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RESEARCH ARTICLE

ALLELOPATHIC EFFECTS OF *HEVEABRASILIENSIS* LEAF EXTRACTON FOUR COMMON LEGUMES

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ABSTRACT

Allelopathic effects of aqueous and methanolic leaf extract of invasive tree para rubber is assayed in the laboratory condition on germination and seedling growth of four commonly cultivated leguminous crops of Tripura. Both the extracts were strongly hinder the seed germination and seedling growth of *Cicerarietinum, Lens culinaris* and *Vignaradiata,* applied in very lower concentration (0.62-2.5% aqueous and 0.1mg/ml – 0.5mg/ml methanol extract). With the increase of concentration, germination, radical and plumule growth of these three species were highly reduced and no germinations were recorded in the experimental sets treated with 5% aqueous solution. Our study reveals that one of the test crop *V. mungo* is resistant to that effect, in which IC_{50} values for germination was recorded very high (25.89%). Our investigation also showed that, aqueous extract has strong allelopathic potentiality than the methanolic extract. This allelopathic potentiality may be the one of the causes and consequences of rubber plantation that does not support the understory vegetation. The result of this investigation may be utilized in the field experiment and the selection of its coexisting species with rubber plantation for the production of herbage and fodder.

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INTRODUCTION

Allelochemicals are thesecondary metabolites like terpenoids, phenolics basically produced in plant body which, interfere or inhibits directly or indirectly to the germination, growth, health andbehaviorof other plants or ecology of neighboring organisms (insects and microorganism) and yield in the agro-ecosystem (Abhilasha *et al*., 2008; Ghafar *et al*., 2000 & Khanh *et al*., 2007). Generally, allelochemicals are the compound that produced at later stage of plant development and influence the growth and reproduction of associated plants (Inderjit, 1996). But all the chemicals present may not always be harmful, as beneficial interactions have also been reported (Foy & Inderjit, 2001). Allelochemicals release from roots, stems and leaves of plants into the environment (Rizvi and Rizbi, 1992) and mainly affect plant at seed emergence and seedling levels (Alam and Islam, 2002). *Heveabrasiliensis*is a deciduous tree belonging to the family Euphorbiaceae, native to the Amazonia, Brazil (Priyadarshan *et al*., 2005). After that rubber has been expanded throughout all of South and Southeast Asia, where it is now grown in countries like Indonesia, India, Malaysia, China, Vietnam, Philippines, Myanmar, Bangladesh, Cambodia and Thailand (Maite *et al*., 2010). These invasive species can survive in a wide range of climatic conditions and rapidly can replace the indigenous diversity. Many native forests have been destroyed by the introduction and large scale plantation of exotic tree species. Consequently, some native species have become endangered and the ecosystem services provided by native forest are deteriorating (Foroughbakhch *et al*., 2001; Isiam *et al*., 1999; Sangha and Jalota, 2005). Rubber tree is introduced in Tripura in 1963 by Tripura Forest departmenton experimental basis for soil conservation, generation of employment for forest dwellers and to use barren land in the state. Ultimately at the end of the 20th century, it becomes very popular and changes the economic structure of the people in the state. Currently Tripura is the second largest rubber producer in India having 61000 hectares of land under rubber plantation (Tripurainfo. 2014). Speedy expansion of rubber monoculture occupied the all proposed reserved forest (PRF) area and degraded forest land, consequently starts to reduce the natural forest gradually. It also reported that monocultures may result in significant losses of above ground (Bunker *et al*., 2005) and belowground (Guo and Gifford, 2002) carbon stocks and biodiversity (Li *et al*., 2008; Ziegler *et al*., 2009 a, b).A number of researchers showed allelopathic effects of various weeds and trees on agricultural crop (Sarkar *et al*., 2012, Kato-Noguchi

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2001). But very little work was available on the alleopathic potentiality of *H. brasiliensis.* Considering the above facts our objective of the present study was a laboratory bioassayof (i) How does *H. Brasiliensis* interfere with the crop field? (ii) Is its allelopathic effects species specific? (iii) Selection of coexisting species in rubber plantation.

MATERIALS AND METHODS

Study area

The experimental area, Suryamaninagar, is situated near the Bangladesh border, about 12 km ahead from the Agartala city. Agriculture is the main livelihood of the local peoples.

