedlings per treatments at random. However, if the germination percentage was less than ten, then all the seedlings were used as the sample. Seedlings sampled from treatments for measurement of root and shoot lengths were used for fresh and dry weight (g) measurements using sensitive electronic balance. Seedlings were dried at 60^0C for 24 h in incubator to get dry biomass.

Germination percentage was calculated using the formula:
Germination percentage (GP) = germinated seeds/total seeds ×100

Inhibition percentage of seed germination (Chatiyanon *et a*l., 2012)
I.P. = C-T/C×100; where
I.P. = Inhibition percentage; C = Seed germination or plant growth in control; T = Seed germination or plant growth in each treatment.

Relative Elongation ratio of Shoot (Rho and Kill, 1986)
RERs = MLSt/MLSc×100; where
RERs = Relative Elongation ratio of Shoot; MLSt = Mean length of shoot of plant under treatment; MLSc = Mean length of shoot of plant under control

Relative Elongation ratio of Root
RERr = MLRt/MLRc×100; where
RERr = Relative Elongation ratio of root; MLRt = Mean length of root of plant under treatment; MLRc = Mean length of root of plant under control

Relative Biomass Ratio
RBR = MBt/MBc×100; where
RBR = Relative biomass ratio; MBt = Mean biomass of plant under treatment; MBc = Mean biomass of plant under control

Seedling Vigour Index (SVI) (Abdul-Baki and Anderson, 1993)
SVI = Germination percentage× Radicle length

Relative Water Content Percentage (RWC %) (Asifa *et al*., 2014)
RWC%= (FW-DW)/FW×100; where
FW= Fresh weight; DW= Dry weight

Statistical analysis

Using statistical analysis (ANOVA, P = 0.05) significance of the difference in seed germination, radicle and plumule length, relative elongation of root and shoot seedling vigour index, fresh and dry weight, relative biomass ratio and relative water content % were tested and compared and revealed that one or more treatments are significantly different. Regression analysis between concentrations of leaf leachates vs, % seed germination, inhibition %, radicle length and plumule length was done.

RESULTS

During the present investigation allelopathy of *R. communis* and *V. negundo* was studied on *H. suaveolens*. The results of percent germination, seedling growth, fresh and dry weight, relative water content percentage of *H. suaveolens* under aqueous leaf leachates treatments of *R. communis* and *V. negundo* is given in Table 1 and Table 2. As shown in Table 1 and 2, leaf leachates of both the plants significantly inhibited germination and seedling growth of *H. suaveolens*. The results were concentration dependent as increasing concentration of leaf leachates increased inhibitory effects on parameters studied. When leaf leachates of both the plants were compared *R. communis* was found to be more effective than *V. negundo* on the test plant studied.

When compared with control (T0) lowest % seed germination of *H. suaveolens* was recorded at T3 (7.5%) followed by T2 (5%) of *R.communis* and then followed by T3 (7.5%) of *V. negundo* while highest % seed germination was observed in 2.5% of leaf leachates of *V. negundo*. Seed germination was completely inhibited by 10% leaf leachates of *R. communis* as well as *V. negundo* (Table 1, 2 and Fig. 1 B, 2 B). The highest inhibitory effect (100%) on percent germination was recorded at T4 (10% concentration) for both the leaf leachates while the lowest inhibitory effect 65.53% and 69.02% was recorded at T1 (2.5%) of *V. negundo* and *R.communis* respectively. Results demonstrated significant ($p<0.05$) allelopathic effect on seed germination and % inhibition.

Growth of future plant is determined by radicle and plumule length at seedling stage. The results presented in Table 1 and 2 showed that radicle and plumule length was reduced with the increase in concentration of leaf leachates. When compared with the control (T0), The highest reduction in radicle (0.85cm) and plumule (1.22cm) length was observed at T3 (7.5%) of *R. communis* leaf leachates while in case of *V. negundo* leaf leachates the highest reduction in radicle (0.67cm) and plumule (1.93cm) length was observed at T3. Lowest reduction was recorded at T1 (2.5%) for leaf leachates of *R. communis* and *V. negundo*. The reduction pattern was quiet similar for leaf leachates of both the plants which is clearly revealed from regression analysis (Fig. 1 and 2).

