



Effect of Leaf Extracts of *Lantana Camara* L. on Germination and Growth of Some Crops Species

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Abstract

Allelopathy is a biotic interaction between plants where one plant inhibits the germination and growth of other plants by releasing certain allelochemicals. In this research we evaluated the allelopathic effect of aqueous leaf extract of Lantana camara on the germination and growth of four crop plants. The experiment was conducted in sterilized petri dishes in Botany lab of Saptagandaki Multiple Campus, Bharatpur, Chitwan, Nepal. The effect of different concentrations of extracts was studied and compared with the control. The results revealed that the germination and seedling growth of all species were significantly inhibited at different concentrations as compared to the control. The lowest germination percentage and seedling growth was found in Brassica campestris and Oryza sativa plant respectively and effect was least in Triticum aestivum plant at 10% concentration. The germination percentages, seedling growth and, biomass in all species were observed to reduce, with the increasing concentration of aqueous extracts. The study concluded that Lantana camara leaf extract allelochemicals have adverse effects on germination, seedling growth and dry matter accumulation of all tested crops.

Keywords: Allelochemicals, *Lantana camara*, allelopathic, germination rate, aqueous extract.

Introduction

Biological invasion is one of the key drivers hindering agriculture production, causing biodiversity loss, and negatively impacting ecosystem services (Rai & Singh 2020). Agricultural lands are one of the heavily invaded areas by an alien (invasive) weeds and its abundant growth has been reported on the croplands (Khatri *et al.*, 2022). Invasive plants species produce large numbers of offspring, with greater chances of spreading widely (Ratnayake, 2015) and liberate allelochemicals into the soil reduce

crop germination and growth (Hoque *et al.*, 2003). Invasive species are considered as rich source of allelochemicals and are released into the rhizosphere of the plants by rainfall leachates, decomposition of plant residues, root exudation, and volatilization from living plants parts (Rich, 1984; Bonanomi *et al.*, 2006). Allelochemicals have various effects including declining rate of germination, reducing the growth of root and shoot of seedling, injury to root tips, reduction the chlorophyll content and, increase in crop sterility (Bhadoria, 2011). The allelopathic action of stimulatory and inhibitory is dependent on the concentration (Hill *et al.*, 2006). Higher concentration of allelochemicals have been observed to have inhibitory effect (Singh, 2019) while lower concentrations exert stimulatory allelopathic impact on seed germination and growth of plant (Sahoo *et al.*, 2010).

Lantana camara is a fast growing woody shrub of verbenaceae family and is native to tropical and subtropical America (Zalucki *et al.*, 2007). It was introduced in Nepal accidentally and spread over large areas of land that has a significant impact on agriculture production (Shrestha, 2016) Several allelochemicals such as phenolic compounds, sesquiterpenes, triterpenes, flavonoid; Lantadene A and Lantadene B were isolated from leaves (Kong *et al.*, 2006) and able to inhibit germination, seedling establishment, and plant growth of agricultural crops (Kato & Kurniadie, 2021). These allelochemicals released from *Lantana camara* may provide the species with a competitive advantage against the native plants and may also suppress the rejuvenation process of native plant species and contribute to establish their habitats as invasive plant species (Kato & Kurniadie, 2021). Allelochemicals has suppressed the growth of plant species grown next to it which is a serious problem in agricultural land and no remedy is yet found. Allelopathic activities of *Lantana camara* on variety of species, have so far been done, however such work is not sufficient yet. This research aims to explore the response of allelochemical on seed germination and seedling growth of common cultivated crop species.

Materials and Methods

This study was done in the Department of Science Laboratory of Saptagandaki Multiple Campus, Tribhuvan University, Bharatpur, Chitwan, Nepal. Bharatpur has tropical monsoon climate with humidity all through the year. The area is located in the south-central lowland area of Nepal in the coordinates of 27°34'N to 27°45'N and

84° 19'E to 84° 29'E with altitudinal variation of 181 to 271 m. The temperature rises up to 34°C in the summer (May) and decreases to 7°C in the winter (January) with 1,993 mm average annual rain fall (<https://www.weather-atlas.com/en/nepal/bharatpur-climate>). The typical vegetation is broadleaf forest with predominantly Sal (*Shorea robusta*) tree covering about 70% of the National Park.