Preparation of sample

H.brasiliensis leaves were collected in the month of December (2014) from the areas surrounding vegetable fields, near Tripura University campus, Tripura. After collection the leaves were thoroughly washed and placed in the shade for drying within the laboratory at room temperature for 130 hours. After that the leaves were pieces into small sizes up to 1cm long and further dried up. Fully dried leaves were grinded into a powder using kitchen grinder.

Aqueous extracts

Twenty five grams of powdered sample were soaked in 500 ml of distilled water within 1 liter conical flask. After that they were kept on a mechanical shaker for 24 hours and filtered through cotton cloth. The supernatant was centrifuged at 10,000 rpm for 5 minutes for separating the extra debris from the solution, which served as a stock solution (5%) for aqueous extract. From that solution various concentrations of extract were prepared by the way of dilution.

Methanol extracts

For the preparation of methanolic extract 20 grams of ground leaf powder were soaked in 200 ml of methanol for 72 hours in 500ml conical flask and mouth stiff up by using aluminium foil. After 3 days the methanolic solution was filtered by using what man No. 1 filter paper within another conical flask. After that the solution was placed in hot water bath with fresh wide mouth glass vessel at 70°C and dried. The dried sample was used as stock for the preparation of the various concentrations (mg/ml) of solution by way of water dilution.

Method of experiments

Healthy seeds of all four selected pulses were surface sterilized with 0.5% sodium hypochlorite solution for 2 min and washed thoroughly with distilled water in such a way that the residue of sodium hypochlorite should not contaminate the inner cotyledons of the pulses. Then sufficient numbers of autoclaved petri dished were prepared; each containing a single layered of No.1 What man filter paper. Each petridishewas wetted with5 ml of test solutions of different concentrations of different extracts used in the experimental set separately. The petridishewetted with distilled water were taken as control and considered to be set 0. In each petridish, 25 surface sterilized pulse seeds were placed separately for each tested species. A total of 3 replications of all the sets of various concentrations was kept undisturbed at room temperature (22 ± 2 °C) in the laboratory for 6 days. The numbers of germinated seeds, length of radical and plumule of each set were recorded for the different experimental set used for both the extracts at 7^{th} days. The emergence of a radical approximately 1 mm in length was taken into considered as germination. For radical and plumule length measurement 10 seedlings were selected randomly from every petri plate and measured by using proper cm scale. To observe the effects of allelochemicals on seedling biomass in aqueous solution of *Heveabrasiliensis* leaf, 10 seedlings of each pulse from every triplicates petri plate were taken and cut separated all of them into radical and plumule. Immediately all of that separated parts of these seedlings were taken separately into trussing papers and freshly weighted by using electronic balance.

Statistical analysis

Percentage of inhibition of germination, radical/plumule growth and biomass (fresh weight) were estimated by using Microsoft excel, 2007 and IC_{50} values were calculated by using a linear regression calculator. In case of graph we used Origin software (version 6.0 professional)

RESULTS AND DISCUSSION

Studies on the allelopathicpotentiality of *Heveabrssiliensis* leaf extract, on germination and seedling growth of four common legumes (*Cicerarietinum, Vignamungo, Lensculinaris* and *Vigna radiate*) have shown species specific effect. Both the aqueous (0.62% -2.5%) and methanol (0.1 mg/ml – 0.7mg/ml) extract of leaf were strongly toxic and hinder the seed germination of three test crops, *Cicerarietinum, Lensculinaris* and *Vignaradiata*, in very low concentration, where IC_{50} values were recorded at 1.61%, 1.78% and 1.65% for aqueous extract and 0.47mg/ml, 0.57mg/ml and 0.19mg/ml in methanol extract respectively (Table 1). With the increase of concentration of both the extract germination, growth of radical and plumule were highly reduced and no germination was recorded in *Cicerarietinum, Lens culinaris* and *Vigna radiate* seeds treated with 5% aqueous extract. Whereas, one of the test crops *Vignamungo* is resistant to that effect having very high IC_{50} value of 25.89% and 0.79mg/ml of aqueous and methanol extract respectively. In 5% aqueous extract *Vignamungo* showed 91.07% germination (Table 1, Plate 1).

Our experimental result showed, aqueous extractstronglyin hibits (75-88%) the radical and plumule growth of three test crops (*Cicerarietinum, Lensculinaris* and *Vignaradiata*) at 2.5% concentration and there is no significant differences in the rate of inhibition of root and shoot growth of these three tested species, although the inhibition of the radical and plumule growth of *V. mungo*is 33.50% and 30.63%,respectively, which is very low in comparison to other three experimental species.
IC_{50} values of aqueous extract for radical and plumule growth of *Cicerarietinum, Lensculinaris* and *Vignaradiata* were recorded from 1.59% to 2.02%. Whereas, IC_{50}value in *V. Mungo* was calculated 4.73% and 5.81% for radical and plumule growth respectively (Table 1), which indicate the less sensitivity of the species tested.