Similar observations were recorded for relative elongation of root and shoot. The relative elongation of root and shoot gradually decreases with the increase in concentration of leaf leachates of both the plants. The relative elongation of root and shoot is maximum in control (100) and least recorded T3 (7.5%) for both the leaf leachates (Table 1 and 2).

Ability of germinating seed to utilize and mobilize the reserved food material is related to vigour index of seedling. Seedling vigour index was higher in control followed by 2.5% and lower in 7.5%. In case of leaf leachates of *R. communis* and *V. negundo* maximum seedling vigour index recorded were 37.03 and 64.92 at T1 and minimum were 6.42 and 7.50 at T3 respectively. When seedling vigour index were compared for the two leaf leachates it was found that *R. communis* was more effective than *V. negundo* on *H. suaveolens*. There was significant ($p<0.05$) decrease in the values when compared with control.

Table 1: The effect of leaf leachates of *Ricinus communis* on seed germination, radicle and plumule length, relative elongation of root and shoot seedling vigour index, fresh and dry weight, relative biomass ratio and relative water content% of *Hyptis suaveolens*

Treatment	T0 (0%)	T1 (2.5%)	T2 (5%)	T3 (7.5%)	T4 (10%)	F values
% seed germination	96.66±3.34	30.00±3.34	10.00±6.67	6.66±3.34	NG	859.7308
I.P.	0	69.02±2.38	89.50±7.28	93.18±3.22	100.00±0.00	365
Radicle length(cm)	4.77±0.16	1.23±0.02	1.05±0.26	0.85±0.33	NG	249.7
Plumule length(cm)	7.02±0.96	4.90±0.13	3.27±1.10	1.22±0.26	NG	53.4
Relative elongation of root(RERr)	100.00±0.00	25.90±1.26	21.88±4.99	18.06±7.48	NG	271.748
Relative elongation of shoot(RER)	100.00±0.00	70.84±11.93	47.76±19.94	17.97±6.46	NG	41.4642
Seedling vigour index	460.43±5.52	37.03±4.55	11.53±9.00	6.42±4.98	NG	3833.34
Fresh wt.(gm)	0.38±0.03	0.19±0.03	0.07±0.05	0.03±0.02	NG	82.611
Dry wt.(gm)	0.14±0.03	0.04±0.01	0.01±0.00	0.01±0.00	NG	54.207
Relative biomass ratio(RBR)	100.00±0.00	49.20±6.33	18.14±13.18	7.13±6.15	NG	100.1
Relative water content %	62.54±5.32	78.95±2.30	89.87±5.81	74.64±35.80	NG	14.08

Values presented are means ± STD and F statistics denoted that values are significantly different at $p < 0.05$.The treatment T0, T1,T2 ,T3, and T4 are % concentrations of leaf leachates wherein T0 = (0% control), T1= (2.5%), T2= (5%), T3= (7.5%), and T4= (10%). NG= No Germination.

Table 2: The effect of leaf leachates of *Vitex negundo* on seed germination, radicle and plumule length, relative elongation of root and shoot seedling vigour index, fresh and dry weight, relative biomass ratio and relative water content% of *Hyptis suaveolens*

