ISSN: 2347-4688, Vol. 12, No.(3) 2024, pg. 1345-1360

Current Agriculture Research Journal

www.agriculturejournal.org

Comparative Impacts of Soaked and Crushed Aqueous Extracts of *Lantana camara* Leaf and Stem on Germination and Early Seedling Length of *Oryza Sativa*

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Abstract

Experiments were performed in laboratory and glasshouse to determine the allelopathic effect of crushed and soaked leaf and stem aqueous extracts of *Lantana camara* L. on germination and early seedling growth of *Oryza sativa*. The aqueous extract of both soaked and crushed aboveground parts (leaf and stem) of *L. camara* with different concentrations (2.5%, 5%, 7.5% and 10%) were used and compared with control (distilled water). Seed germination, length, biomass, moisture content and seed vigor index of rice crop were documented in different treatments. Maximum suppression in germination and other parameters i.e., length and biomass were recorded in soaked leaf extract while, crushed leaf extract promoted the germination and growth at highest (10%) concentration. However, relative moisture content and seed vigor index exhibited more inhibitory effects in crushed leaf extract in comparison to soaked leaf treatment. Higher amount of allelochemicals released from the soaked leaf extracts of *L. camara* may be one of the reason in variations of allelopathic effect while the stimulatory effects of crushed leaf extract on measured plant traits may be possibly caused by increase in nutrient concentration in the soil. Contrasted with control (C_0), the lower concentration depicted promotion in the studied plant traits while higher concentrations suppressed the germination and early seedling growth. Though laboratory analysis research in allelopathy is highly significant, a field study is suggested to confirm the allelopathic impacts of *L. camara* on cropland ecosystems in various field conditions. This will play role in understanding the underline causes and physiological processes involved in the different consequences of the leaf and stem allelochemicals on different crop species in agriculture.

Article History
Received: 03 September 2024
Accepted: 20 November 2024

Keywords
Agricultural Crops; Allelopathic Effect; Allelochemicals; *Lantana camara*; Phenolic Content.

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Doi: http://dx.doi.org/10.12944/CARJ.12.3.27

Introduction

Weeds constitute the most significant biotic limitations on both developing and developed nations for agricultural production. Weeds have the tendency to compete with cultivated and native plants for moisture, light, nutrients, and space.[1] They can also possess a serious threat by hosting the pathogens that cause diseases in cultivated plants[2] that could reduce the yield of the crops by 20–50%.[3] As a result, impact of weed poses a serious concern for crop productivity, and contemporary agriculture so it must be managed effectively to prevent yield losses and guarantee food security.[4]

Alien invasive species have the tendency to suppress germination, emergence and growth of other plants by secreting allelopathic compounds which resulted into promotion of the invasion success of alien plants via regulating the sprouting, proliferation and developing stages of the native plant species.[5] When allelopathic substances enter into the soil they retard the growth and development of native species, affecting their occurrence and thus biodiversity released by different vegetative and floral parts of plants.[6] Allelochemicals released are the different types of secondary metabolites like phenolics, terpenoids, alkaloids and their derivatives by which alien invasive plants communicate. Therefore, allelopathy is important plant interaction mechanism for the successful establishment of invasive exotic weeds. According to[7] allelopathy may have the ability to reduce the potential of native plants by 25% and thereby play key role in contribution for invasion of alien plants. Himalayan forests are facing high risk of invasion; previous studies reported that there is allelopathic effect of invasive alien species on agriculturally used traditional crops and weeds.[8-9] One of them is *L. camara* which is worldwide known invasive plant and perennial aromatic shrub belongs to Verbenaceae family.

In 1809, *L. camara* was brought to India for its ornamental role at National Botanical Garden but now this plant has spread across all open areas along roadsides, railway tracks, edges of crop fields and open forests all over the country. *L. camara* is an alien invasive species hence allelopathy has a significant impact in its invasion and establishment by releasing differential amount of allelochemicals into the soil beneath its canopy cover. These secondary metabolites i.e., allelochemicals are released into surrounding environment and biogeochemical interface of plants by precipitation leachates, decomposition of different plant parts, root derived nutrients and volatilization which may results into the reduction of germination and early seedling length of native plant species.[10-12] According to Sharma and Raghubanshi,[13] *L. camara* interferes with the growth of surrounding vegetation by outcompeting for soil nutrients and altering microenvironment (e.g., light and temperature) by forming dense thickets. *L. camara* as an invasive plant releases certain amount of chemicals to discourage the growth of native plants, the phenomenon called as allelopathy.[14] Aqueous extract of *L. camara* shows effect on germination and growth of five crops (Chinese cabbage, spinach, rapeseed, cucumber and chili) in laboratory, greenhouse, and field conditions.[15] *L. camara* is one of the invasive species of Kumaun Himalaya that infest rice-based agroecosystems.[16] Rice is one of the staple food crops of India which is negatively affected by this invasive species.[17]

In the Himalayan belt, rice seeds are often directly sown, resulting in simultaneous growth with weeds, which compete for essential nutrients and space. Allelochemicals released by these weeds also contribute towards this competition. Himalayan economy is flourished by agriculture and related activities which plays a significant role in the lives of this region.[18] Mostly the agriculture and allied activities are centered between 1200 and 2000 m above sea level (mid–hill zone), which accounts for about 80% of the rural economy. Further, as a result of climate change, rising global temperatures and cropland damage, the invasive weeds are increasing and causing considerable decline to the agricultural productivity.[17] Therefore, the objectives for present study were: i) To evaluate the comparative effect of leaves and stem of *Lantana camara* and ii) To evaluate the comparative effect of soaked and crushed extract of *Lantana camara* against one of the rice variety (Chandan-21).

Materials and Methods

Collection of Plant Material and Rice Seeds

Plant material (leaves and stem) are collected from fields located in Bhowali near Nainital in Kumaun Himalaya. Certified rice seeds of variety Chandan-21

were used in experiment. These seeds were then tested for viability, washed and sterilized thoroughly. Experiments regarding this present study were conducted in the Department of Botany, D.S.B. Campus, Nainital.

Preparation of Aqueous Extracts

Collected leaves and stem were separated from healthy plants and brought to the laboratory. 10 g of different plant material were crushed and soaked in 200 mL of distilled water for 24 h at room temperature. Plant material is crushed into mortar and pastel. The extract was then filtered through double layered muslin cloth followed by Whatman no. 1 filter paper and was considered as stock solution (100%). This extract was then diluted to prepare different concentrations of crushed and soaked treatments. Different concentrations i.e., 2.5% (C_1), 5% (C_2), 7.5% (C_3) and 10% (C_4) were prepared from crushed and soaked plant material using distilled water as dilution factor. Control (C_0) was also used to compare the results. Fresh stock solutions were prepared for each crushed and soaked extract preparation after one week of time period to maintain the chemical nature of phytotoxins and their viability.

Experimental Design

Experiments for investigation of germination and seedling growth of rice were conducted on root trainer having the height of 4 cm and diameter of 5 cm each. Soil for the experiment was collected from *L. camara* free oak forest around D.S.B. Campus, Nainital. Soil was oven sterilized for 6-8 hrs prior to the experiment. 10 seeds were sown in each root trainer and replicated 10 times per concentration per treatment. 10 mL aqueous extract of each concentration (C_1, C_2, C_3 and C_4) were added to each of the replicates separately. Similarly, the control was treated with distilled water. Germination test was conducted under condition of 12 h light/dark cycle for 15 days with 15°C minimum and 25°C maximum temperature. A seed was considered germinated when radical was 2 mm long. The root and shoot length were measured on the first 15 days then further harvestings were done after 30 days and 45 days. Prior to each harvesting, seedlings were thinned to equal numbers in each pot to maintain the intraspecific competition.