Table 1. Percentage of inhibition of seed germination and seedling growth of four legumes treated with aqueous leaf extract of *H. brasiliensis*

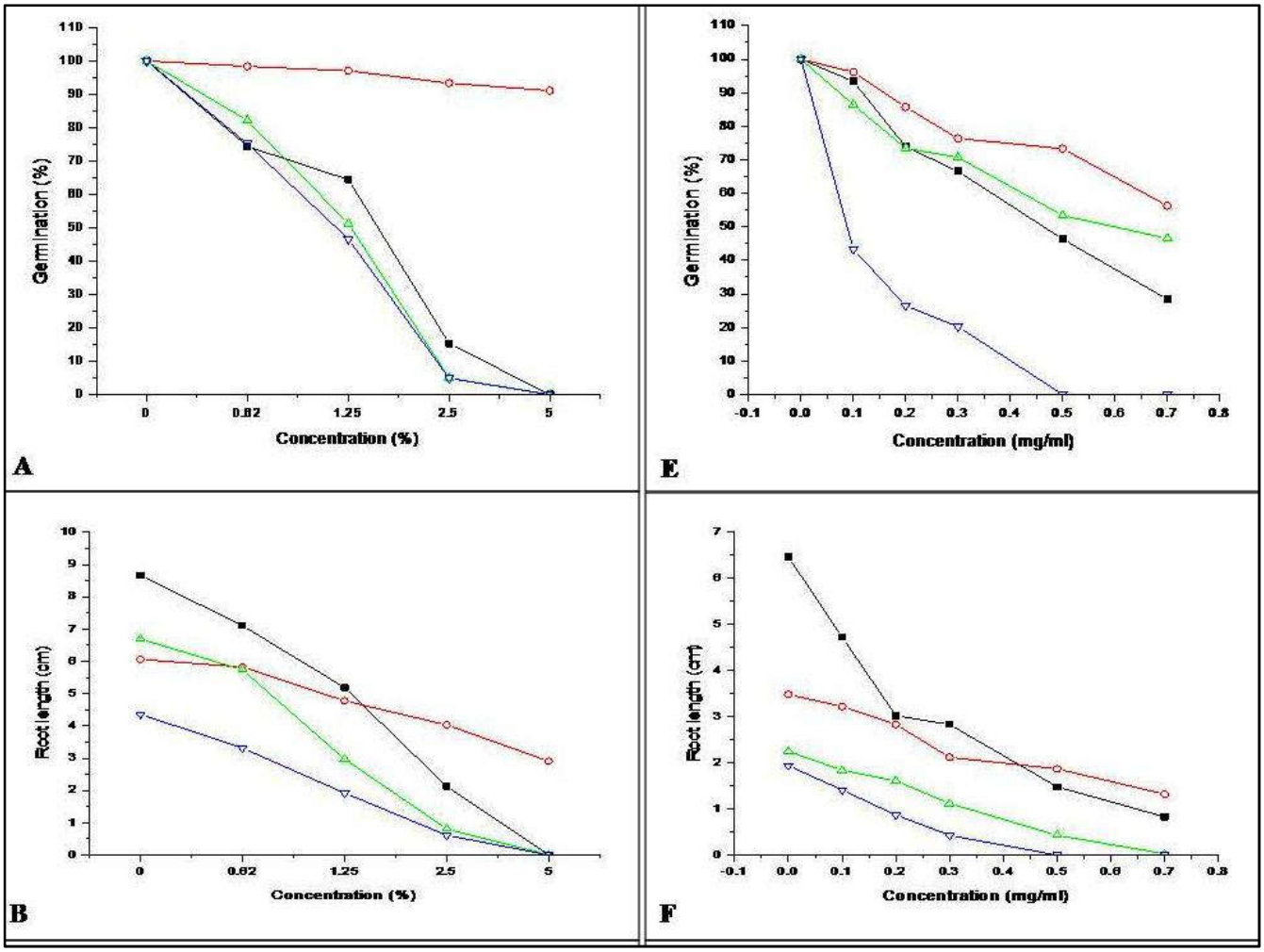
Conc. (%)	Percentage of inhibition															
	C. arietinum				*V. mungo*				*L. culinaris*				*V. radiata*			
	G	Ra	Pl	Bm	G	Ra	Pl	Bm	G	Ra	Pl	Bm	G	Ra	Pl	Bm
Control	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
0.62	25.62	17.99	14.40	23.90	1.67	3.79	8.11	5.38	7.84	14.33	17.29	17.24	24.81	24.08	35.05	30.03
1.25	35.59	40.25	52.96	50.07	3.00	21.12	12.87	15.86	48.82	55.67	40.50	41.37	53.40	55.96	61.62	47.48
2.50	84.68	75.43	72.88	83.19	6.66	33.50	30.63	32.01	95.00	87.91	88.81	100	95.11	86.00	85.36	100
5.00	100	100	100	100	8.93	51.98	36.29	53.54	100	100	100	100	100	100	100	100
IC_{50} (%)	1.91	2.02	1.96	1.80	25.89	4.37	5.81	4.46	1.78	1.80	1.90	1.80	1.68	1.73	1.59	1.65