Treatment	T0 (0%)	T1 (2.5%)	T2 (5%)	T3 (7.5%)	T4 (10%)	F value
% seed germination	96.66±3.34	33.33±3.33	14.44±1.92	11.11±1.92	NG	753.4382
I.P.	0	65.53±3.01	85.08±1.53	88.53±1.62	100.00±0.00	1701
Radicle length(cm)	4.77±0.16	1.95±0.03	1.51±0.14	0.67±0.14	NG	739.6
Plumule length(cm)	7.02±0.96	4.40±0.05	3.35±0.92	1.93±0.27	NG	56.56
Relative elongation of root(RERr)	100.00±0.00	40.88±1.83	31.72±2.77	13.97±2.93	NG	1129.6
Relative elongation of shoot(RERs)	100.00±0.00	63.51±9.76	49.10±18.16	27.84±5.68	NG	46.3718
Seedling vigour index	460.43±5.52	64.92±7.13	21.66±0.84	7.50±2.50	NG	6598.06
Fresh wt.(gm)	0.38±0.03	0.25±0.02	0.15±0.02	0.06±0.01	NG	82.611
Dry wt.(gm)	0.14±0.03	0.05±0.02	0.01±0.01	0.003±0.01	NG	42.827
Relative biomass ratio(RBR)	100.00±0.00	66.72±3.92	38.56±3.83	14.83±2.02	NG	710.6
Relative water content %	62.54±5.32	80.55±5.56	90.81±2.02	95.14±3.37	NG	303.7

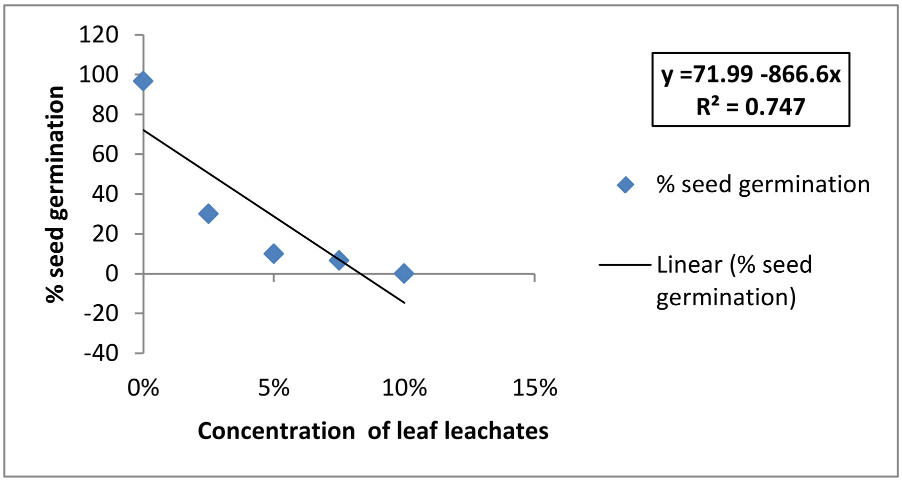
Values presented are means ± STD and F statistics denoted that values are significantly different at $p < 0.05$. The treatment T0, T1, T2, T3, and T4 are % concentrations of leaf leachates wherein T0 = (0% control), T1= (2.5%), T2= (5%), T3= (7.5%), and T4= (10%). NG= No Germination.

Maximum fresh and dry weight was recorded in control over all the treatments. There was a significant ($p<0.05$) gradual decrease with increase in concentration. Maximum and minimum inhibitory effect on fresh and dry weight was obtained at 7.5% and 2.5% concentrations of both the leaf leachates respectively (Table 1 and 2). All the concentrations of leaf leachates of *R. communis* and *V. negundo* exhibited significant decrease in fresh and dry weight of seedlings of *H. suaveolens*. In case of leaf leachates the lowest fresh weight and dry weight values were 0.03gm and 0.003gm respectively compared with control. There was significant decrease in fresh and dry weight with increased concentration of leaf leachates (fig. 3).