Data Analysis

Seed germination was recorded and observed on a daily basis for data analysis. Percentage and rate of germination were calculated.[19] The length of plumule and radicle was measured after final count and was taken through ruler (0.1 cm accuracy). Simultaneously, the fresh weight of plumule and radicle was taken with the help of electronic weighing machine (0.0001g accuracy). Shoot and root dry weights were recorded after oven drying at 60 °C for 48 h. The moisture content respective to the fresh weight was calculated.[20] Seed Vigor Index (SVI) was measured by following Kumar and Garkoti.[21] The Response Index was calculated as per the formula given by[22] for the magnitude of inhibition versus stimulation by imposed stress on seed germination and seedling growth.

Statistical Analysis

The measured parameters were analyzed by Analysis of Variance (ANOVA). Statistical analysis was performed by SPSS version[25]

Results and Discussion

Statistical analysis showed that *L. camara* plant parts (stem and leaves) and process (crushed and soaked) significantly affected germination and early seedling growth phases of rice in laboratory bioassay. Statistical analysis done by two-way ANOVA showed significant effects of leaf and stem extracts, extract concentrations and their interactive effects on root length, plant biomass and relative moisture content of rice seedlings (Table1). The studied plant traits at three different harvesting periods performed after 15 days of time interval showed an inconsistent pattern. However, the overall results depicted that soaked leaf extract was more suppressive as compared to crushed leaf extract and similar to these lower concentrations promoted the trait values and higher concentration suppressed the same. Similar to our results,[23] reported that soaked leaves of *L. camara* exhibited more inhibitory as well as fluctuating results on germination and early growth of three agricultural crops i.e., maize, finger millet and teff (William love grass). Allelochemicals present in plants can influence germination and early seedling growth of other plants in concentration dependent manner; and alteration of these chemicals are selective and can vary from species to species.[24-28]

Impact of Plant Part and Extract type on Seed Germination

When control (C_0) was compared with other concentrations (C_1, C_2, C_3 and C_4) of crushed leaf extract, all of them showed increased seed germination by 6%, 4%, 3.9% and 3.7%, respectively. Soaked leaf treatment recorded inhibition of 6.6%, 1.2% and 0.3% in C_2, C_3 and C_1 while, increment of 1.8% in germination percentage of C_4, respectively. The inhibitory effect on the germination of the rice was proportional to the concentration of the extract and the higher concentration had the strongest inhibitory effect for both crushed and soaked extracts.

When compared with Control (C_0), C_1, C_2 and C_3 levels of crushed stem treatment inhibited the seed germination by 0.1%, 3.3% and 5.4%, respectively, whereas, C_4 promoted the seed by 2% (Table 2). In soaked stem treatment, all the concentrations inhibited seed germination in comparison to control i.e., the recorded inhibition was as: C_1 (1.13%) < C_3 (1.39%) < C_2 (3.38%) < C_4 (3.38%) [Fig.1]. The inhibitory effect on the germination was inversely proportional to the concentration of the extract and the higher concentration had the strongest inhibitory effect for both crushed and soaked extracts. Plants have the capacity to sense the environmental stimuli and respond to the environment either by altering its functional traits or via adaptation in response to its environmental factors.[29] In conformity with our results, prior studies on allelopathic influence of *L. camara* also demonstrated its potential impact on seed germination and growth of many plant species including agricultural crops.[30-31,25]

Effect of Plant Part and Extract type on Seedling Growth

At 1st harvest, crushed leaf extract [Fig. 2] showed inhibition in root length and the recorded order was as: C_2 (45%) > C_1 (40%) > C_3 (22%) > C_4 (2%). Soaked leaf extract showed increment in the order: C_2 (35%) > C_1 (23%) > C_3 (22%) > C_4 (20.7%). Crushed leaf extract showed 11%, 7.9%, 7.1% and 6.2% inhibition in shoot length at C_4, C_1, C_3 and C_2, respectively, whereas, in case of soaked leaf extract, recorded inhibition was 13%, 8.6%, 2.8% and 2.7% at C_4, C_3, C_1 and C_2, respectively. Total shoot length in crushed leaf extract was suppressed by 21.9%, 23.07%, 13.6% and 16.8% in C_1, C_2, C_3 and C_4, respectively, while, soaked leaf extract showed 1.6%- 16.8% increment. Crushed leaf extract inhibited shoot diameter by 0.39% in both C_4 and C_1, 0.16% in C_3, and increased by 3.03% in C_2. The soaked leaf extract showed inhibition of 8.2% in C_4 and 6% in C_3 level and increment of 3% in C_2 and 2.6% in C_1 level, respectively. After 2nd harvesting in [Fig. 3] crushed leaf extract, root length showed 13% and 4%increment in C_4 as well as C_3 level and 5% inhibition in both C_1 and C_2 level. Soaked leaf extract imposed maximum inhibition of 37% in C_2, 19% in C_3; while C_4 and C_1 promoted the root length by 11% and 3.6%. Shoot length in crushed leaf extract was inhibited in the order: C_4 (8.8%) > C_1 (3.3%) > C_2 (2.3%) > C_3 (0.9%), while in soaked leaf extract, maximum inhibition was shown by C_2 (8%) followed by C_4 (5.1%) and increment was observed in C_1 (2.3%) and C_3 (1.6%). The crushed leaf extract increased the total shoot length by 11% and 0.7% in C_4 and C_2, and inhibition of 2.9% and 1.4% was recorded in C_3 and C_1, respectively. In the soaked leaf extract, C_4 and C_1 promoted the total shoot length by 11% and 4.8% and suppressed the same by 24% and 9.9% in C_2 and C_3, respectively. Shoot diameter in crushed leaf extract showed increment of 6%, 5.2%, 4.6% and 1.7% at C_4, C_3, C_2 and C_1, respectively, whereas, in soaked leaf extract inhibition of 13%, 5.5%, 1.4% and 0.8% were recorded at C_4, C_3, C_2 and C_1 concentrations, respectively. At 3rd harvest, root length [Fig. 4] in crushed leaf extract showed increment in C_4, C_1 and C_2 of 16.9%, 11% and 7.7%, respectively and inhibition of 4% in C_3, while in soaked leaf extract, C_1 showed maximum inhibition of 40%, C_2 10% whereas, C_4 and C_3 showed increment of 33.3% and 29%, respectively.

Shoot length in crushed leaf extract showed inhibition of 8.9%, 6% and 2.9% in C_4, C_3 and C_2, respectively, and C_1 showed increment of 0.5%, while in soaked leaf treatment, there was increment of 10.8% and 3.8% for C_3 and C_1 and inhibition of 4.1% and 3.7% in C_2 and C_4.Total shoot length of crushed leaf treatment showed inhibition in both C_1 (23%) and C_3 (4.7%) and increment in C_4 (10%) and C_2 (5.3%), while, for soaked leaf treatment, the order of increment was: C_3 (27%) > C_4 (22%) > C_1 (20.9%) > C_2 (2.1%). Crushed leaf extract showed increment in shoot diameter by 8%, 4%, 2.3% and 1.9%at C_3, C_4, C_2 and C_1, respectively, and in

soaked leaf extract C_2 showed inhibition of 5.3% and promotion at C_3 (5.6%), C_1 (3.1%) and C_4 (1.6%). At 1st harvest, the root length in crushed stem extract showed increment of 1.2% (C_3) and 17% (C_4). In contrast, soaked stem extract showed inhibition of 30% in root length for higher concentration (C_4) [Fig. 5]. However, crushed (14.8%) as well as soaked (11.63%) aqueous extract of *L. camara* stem suppressed shoot length of rice seedling at higher concentrations. Total plant length was stimulated by crushed stem extract and inhibited by soaked stem extract. An increasing pattern of inhibition in shoot diameter was recorded in crushed stem extract with the increasing extract concentrations: 3.18% at C_1, 4.67% at C_2, 5.38% at C_3 and 6.2% at C_4 and soaked stem extract showed increment in shoot diameter at lower concentration (C_1 by 1.43%) and inhibition was recorded at higher concentrations: C_2(0.03%), C_3 (3.32%) and C_4 (9.4%). As compared to 1st harvest, at 2nd harvest [Fig. 6] the application of crushed stem extract showed considerable inhibition in root length i.e., C_1 (8.7%), C_2 (17.1%), C_3 (5.4%) and C_4 (24.7%), whereas, soaked stem extract showed increment at C_1 by 9.27%, C_3 by 0.86%, C_4 by 1.28% and suppression at C_2 by 11.44%.