G- Germination; Ra- Radical; PL- Plumule; Bm- Biomass (Fresh)

Table 2. Percentage of inhibition of seed germination and seedling growth of four legumes treated with methanolic leaf extract of *H. brasiliensis*

Conc. (mg/ml)	Percentage of inhibition															
	C. arietinum				*V. mungo*				*L. culinaris*				*V. radiata*			
	G	Ra	Pl	Bm	G	Ra	Pl	Bm	G	Ra	Pl	Bm	G	Ra	Pl	Bm
Control	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
0.1	6.66	26.93	22.60	11.34	3.86	7.47	28.41	19.58	13.61	18.22	6.66	26.66	56.67	27.31	16.25	33.93
0.2	26.12	53.25	33.33	22.60	14.27	18.67	39.92	37.11	26.56	28.44	12.59	47.61	73.51	55.15	48.12	63.34
0.3	33.45	56.19	48.29	33.58	23.66	39.08	49.82	55.15	29.29	50.22	16.29	64.76	79.77	77.83	78.12	79.48
0.4	53.67	77.08	66.07	66.27	26.68	46.26	63.66	62.37	46.62	80.88	25.18	80.00	100	100	100	100
0.5	71.59	87.15	86.30	48.13	43.75	62.06	67.44	77.31	53.48	98.60	35.55	89.52	100	100	100	100
IC_{50} (mg/ml)	0.47	0.31	0.37	0.42	0.79	0.54	0.40	0.38	0.57	0.34	1.00	0.30	0.19	0.25	0.27	0.23

G- Germination; Ra- Radical; PL- Plumule; Bm- Biomass (Fresh)