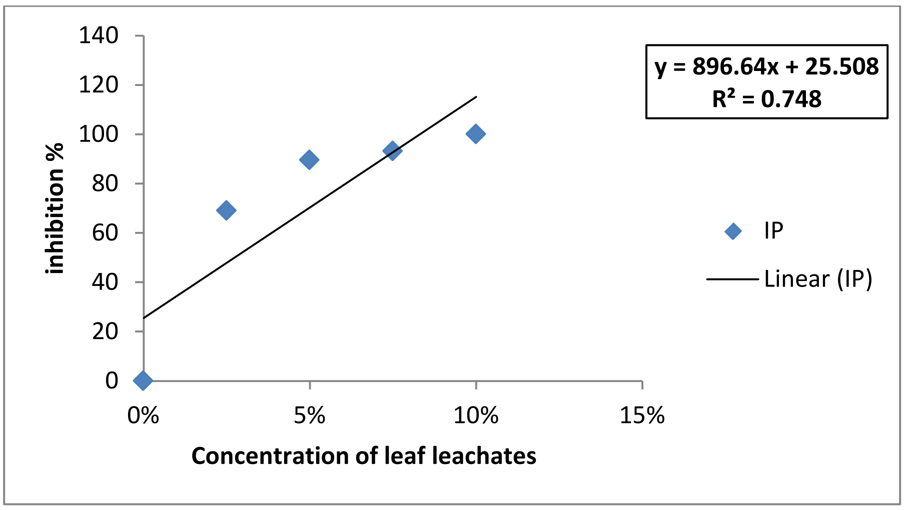
Relative biomass ratio of treatments was significantly different ($p<0.05$) from that of control. Maximum reduction in relative biomass ratio was observed at T3 (7.5% *R. communis*) while minimum reduction was observed at T1 (2.5% *V. negundo*) over control.

Relative water content % was found to be increased with the decrease in concentrations of leaf leachates of *V. negundo* (Table 2) while there was no specific trend recorded in case of *R. communis* (Table 1). Maximum relative water content % was recorded at T3 (95.14) of *V. negundo* while minimum value was observed at T0.