Shoot length was increased at lower concentration i.e., C_1 (5.05%) of crushed stem while inhibited towards increasing concentrations: C_2 (1.48%) < C_3 (2.29%) < C_4 (11.19%). Soaked stem extract also recorded increment in shoot length at C_1 (6.36%), C2 (5.29%), C_3 (3.9%) and inhibition at C_4 (11.63%). Crushed stem extract suppressed total plant length at higher concentration (19.32%), whereas, soaked stem treatment promoted total plant length (12.02%) in C_4 concentration. Shoot diameter showed an inconsistent pattern, it was promoted at C_1 (2.85%) and C_3 (0.74%) and inhibited at C_2 (3.18%) and C4 (6.02%) in crushed stem extract. Similarly, it showed increment at C_1 (1.71%) and C_3 (3.95%) while, inhibition at C_2 (2.83%) and C_4 (0.33%) in soaked stem extract. At third harvest, crushed stem [Fig. 7] extract increased root length at lower concentrations i.e. C_1 (22.63%) and C_2 (11.6%) and inhibited at higher concentrations: C_3 (22.44%) and C_4 (2.63%). Soaked stem treatment showed inhibition in root length at all concentrations: C_1 (13.9%), C_2 (4.99%), C_3 (20.58%) and C_4 (21.46%). The shoot length showed inhibition at C_2 (7.3%), C_3 (8.69%), C_4 (15.18%) and increment at C_1 (2.5%) in crushed stem treatment, whereas, soaked stem extract showed inhibition at C_2 (1.07%) and C_4 (7.92%) and increment at C_1 (10.62%) and C_3 (1.07%). Total plant length showed inconsistent pattern along the concentration gradient, in crushed stem treatment, it increased at lower concentrations i.e. C_1 (17.74%) and C_2 (6.67%) and decreased at higher concentrations: C_3 (14.48%) and C_4 (5.48%) whereas, in soaked stem treatment it decreased at all concentrations: C_1 (8.9%), C_2 (2.8%), C_3 (12.58%) and C_4 (17.16%). Crushed stem treatment inhibited shoot diameter at C_1 (1.47%) and C_4 (4.04%) level and promoted at C_2 (0.1%) and C_3 (0.72%), in contrast, soaked stem treatment increased shoot diameter at C_1 (0.96%) and C_4 (0.37%) and decreased at C_2 (2.81%) and C_4 (83.34%).

Effect of plant part and extract type on Dry Biomass During 1st harvest, dry root weight in crushed leaf extract showed inhibition and the order was: C_4 (63.5%) > C_1 (44.6%) > C_2 (26%) > C_3 (10%), whereas, in soaked leaf extract, order of increment was: C_2 (24.1%) > C_1 (23%) > C_3 (20.5%) > C_4 (20.4%). Dry shoot weight in crushed leaf extract showed increment in C_3 (14.8%), C_1 (4.4%), and C_4 (2.6%), whereas, inhibition of 2.6% was recorded in C_2. In soaked leaf extract, the increment of 10%, 6.2% and 3.8 % was shown by C_1, C_3 and C_4 and inhibition of 4.1% by C_2. Total dry weight in crushed leaf treatment was increased in C_3, C_1, and C_2 by 15.4%, 12.8%, and 0.09%, respectively, and inhibition of 4.1% in C_2 was observed. In soaked leaf extract, the increment was observed in C_3, C_2, C_4 and C_1 by 9.7%, 9.6%, 8.1% and 7.9%, respectively. After 2nd harvesting, dry root weight of crushed leaf extract showed inhibition of 65%, 46% and 7.4% in C_1, C_2 and C_3 and increment of 7.6% in C_4,while in soaked leaf extract, increment was observed in C_4 (31.7%), C_3 (8.6%) and C_1 (4.3%), however, inhibition of 32% was recorded at C_2. Dry shoot weight of crushed leaf extract had inhibition of 65%, 46% and 7% in C_1, C_2 and C_3, respectively, while promotion of 7.6% in C_4, whereas, in soaked leaf extract, increment of 31%, 8.6% and 4.3% at C_4, C_3, and C_1, respectively and inhibition of 32% at C_2 was observed. Total dry weight of crushed leaf extract showed increment in the order: C_2 (14.7%) > C_3 (13.4%) > C_4 (8.8%) > C_1 (3.5%), while in soaked leaf treatment, C_3 and C_4 showed increment by 7.5% and 3.1% and inhibition by 4.5 and 2.5% in C_1 and C_2, respectively.

Table 1: Two-way ANOVA showing the impact of L. camara stem extracts on the seed germination and early seedling growth of rice.