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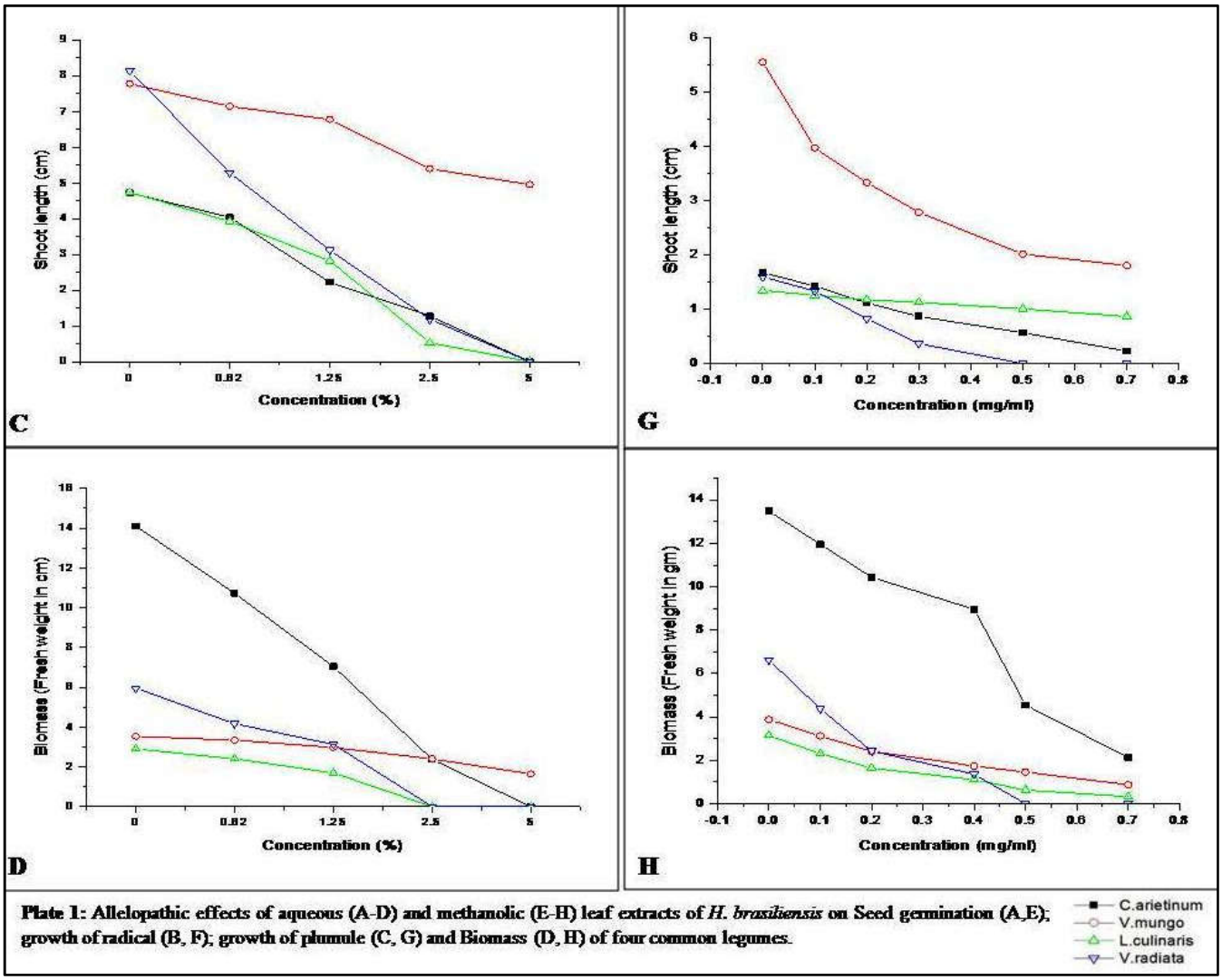

Plate 1: Allelopathic effects of aqueous (A-D) and methanolic (E-H) leaf extracts of *H. brasiliensis* on Seed germination (A,E); growth of radical (B, F); growth of plumule (C, G) and Biomass (D, H) of four common legumes.

While, methanol extract showed the similar result of the radical and the plumule growth of *C. Arietinum, V. mungo and V. Radiate* where, IC_{50} values ranges from 0.25 mg/ml to 0.54 mg/ml for radical growth and 0.27 mg/ml to 0.4 mg/ml for plumule growth. The differential inhibitory response is observed on radical (IC_{50}= 0.34 mg/ml) and the plumule (IC_{50}=1 mg/ml) growth of *L. culinaris.* This result indicates the root growth of *L. Culinaris* is more sensitive than its shoot growth (Table 2). 100% inhibition of seed germination and seedling growth is observed in *V. radiata* at 0.5 mg/ml methanol extract.

Our study also reveals seedling growth of *L. Culinaris* and *V. radiata* in terms of fresh weight is lethal when treated with 2.5% aqueous extracts whereas, 100% inhibition of seedling growth is observed in *C. arietinum* treated with 5% aqueous solution, but, the seedling growth of the *V. mungo* was continued in the same treatment with lower rate. All concentration of methanol extracts reduces the biomass in fresh weight, but, highest percent of inhibitions were observed in the experiment treated with 0.5 mg/ml to 0.7 mg/ml concentration of the extract. Our investigation result is supported by the finding of other workers (Marinov-Serafimov 2010). The result showed that aqueous extract is more toxic than the methanol extract. It may be the indication of water solubility of allelochemicals. The species specific effects of leaf extract may be the results of genetic constituent diversity which may be utilized for coexisting species selection of rubber plantation.

Conclusion

As the rubber is well-known as liquid gold in the rural as well as the state economy in Tripura, expansion of rubber plantation cannot be accessed. So for the conservation of threatened biodiversity due to rubber plantation, integrated plantation management system has to develop. Our experimental result may help the plantation manager to develop the integrated management system. It is also evident from our present study that the rubber leaf contains water soluble allele chemicals which can interfere the growth and yield in the nearby agro-ecosystem. *Vignaradiate,* which is moderately resistant to that effect could be used to develop understory vegetation in the rubber plantation which can support other species thus conserve biodiversity. This result may also be used for the production of herbage and fodder for the cattle, thus to develop an integrated farming system in the state.

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