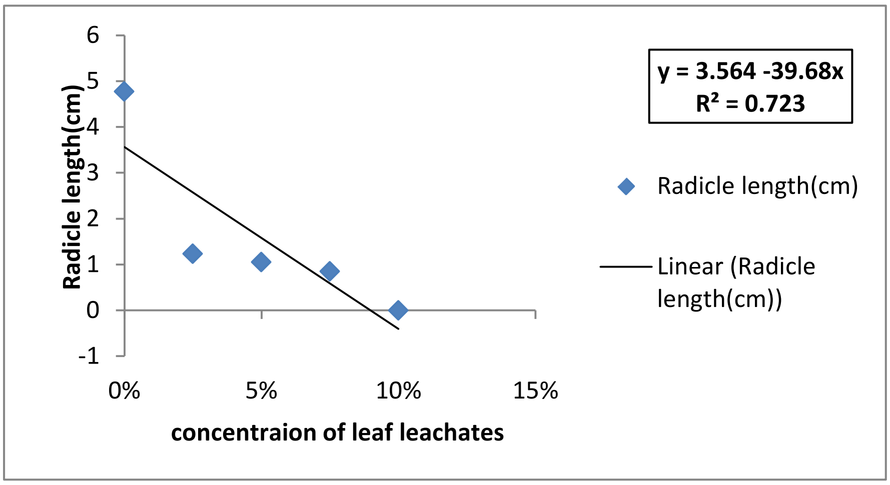
A



B



C



D

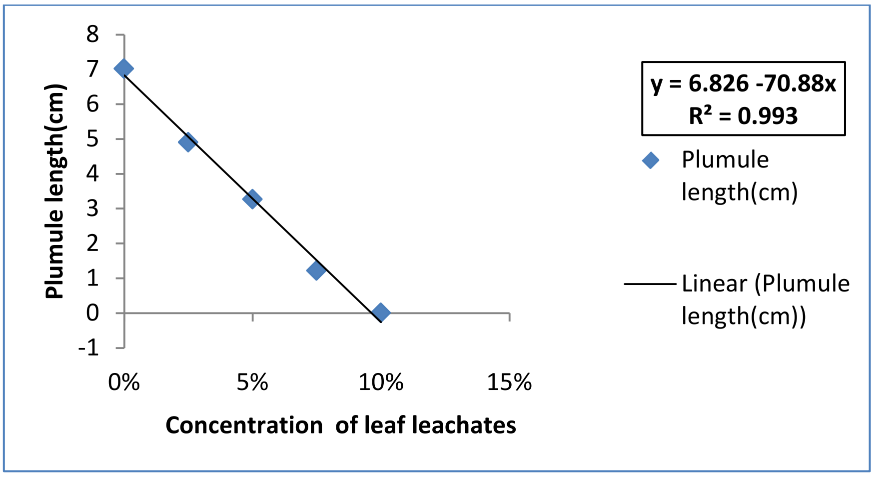
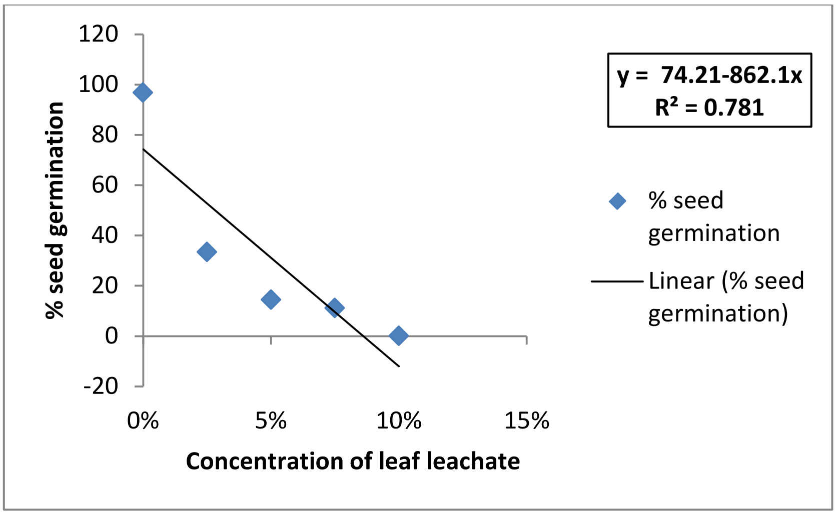


Figure 1: Regression analysis showing relationship between concentrations of leachates of *Ricinus communis* and (A) % seed germination (B) inhibition percent (C) radicle length (D) plumule length of *Hyptis suaveolens.*

A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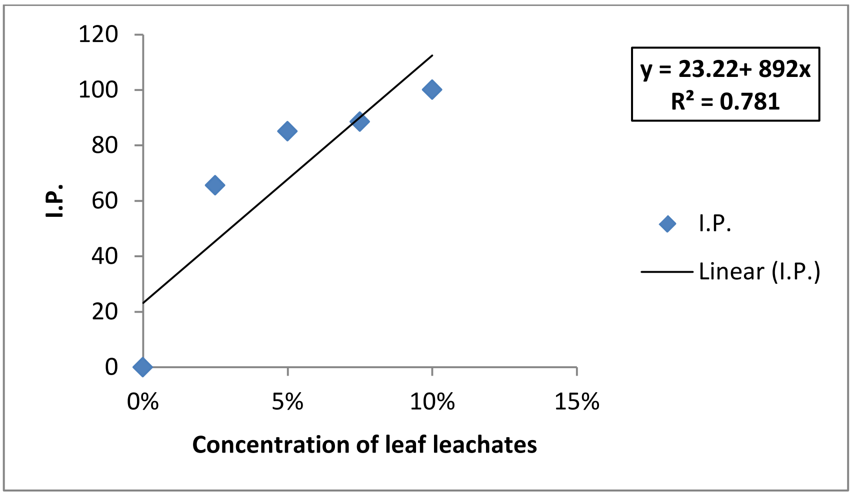
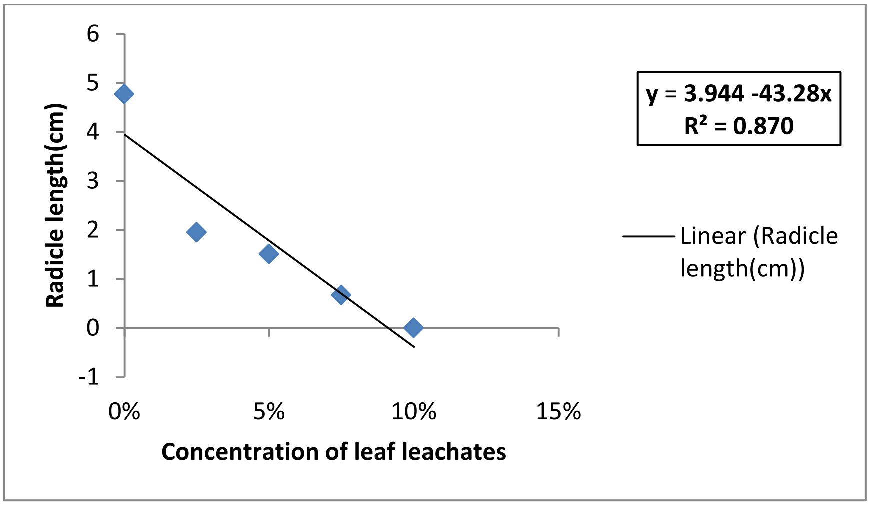
R² = 0.781
% seed germination
120
100
80
60
40
20
0
-20
0%
5%
10%
15%
Concentration of leaf leachate
% seed germination
Linear (% seed germination)