	Variables	Extract type					Concentration					Extract type x Concentration				
	Plant traits	SS	df	MS	F	Sig.	SS	Df	MS	F	Sig.	SS	df	MS	F	Sig.
Germination	GP	169	1	169	1.703	0.195	160	4	40	0.403	0.806	216	4	54	0.544	0.704
	GR	0.16	1	0.16	3.404	0.068	0.503	4	0.126	2.668	0.037	0.097	4	0.024	0.514	0.726
1st Harvest	RL	0.548	1	0.548	0.152	0.698	11.928	4	2.982	0.827	0.511	24.012	4	6.003	1.665	0.165
	SL	0.084	1	0.084	0.549	0.461	4.541	4	1.135	7.407	0	0.857	4	0.214	1.398	0.241
	TPL	0.203	1	0.203	0.053	0.819	14.983	4	3.746	0.971	0.427	26.917	4	6.729	1.745	0.147
	SD	0.004	1	0.004	52.41	0	0.001	4	0	4.494	0.002	0	4	0	1.245	0.298
	DRW	2.28E-06	1	2.28E-06	1.428	0.235	2.36E-06	4	5.89E-07	0.369	0.83	1.05E-05	4	2.62E-06	1.64	0.171
	DSW	4.36E-05	1	4.36E-05	3.587	0.061	2.67E-05	4	6.67E-06	0.549	0.7	7.14E-06	4	1.79E-06	0.147	0.964
	TDW	6.56E-05	1	6.56E-05	4.406	0.039	3.98E-05	4	9.94E-06	0.668	0.616	1.78E-05	4	4.46E-06	0.3	0.878
	RMC	971.444	1	971.444	13.355	0	765.472	4	191.368	2.631	0.039	101.061	4	25.265	0.347	0.845
	SVI	0.899	1	0.899	0.22	0.64	19.07	4	4.768	1.167	0.331	25.341	4	6.335	1.551	0.194
2nd Harvest	RL	16.16	1	16.16	0.73	0.395	57.23	4	14.308	0.646	0.631	35.262	4	8.815	0.398	0.81
	SL	1.513	1	1.513	1.468	0.229	7.121	4	1.78	1.728	0.151	1.047	4	0.262	0.254	0.907
	TPL	27.563	1	27.563	1.177	0.281	89.405	4	22.351	0.955	0.436	44.389	4	11.097	0.474	0.755
	SD	0.005	1	0.005	72.295	0	0.001	4	0	4.78	0.002	0	4	8.00E-05	1.18	0.325
	DRW	2.51E-05	1	2.51E-05	1.816	0.181	1.47E-05	4	3.67E-06	0.265	0.9	1.84E-05	4	4.59E-06	0.332	0.856
	DSW	0	1	0	6.452	0.013	4.12E-05	4	1.03E-05	0.284	0.888	0	4	8.15E-05	2.247	0.07
	TDW	0	1	0	8.587	0.004	1.97E-05	4	4.94E-06	0.103	0.981	0	4	6.53E-05	1.36	0.254
	RMC	84.566	1	84.566	3.404	0.068	176.473	4	44.118	1.776	0.141	206.536	4	51.634	2.079	0.09
	SVI	18.827	1	18.827	0.798	0.374	82.143	4	20.536	0.87	0.485	25.914	4	6.479	0.274	0.894
3rd Harvest	RL	157.001	1	157.001	9.946	0.002	88.043	4	22.011	1.394	0.242	47.401	4	11.85	0.751	0.56
	SL	2.016	1	2.016	4.025	0.048	4.765	4	1.191	2.378	0.058	1.451	4	0.363	0.724	0.578
	TPL	1.392	1	1.392	0.25	0.618	113.766	4	28.441	5.113	0.001	18.296	4	4.574	0.822	0.514
	SD	6.40E-05	1	6.40E-05	0.159	0.691	0	4	0	0.282	0.889	0.001	4	0	0.861	0.49
	DRW	0	1	0	3.112	0.081	0	4	5.16E-05	1.214	0.31	0	4	9.67E-05	2.275	0.067
	DSW	0.003	1	0.003	19.682	0	0	4	5.21E-05	0.338	0.852	0	4	6.92E-05	0.449	0.773
	TDW	0.002	1	0.002	7.304	0.008	0.001	4	0	0.639	0.636	0.001	4	0	1.217	0.309
	RMC	945.132	1	945.132	7.897	0.006	205.157	4	51.289	0.429	0.788	195.996	4	48.999	0.409	0.801
	SVI	135.839	1	135.839	8.332	0.005	107.232	4	26.808	1.644	0.17	59.664	4	14.916	0.915	0.459

Table 2: Response/allelopathy Index (RI) showing responses of rice to L. camara leaf and stem aqueous extracts.

Parameters	Traits	Leaf Extract								Stem Extract							
		Crushed				Soaked				Crushed				Soaked			
		C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4
Germination	GP	0.0606	0.0474	0.0379	0.0396	-0.0035	-0.0666	-0.013	0.0183	-0.001	-0.033	-0.054	0.02	-0.024	-0.033	-0.013	-0.033
	GR	0.0293	0.0584	-0.0364	0.0727	-0.0882	-0.1211	-0.0486	0	-0.0377	-0.1365	-0.0752	0.0099	-0.0782	-0.0831	-0.0666	-0.0284
1st harvest	RL	-0.4025	-0.4506	-0.2203	-0.2119	0.2353	0.358	0.2201	0.208	0.1397	0.0669	0.0199	0.17	-0.281	-0.2042	-0.3516	-0.2171
	SL	-0.0796	-0.0628	-0.0719	-0.1157	-0.0281	-0.0276	-0.0863	-0.1367	-0.0331	-0.0175	-0.0372	-0.148	-0.0665	-0.0913	-0.0838	-0.1164
	TSL	-0.2198	-0.2308	-0.1363	-0.1681	0.0593	0.1681	0.0512	0.0164	0.0813	0.0254	0	0.0125	-0.1681	-0.1395	-0.2179	-0.1493
	SD	-0.0039	0.0304	-0.0016	-0.0039	0.0264	0.0305	-0.0602	-0.0827	-0.0319	-0.0467	-0.0538	-0.0621	0.0129	-0.0003	-0.0333	-0.0944
	DRW	-0.4467	-0.26	-0.106	-0.635	0.231	0.2412	0.2058	0.2043	0.1216	0.1837	0.2762	0.2494	-0.2277	-0.0759	-0.2788	-0.1752
	DSW	0.0443	-0.0262	0.1489	0.0269	22.0583	0.1006	-0.0417	0.0624	-0.0906	-0.0122	0.0236	-0.0306	-0.0154	0.063	0.02	-0.018
	TDW	0.0288	0.0009	0.1546	-0.0429	0.0796	0.0965	0.0971	0.0812	-0.0702	0.0211	0.073	0.0186	-0.0416	0.031	-0.0238	-0.0401
	RMC	0.0074	0.0234	-0.0935	-0.043	0.1077	0.133	0.0702	-0.103	-0.001	-0.0197	-0.0998	-0.1145	0.02	-0.0123	-0.0235	-0.0898
	SVI	-0.172	-0.1953	-0.1044	-0.1373	0.083	0.101	0.0305	0.0323	0.0917	-0.0371	-0.0588	0.0225	-0.1922	-0.1611	-0.2259	-0.1754
2nd harvest	RL	-0.0544	-0.0548	0.0431	0.1312	0.0366	-0.3707	-0.1952	0.1179	-0.0874	-0.171	-0.0543	-0.2473	0.0928	-0.1145	0.0087	0.0129
	SL	-0.0337	-0.0236	-0.0094	-0.0883	0.0231	-0.0805	0.0164	-0.0518	0.0506	-0.0149	-0.023	-0.1119	0.0636	0.053	0.0392	-0.0064
	TSL	-0.0141	0.007	-0.0298	0.118	0.0487	-0.2409	-0.0997	0.1123	-0.0285	-0.0897	-0.0175	-0.1933	0.1203	-0.0243	0.0576	0.0188
	SD	0.0171	0.0469	0.0526	0.0607	10.4646	-0.0147	-0.0086	-0.0557	0.0285	-0.0318	0.0074	-0.0603	0.0171	-0.0284	0.0396	-0.0033
	DRW	-0.653	-0.4607	-0.0743	0.0768	0.0437	-0.3206	0.0869	0.3172	-0.0523	0.001	0.0042	-0.0673	0.0199	-0.1563	-0.0686	0.0213
	DSW	-0.653	-0.4607	-0.0743	0.0768	0.0437	-0.3206	0.0869	0.3172	0.1758	-0.0543	0.0209	0.0217	-0.1576	-0.0009	-0.1377	-0.127
	TDW	0.0353	0.1474	0.1349	0.0887	-0.0452	-0.0259	0.0755	0.0316	0.143	-0.0191	0.0537	0.031	-0.1061	-0.0212	-0.1082	-0.0888
	RMC	-0.0815	-0.0011	-0.0054	-0.0207	0.036	0.009	-0.0072	0.0088	0.0274	0.0066	0.0128	-0.0766	0.0339	-0.004	0.0512	0.0357
	SVI	0.0302	0.0312	0.1688	0.5699	0.0363	-0.2907	-0.1156	0.1088	-0.0138	-0.1047	-0.0501	-0.1661	0.0947	-0.0559	0.0385	-0.0145
3rd harvest	RL	0.1194	0.0774	-0.0494	0.1691	-0.4007	-0.1051	0.2957	0.3333	0.2264	0.116	-0.2244	-0.0264	-0.1391	-0.0499	-0.2059	-0.2146
	SL	0.0053	-0.0292	-0.0605	-0.0895	0.0384	-0.0419	0.1085	-0.0378	0.025	-0.0739	-0.087	-0.1519	0.1063	-0.0108	0.0107	-0.0793
	TSL	-0.2301	0.0538	-0.0475	0.1076	0.2098	0.0218	0.2736	0.2206	0.1774	0.0667	-0.1448	-0.0549	-0.0891	-0.0281	-0.1259	-0.1716
	SD	0.0192	0.0237	0.0802	0.0401	0.0312	-0.0518	0.056	0.0162	-0.0174	0.001	0.0072	-0.0405	0.0096	-0.0281	-0.8384	0.0038
	DRW	0.0385	0.0304	0.0254	-0.088	0.3121	-0.1761	0.3564	0.2248	0.1558	-0.0964	-0.2873	-0.3167	-0.0774	0.0234	0.0947	-0.0464
	DSW	0.0088	0.0586	0.1241	0.1332	0.0522	-0.2142	0.1726	-0.0403	0.1021	-0.0033	-0.1259	-0.0591	-0.051	0.0198	-0.0658	-0.0385
	TDW	0.0451	0.2391	0.1539	0.0946	0.1254	-0.2061	0.2159	0.0149	0.1578	-0.0093	-0.1459	-0.1188	-0.0532	0.0233	-0.0267	-0.0351
	RMC	-0.0526	-0.0314	-0.0626	-0.0624	0.0088	0.0682	0.0074	-0.0351	-0.009	-0.0001	-0.0114	-0.0508	-0.1007	-0.0146	0.0058	-0.0203
	SVI	0	-0.0343	-0.0272	0.109	-0.0523	0.0093	0.1037	0.2026	0.1706	0.0341	-0.194	-0.0411	-0.0964	-0.0543	-0.1283	-0.1844

At 3rd harvesting, promotion of 3.8%, 3% and 2.5% in C_1, C_2 and C_3 concentration in dry root weight of crushed leaf extract and inhibition of 8.7% in C_4 was reported, whereas, in soaked leaf extract, the increment was observed to be: C_3 (35%), C_1 (31%) and C_4 (22%) and inhibition of 17.6% in C_2. Dry shoot weight of crushed leaf extract showed increment in order: C_4 (13.3%) > C_3 (12.4%) > C_2 (9.4%) > C_1 (4.5%), whereas, in soaked leaf extract inhibition of 21% and 4% in C_2 and C_4 was observed and increment of 17.2% and 5.2% in C_3 and C_1.Total dry weight in crushed leaf extract showed increment in order of 23.9%, 15.3%, 9.4% and 4.5% for C_2, C_3, C_4 and C_1, respectively, while in soaked leaf extract, the increment was recorded to be 21%, 12% and 1.4% in C_3, C_1 and C_4, respectively, and inhibition was 20.6% in C_2. During 1st harvest, crushed stem extract showed increase in dry root weight at all concentrations: 12.15% at C_1, 18.3% at C_2, 27.6% at $C_3$and 24.9% at C_4, whereas, soaked stem treatment showed inhibition at all concentrations: by 22.7% at C_1, 7.5% at C_2, 27.8% at $C_3$and 17.5% at C_4. Dry shoot weight was suppressed at C_1 (0.09%), C_2 (0.01%) and C_4 (0.03%) and promoted it at C_3 (0.02%) in crushed stem extract, whereas, soaked stem extract showed inhibition at C_1 (1.5%) and C_4 (1.8%) and increment in C_2 (6.3%) and C_3 (1.99%). Total dry weight was repressed at lower concentration: C_1 (7.02%) and enhanced at higher concentrations: C_2 (2.11%), C_3 (7.29%) and C_4 (1.86%) in case of crushed stem treatment, whereas, soaked stem treatment inhibited the total dry weight by 4.16% at C_1, 2.3% at C_3 and 4% at C_4 and promoted at C_2 by 3.09%. At 2nd harvest, crushed stem extract showed inhibitory effect on dry root weight at C_1 (5.22%) and C_4 (6.73%) and promoted it at C_2 (2.5%) and C_3 (0.41%), whereas, soaked stem extract showed inhibition at C_2 (15.63%) and C_3 (6.85%) and increment at C_1 (1.99%) and C_4 (2.12%). Dry shoot weight was promoted by crushed stem extract at C_1 by 17.58%, C_3 by 2.09% and C_4 by 2.16% and inhibited at C_2 by 5.43%, however, soaked stem treatment showed considerable inhibition at all concentration: C_1 (15.75%), C_2 (0.08%), C_3 (13.76%) and C_4 (12.7%)

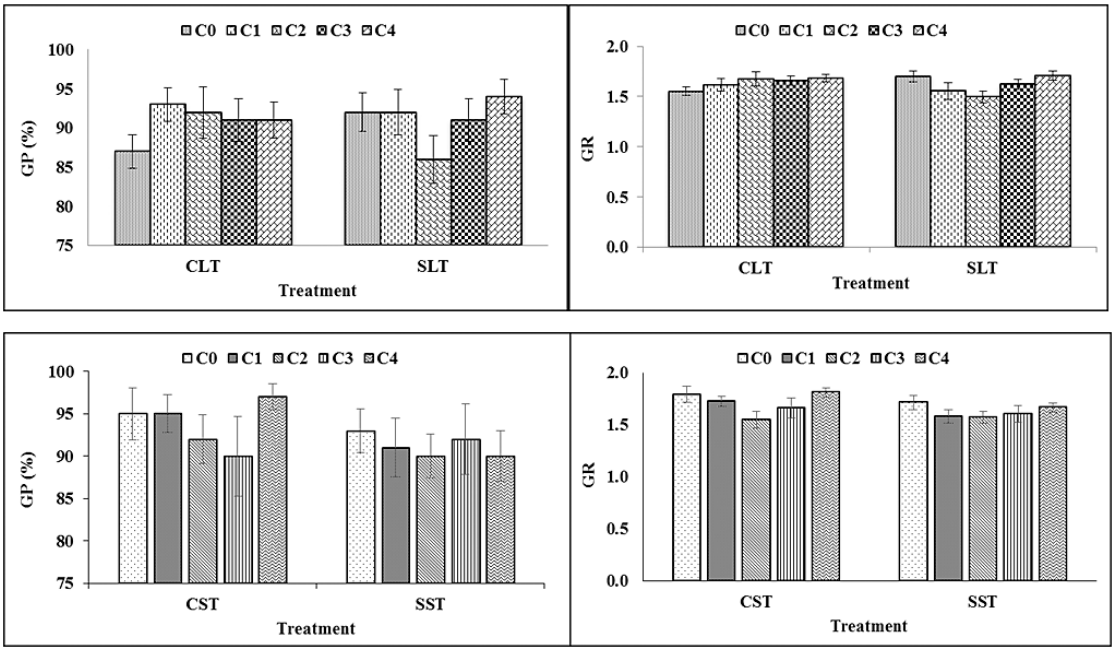

Fig. 1: Effect of crushed and soaked leaf and stem extract on seed germination of rice.