B


y = 23.22+ 892x
R² = 0.781
I.P.
120
100
80
60
40
20
0
0%
5%
10%
15%
Concentration of leaf leachates
I.P.
Linear (I.P.)

C



D

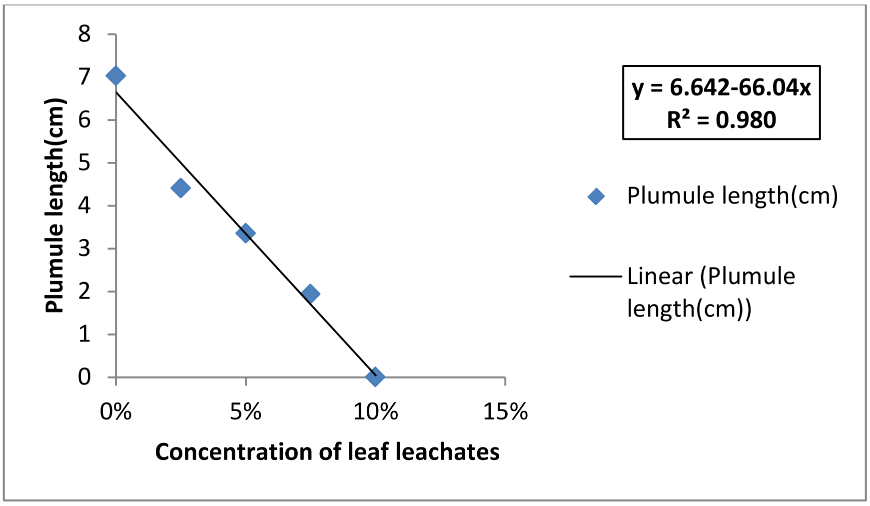
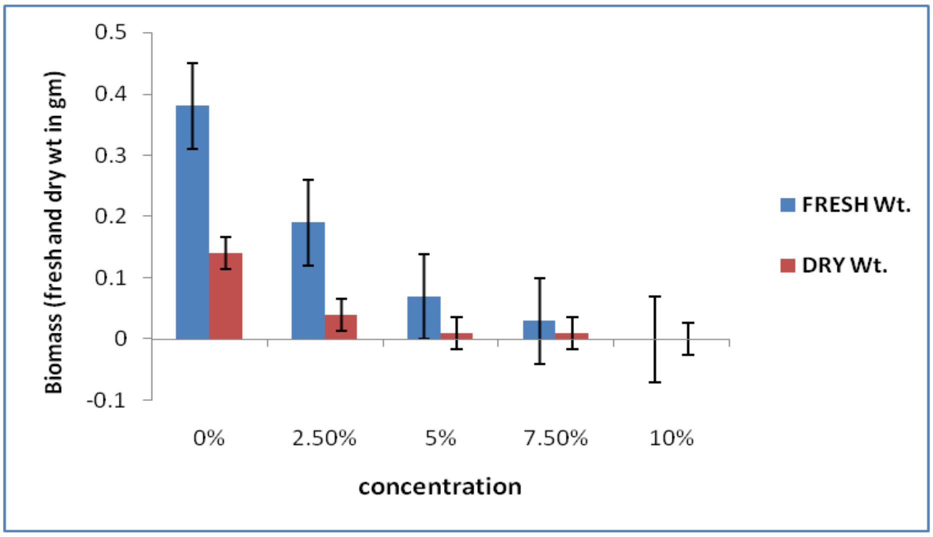