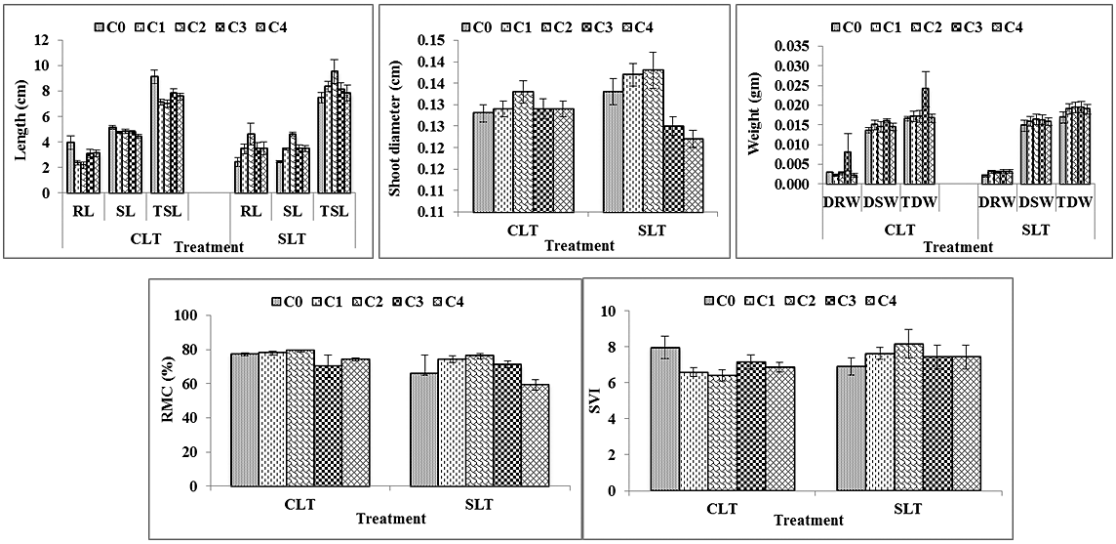

Fig. 2: Effect of crushed and soaked leaf extract on plant traits at first harvest.

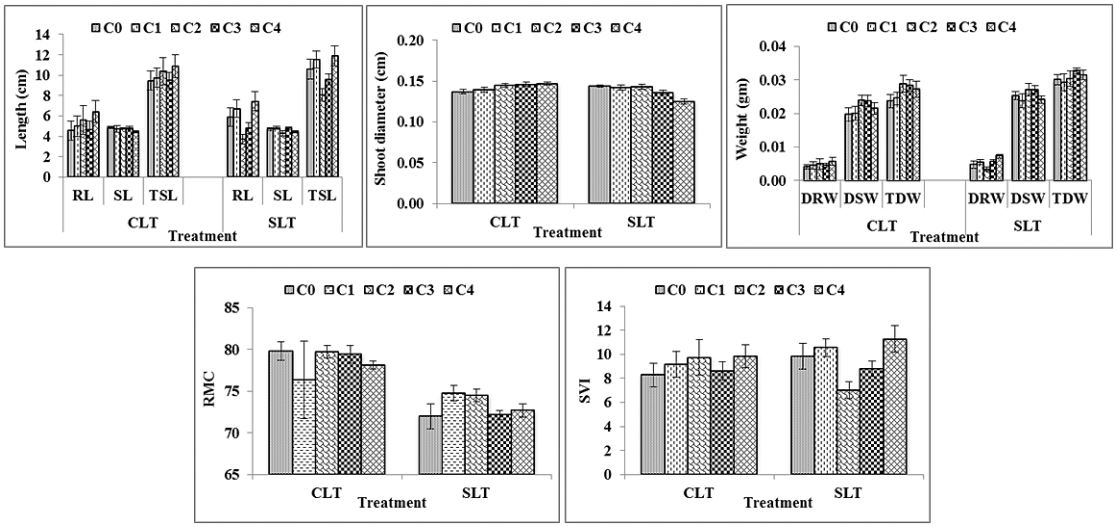

Fig. 3: Effect of crushed and soaked leaf extract on plant traits at second harvest.

Crushed stem extract showed increment in total dry weight at C_1 (14.3%), C_3 (5.37%) and C_4 (3.09%) and inhibition at C_2 (1.9%), whereas, soaked stem extract suppressed total dry weight at all concentrations: C_1 (10.6%), C_2 (2.11%), C_3 (10.81%) and C_4 (8.87%). At 3rd harvesting, the dry root weight was observed to be promoted at lower concentration in crushed stem extract i.e. C_1 (15.57%) and inhibited at higher concentrations: C_2 (9.63%), C_3 (28.72%) and C_4 (31.6%), whereas, soaked stem treatment showed inhibition at C_1 (7.73%) and C_4 (4.64%) and increment at C_2 (2.34%) and C_3 (9.47%) at 3rd harvest. Dry shoot weight was inhibited at higher concentrations: C_2 (0.33%), C_3 (12.5%) and C_4 (5.9%) and promoted at lower concentration: C_1 (10.21%) in crushed stem extract, whereas, soaked stem extract showed inhibition at C_1 (5.1%), C_3 (6.57%) and C_4 (3.84%) and increment at C_2 (1.98%).

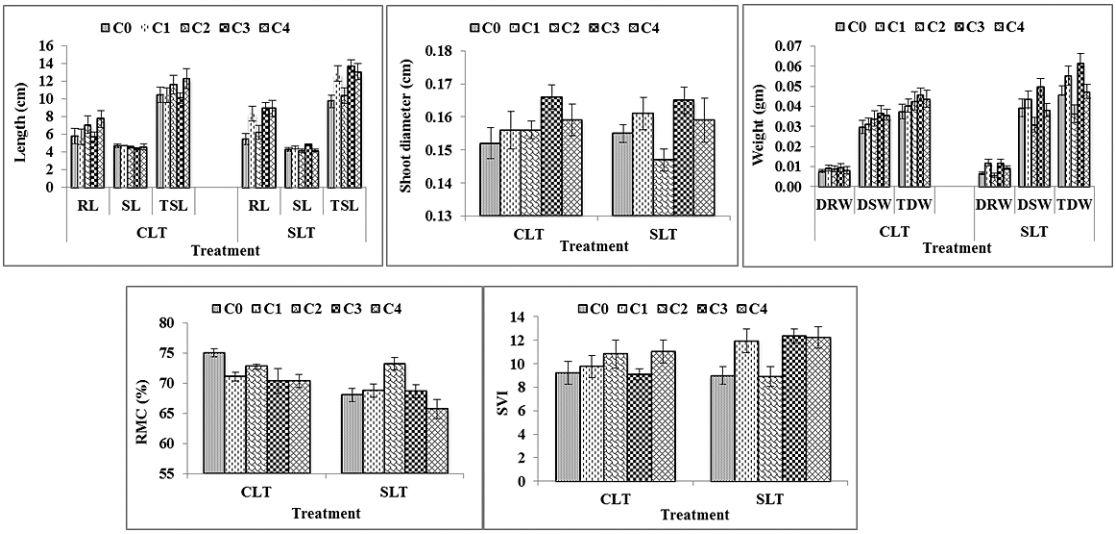

Fig. 4: Effect of crushed and soaked leaf extract on plant traits at third harvest.

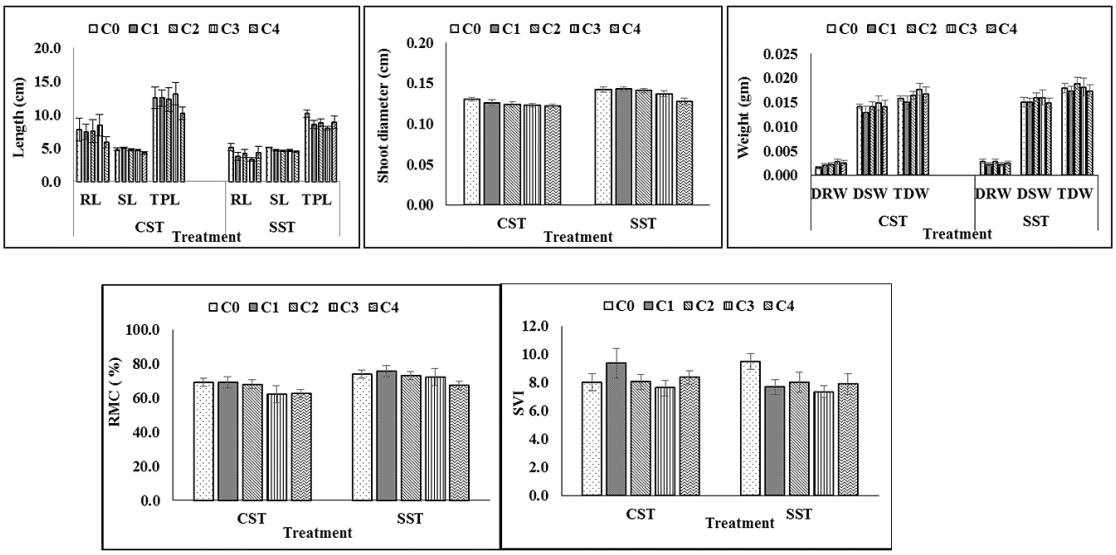

Fig. 5: Effect of crushed and soaked stem extract on plant traits at first harvest.