Figure 2: **Regression analysis showing relationship between concentrations of leachates of *Vitex negundo* and (A) % seed germination (B) inhibition percent (C) radicle length (D) plumule length of *Hyptis suaveolens.***

A



B

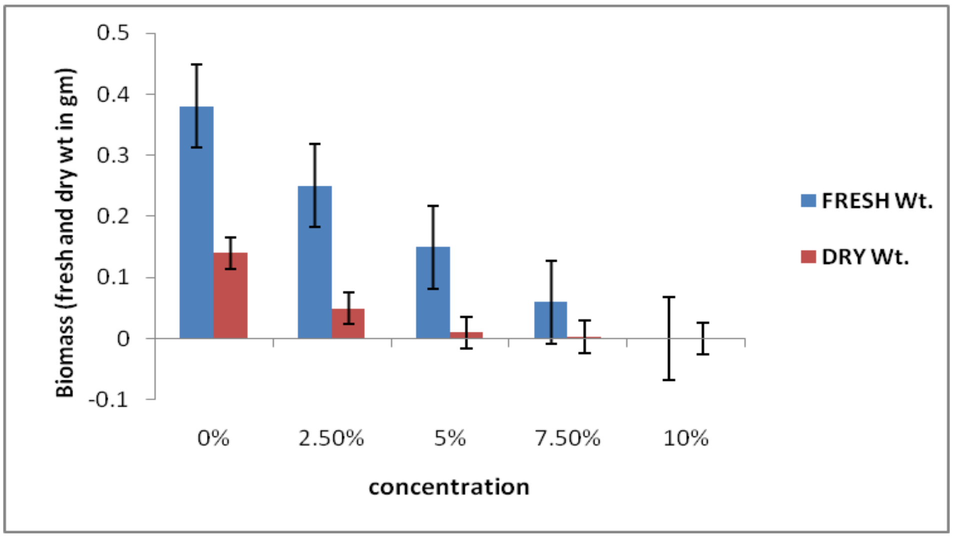


Fig 3: Biomass of seedlings of *Hyptis suaveolens* at different concentrations of leaf leachates of (A) *Ricinus communis* and (B) *Vitex negundo*

DISCUSSION

Allelopathic potential of a plant species is attributed to their secondary metabolites which may be stimulatory or inhibitory in action. Determination of allelopathic potential of a plant species may help in the formulation of biological herbicides or biological growth regulators. Poornima *et al*., (2015), reported inhibitory effect of *H. suaveolens* on seed germination of Wheat and Ragi. *H. suaveolens* which is an obnoxious weeds and posing threat to the agricultural system, should be controlled by natural and ecofriendly methods.