Same trend was observed in case of total dry weight as crushed stem treatment showed inhibition in at C_2 (0.92%), C_3 (14.58%) and C_4 (11.87%) and increment at C_1 (15.78%), whereas, soaked stem extract inhibited the total dry weight at C_1 (5.31%), C_3 (2.67%) and C_4 (3.51%) and promoted at C_2 (2.32%). Roots showed stronger and sensitive responses towards *L. camara* leaf extracts than shoots. This could be due to the close contact of roots with the extract solution which was added to the soil.[15] Individual plant roots grow through soil following beneficial mechanical and moisture gradients in order to obtain water and nutrients.[32-33] By applying localized suction around the root-soil interface, plant roots pull water towards them.[34] Cell growth is a biomechanically irreversible process in which wall stress relaxation, water uptake, wall expansion and turgor restoration are closely connected.[35] There may be possible damage to plasma membrane due to seed pretreatment with the leaf extracts and leachates of *L. camara* can be demonstrated from the higher dissolution of amino

acids and soluble carbohydrates from the water imbibed seeds.[36] However, without any deposition of new materials into the existing wall during growth, the cell would eventually burst as a result of wall thinning and subsequent mechanical failure.[35] Abiotic stress causes changes to cell wall structure,[37-38] which can be sensed by cell wall integrity sensors. The reason behind the fluctuating results was the various phytochemicals like aromatic alkaloids and phenolic content released from soaked and crushed leaf extracts which may alter the physiochemical properties of soil that may have influenced the plant traits (root length, shoot length, fresh and dry biomass).[39] The secondary metabolites present in *L. camara* such as phenolics, with umbelliferon, methyl coumarin and salicylic acid being the most toxic one.[40]

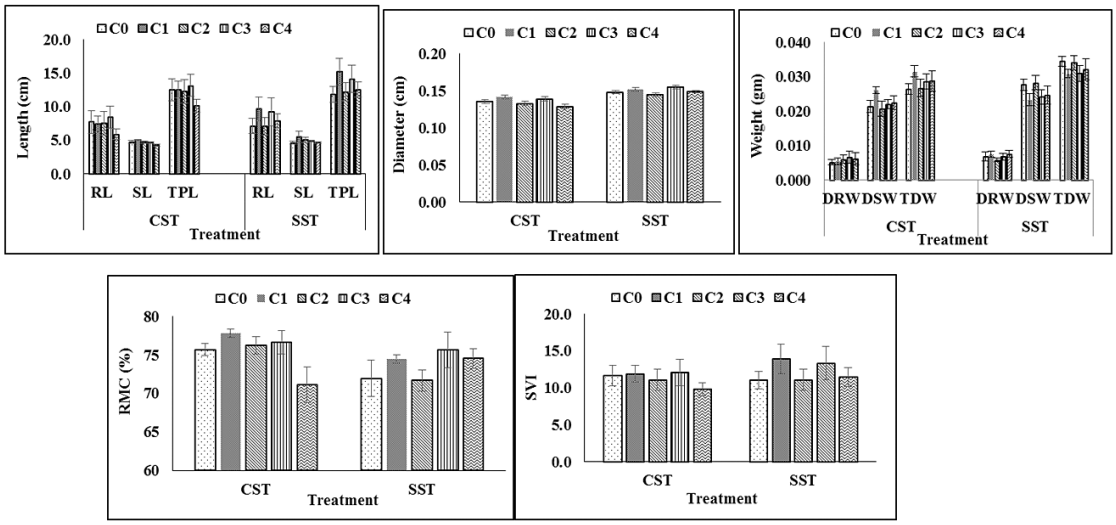

Fig. 6: Effect of crushed and soaked stem extract on plant traits at second harvest.

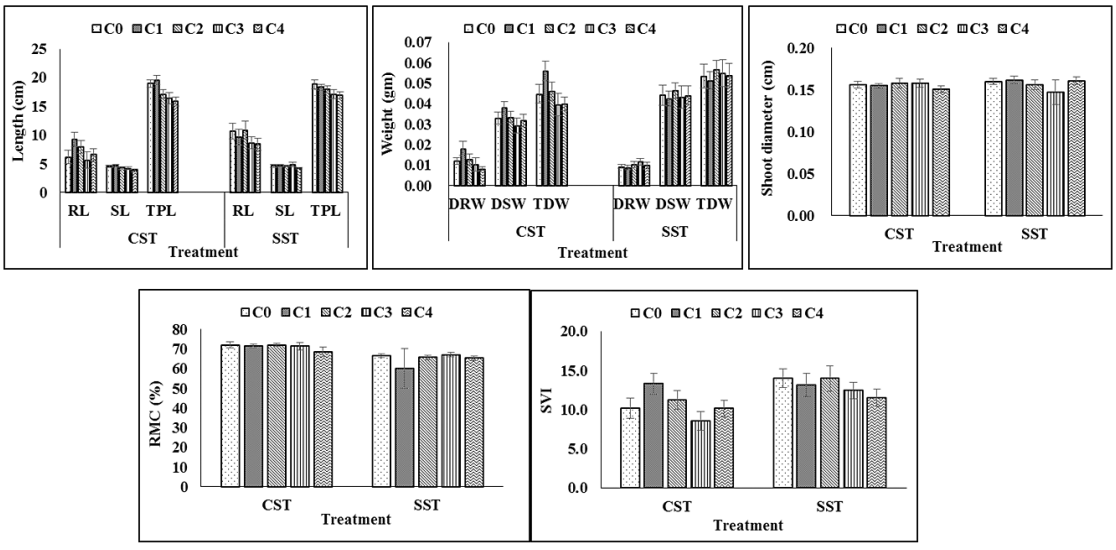

Fig. 7: Effect of crushed and soaked stem extract on plant traits at third harvest.

Some of them are: lantadene a and b, icterogenin, oleanoic acid, ursonicacid, 4-epihederagonic acid, 24-hydroxy-3-oxours-12-en-28-oic acid, lantanolic acid, lantanone, lantanilic acid, lantic acid, camarilic acid, camaracinic acid, camarinic acid, camaryolic acid,ursoxy acid, camarolide, linaroside, lantanoside, martynoside, α-phellandrene, dipentene, α-terpinol, geraniol, cineol, eugenol, citral, furfural, phellandrone, linalool.[13,15] There are wide range of phenolic compounds which are often mentioned as assumed allelochemicals, in plants and soils. These phenolic compounds depicted inhibition in concentration dependent manner.[18,41] Previous researchers[27] assessed that plant phenolic acids occurrence and behavior in soil microenvironments results into its potential involvement in allelochemical interference interactions. Phenolic compounds have the ability to alter and influence several enzymatic activities and major physiological processes, such as plant hormone functions, nutrients uptake, water balance and stomatal functions, photosynthesis, respiration, and the metabolism of certain compounds and carbon flow.[42-43] Aqueous leaf extract recorded maximum inhibitory effect followed by stem and root extract to selected crop species.[44] Allelochemicals released into the soil can change soil properties, in turn affecting the composition and diversity of soil microbial community.[45-46] They alter soil pH and change the microbial community activity, thereby can modify the native plant nutrient uptake.[2,9]

At 1st harvest, crushed stem extract showed decrease in relative moisture content in comparison to control and showed proportional relation in inhibition with increasing extract concentrations i.e., C_1 (0.1%) < C_2 (1.97%) < C_3 (9.97%) < C_4 (11.4%), in contrast, soaked stem extract promoted relative moisture content at C1(1.99%) and inhibited at C_2, C_3 and C_4 by 1.22%, 2.34% and 8.9%, respectively. At 2nd harvest, relative moisture content was promoted by crushed stem extract at lower concentrations i.e., C_1 (2.47%), C_2 (0.65%), C_3 (1.27%) and inhibited at the highest concentration: C_4 (7.65%), whereas, soaked stem extract promoted relative moisture content at C_1 (3.38%), C_3 (5.11%), C_4 (3.57%) and inhibited at C_2 (0.39%). Crushed stem extract showed inhibition in relative moisture content at 3rd harvest at all concentrations- C_1 (0.9 %), C_2 (0.01%), C_3 (1.13%) and C_4 (5.08%), whereas, soaked stem extract promoted relative moisture content at C_3 by 0.58% and inhibited at C_1, C_2 and C_4 by 10.06%, 1.46% and 2.02%, respectively.

Effect of Plant Part and Extract type on Seed Vigor index

Seed vigor index in crushed leaf extract showed inhibition of 19.5%, 17.1%, 13.7% and 10.4% in C_2, C_1, C_4 and C_3, respectively.

The soaked leaf extract increased seed vigor index of rice by 10%, 8.3%, 3.2% and 3% in C_2, C_1, C_4 and C_3, respectively. When compared to 1st and 2nd harvest, crushed leaf extract showed increment in order of C_4 (56.9%) > C_3 (16.8%) > C_2 (3.1%) > C_1 (3%), while, soaked leaf extract showed inhibition at C_2 (29%) and C_3 (11.5%) and increment at C_4 (10.8%) and C_1 (3.6%) for seed vigor index. At 3rd harvest, crushed leaf extract showed inhibition in C_2 (3.4%), C_3 (2.7%) and C_1 (0.0%) and increment in C_4 (10.9%), while, in soaked leaf extract, C_4 (20%), C_3 (10.3%) and C_2 (0.9%) showed increment while suppression of 5.2% was observed in C_1 for seed vigor index. At 1st harvest, seed vigor index was promoted by crushed stem extract at C_1 (9.17%) and C_4 (2.24%) and inhibited at C_2 (3.7%) and C_3(5.8%), whereas, soaked stem extract inhibited seed vigor index at all the concentrations- C_1 (19.21%), C_2 (16.1%), C_3 (22.5%) and C_4 (17.5%). At 2nd harvest, crushed stem extract suppressed seed vigor index at all the concentrations: C_1 (1.37%), C_2 (10.46%), C_3 (5%) and C_4 (16.61%), whereas, soaked stem extract promoted seed vigor index in C_1 (9.46%) and C_3 (3.85%) and inhibited in C_2 (5.59%) and C_4 (1.44%). After 3rd harvesting, seed vigor index was observed to be promoted by crushed stem extract at lower concentrations i.e. C_1 (17.05%) and C_2 (3.4%) and inhibited it at higher concentrations: C_3 (19.4%) and C_4 (4.1%), however, soaked stem extract inhibited seed vigor index at all concentrations: C_1 (9.64%), C_2 (5.42%), C_3 (12.83%) and C_4 (18.44%).

The soaked leaf extract suppressed rice seed germination at higher (C_4) concentration, while, in crushed leaf extract stimulated. If we compare the results according to harvesting time in leaf either the crushed or soaked extracts all the measured plant traits i.e., length, biomass, relative moisture content and seed vigor index exhibited differential pattern of inhibition or increment. As also reported by[47] all the plant traits such as length, biomass, moisture

content and seed vigor index illustrated fluctuating pattern along the concentration gradient. At second and third harvest, relatively lower suppression was recorded in the majority of studied plant traits as compared to first harvesting. This could be due to the adaptation of seedlings towards different concentrations of both crushed as well as soaked leaf treatment after experiencing its environment during the initial developmental phases.[48] On the other hand, soaked stem extract had more inhibitory effects on seed germination, seedling biomass and seed vigor index, whereas, crushed stem treatment was more suppressive on the traits like seedling growth and relative moisture content.

Soaked treatment resembles the allelopathic nature of plants in soil, since many allelochemicals are leached out of living plants or plant residue by rain or dew.[49-50] The soaked stem extract demonstrated with maximum suppression in comparison to crushed stem extract could be due to the storage function of stem. As stem stores the nutrients and these nutrients would leach out during crushing, whereas, soaking would only release the phytochemicals which are present in either extracellular matrix or cell wall components without disrupting the cell organelles which store the photosynthetic and bioactive compounds.[28] This study demonstrated that the aqueous soluble allelochemicals of *L. camara* decrease the initial growth of rice variety depicts that the effects are concentration-dependent.[51,52] According to three harvestings performed the last showed more inhibitory effects for plant traits, plant growth, biomass, moisture content and vigor index either for crushed or for soaked stem treatment. Previous experiments conducted indicate that invasive plants increase utilization of nutrients with allelopathic substances that also inhibit native plant growth and decrease the plant biomass.[8,53,54]

Conclusion

Experimental results indicated suppressive as well as promoting effects of aqueous stem extracts of *L. camara* on seed germination and seedling growth of rice plants. More pronounced inhibitory effects on measured traits were observed for soaked stem extract as compared to the crushed stem extract. In general, the lower aqueous extract concentration recorded increment in the studied traits while higher aqueous extract concentrations suppressed the germination and early seedling growth of selected rice variety. In this study with increasing time, the rice crop showed adaptation towards the allelochemical released from the aqueous extracts of leaves as indicated by insignificant effect at 2nd harvest. But in case of stem after second harvesting both crushed and soaked aqueous extract showed inhibition for every plant trait. Even though, laboratory experiments are important to demonstrate the allelopathic affects it necessitates in investigating the significance of these results under field conditions. Besides, isolation and identification of allelochemicals released by the *L. camara* plants may help in assessing the distinctive role of specific chemical on crop plant. Further field investigations are required to test the crops against the *L. camara* weed extracts considering other growth parameters and yield attributes. Ultimately this research will help to contribute the deeper understanding of the key factors influencing the allelochemical interactions and actual mechanisms involved in the differential effects of the stem allelochemicals on different agricultural fields.

Acknowledgement

We are thankful to the Head, Department of Botany, D.S.B. Campus, Nainital for providing necessary lab facilities.

Funding Sources

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors have no conflict of interest to disclose.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that required ethical approval.

Author contributions

- **Vartika Joshi:** Methodology, Writing – Original Draft, Data Collection, Analysis, Writing – Review & Editing.
- **Charu Joshi:** Methodology, Writing – Original Draft, Data Collection, Analysis, Writing – Review & Editing.

- **Archana Fartyal:** Writing – Review & Editing, Analysis.
- **Kiran Bargali**: Analysis, Visualization and Supervision.
- **Surendra Singh Bargali:** Resources, Analysis, Visualization and Supervision.

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