During the present investigation allelopathic effect of *R. communis* and *V. negundo* on *H. suaveolens* was evaluated and results of findings indicate that both plants leaf leachates have allelopathic potential which is because of allelochemicals responsible for their growth retarding activity. Chou and Yao (1983) and Kuo *et al*., (1998), reported the presence of different levels of allelochemicals in the leaf extracts of *V. negundo*, namely p-hydroxy benzoic acid, p-coumaric acid, ferulic acid, vanilic acid, syringic acid and more than 10 flavonoids which may the reason for its allopathic effect. Islam and Kato-Noguchi, (2013) recommended *R. communis* as a bioherbicide on the basis of its stronger inhibitory allelopathic activity. Furthermore, inhibitory compounds or allelochemicals of both plant leaf leachates inhibited seed germination and other growth parameters were concentration dependent. Similar to our findings Nekonam *et al*., (2014) also found that germination of test plant was significantly reduced with the increase in the concentration of extract of *R. communis* while Kavitha *et al*., (2012) reported inhibitory effect at 10% aqueous leaf extract of *V. negundo* on green gram and black gram on the other hand its stimulatory effect on seed germination at lower concentration which is in contrast to our results. Swaminathan *et al.*, (1989) reported that inhibitory effect on seed germination is induced by phenolic acids. Compared to the leaf leachates of *V. negundo, R. communis* showed stronger inhibitory activity against test plant. In line with our results, Islam and Kato-Noguchi, (2013) reported complete inhibition of growth of lettuce, Italian ryegrass and barnyard grass by *R. communis*. Khan *et al*., (2011) showed that *V. negundo* was more effective at 10mg/ml than at 1mg/ml when tested on radish.

R. communis and *V. negundo* leaf leachates retarded seedling growth, reduced seedling vigour index, fresh and dry weight. Joshi *et al*., (2015) reported inhibitory effect at 5% concentration of aqueous leaf extract of R. communis on growth parameters of *Vigna radiata*. Jadhav (2003) also reported inhibition of growth of field crops by *Terminalia tomantosa, Sapindus emarginatus* and *Vitex negundo*.

Ahmed *et al.*, (2007) reported significant inhibitory effect of various concentration of leaf aqueous extract of *Lantana camara* on germination, root and shoot elongation and development of lateral roots of *Brassica juncea*, *Cucumis sativus*, *Phaseolus mungo*, *Raphanus sativus*, *Vigna unguiculata* and *Cicer arietinum*. Sahu and Devkota (2013), reported that seed germination and seedling growth of *Oryza sativa* L. and *Raphanus sativus* L. were inhibited by concentrated aqueous extract of *Mikania micrantha,* are also in line with our results.

In present investigation only leaf leachates of both the plants tested against *H. suaveolens* and showed strong inhibition of all the parameters. Suwal *et al*., (2010) findings of strong inhibitory effect of leaf extract of *Chromolaena odorata* on various growth parameters of paddy and barnyard grass is in agreement with present findings.

Fresh and dry weight of *H. suaveolens* was also significantly reduced by all concentration of leaf leachates when compared with control. In line with this Gella *et al*., (2013) also reported negative effect of major weed species plant parts on biomass production of wheat cultivars.

CONCLUSION

In the present experiment, it was observed that both *R. communis* and *V. negundo* leaf leachates significantly inhibited seed germination, seedling growth, relative elongation of root and shoot, reduced vigor index, fresh and dry weight and relative biomass ratio of *H. suaveolens*. Complete inhibition of seed germination was recorded at 10% (T4) leaf leachates of both the plants. The inhibitory activity on the germination and other growth parameters of

H. suaveolens by *R. communis* and *V. negundo* indicates that both have allelopathic potential. However compared to *V. negundo*, *R. communis* had stronger inhibitory activity on seed germination and seedling growth of *H. suaveolens*, a ruderal weed and emerging invader of central India and thus can be used as pre-emergence bioherbicides. Leaf leachates of both plants may be favourably used as natural bioherbicides for invasive alien species like *H. suaveolens*. Although laboratory experiments are important to find out allelopathic effects but it is necessary to investigate and figure out the significance of these preliminary findings under field conditions for the formulation of natural biocontrol against invasive weed management.

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