Seed Collection and Sterilization

Healthy uniform seeds of *Oryza sativa* L. (Rice), *Triticum aestivum* L. (Wheat), *Brassica campestris* L. (Mustard), *Lens culinaris* Medik. (Musuro) were used as test plants for initial screening of species to check allelopathic effect. Before germination test the seeds were surface sterilized with 5% alcohol for 20 min, then rinsed with the distilled water for several times to remove excess alcohol. The seeds varieties for experiments were verified by Agro Ecology Lab of the AFU (Agriculture and Forestry University), Rampur, Chitwan, Nepal.

Study Species Characters

Lantana camara L. (Verbenaceae) is a woody shrub native to Central and South America and is regarded as one of the ten worst invasive species in the world (Richardson & Rejmanek 2011). It was introduced as an ornamental shrub berry plant in India, from where it escaped and became invasive in India and Nepal. The plant is profusely branched and grows up to 2–4 meters high in open un-shaded sunny environments, and as a liana up to 15 m when light intensity is low (Lowe *et al.*, 2000). It is shade-tolerant with long petioles, oblong blades, hairy, and serrate leaves. Flowers are tiny, multicolored, and densely clustered in flat-topped clusters and produce 10,000–12,000 fruits and are very commonly distributed across the forests and agricultural land of Nepal.

Collection of Plant Material

Fresh *Lantana camara* leaves in vegetative stage were collected in March, 2022 from the edge of agricultural field around the Bharatpur Metropolitan city -7 Prembasti, Chitwan (GPS Location: latitude 27. 3857°N and longitude 84. 2445° E.). The leaves were washed thoroughly with tap water to remove dust particles and allowed to air dried at room temperature in the shade for 60 hours. The dried leaves were then sealed in a Zipper bag to keep them air tight. The air dried leaves were ground into a

fine powder with the help of grinder and then was stored in air tight plastic bag at room temperature before used for experiments.

Preparation of Aqueous Extract

Fifty grams of air-dried grounded leaves of *Lantana camara* were soaked in 500 ml of distilled water (considered as 100% concentration) and kept airtight and left for 24 hrs. in the existing dark room temperature (average during the day, 25°C) for extraction. The aqueous extract was obtained as filtrate by using Whatman's filter paper of the mixture and the final volume of the collected filter was adjusted to 500ml; this gave 10% aqueous extract. The extract was considered as stock solution and a series of solutions with different strengths (2, 4, 6 and 8%) were prepared by dilution.

The following treatments were followed during the experiment:

- Control:** Seeds grown in distill water only;
- T₁:** Seeds grown in extracts of 2% concentration;
- T₂:** Seeds grown in extracts of 4% concentration;
- T₃:** Seeds grown in extracts of 6% concentration;
- T₄:** Seeds grown in extracts of 8% concentration;
- T₅:** Seeds grown in extracts of 10% concentration.

Germination Test and Growth Records

For the germination and growth record, selected healthy seeds were rinsed with distilled water. Seeds were evenly distributed on the sterilized petri dish (150 mm×15 mm) which was double-lined with Whatmann's filter paper. The adjustment was wetted with 10 ml of different concentrations of stock solution (2 to 10%), including one as control with only distilled water. Ten uniform and surface sterilized seeds of *Oryza sativa* (Rice), *Triticum aestivum* (Wheat), *Brassica campestris* (Mustard) and, *Lens culinaris* (lentil) were placed for germination. Each treatment had three replicas (total number of seeds 10×3=30). The entire experiment was conducted in a laboratory setting (Room temperature of 25°C during the day). When the moisture of the filter paper began to decline, an equivalent volume of distilled water was added to the dishes. The experiment was extended over a period of seven days (1 week) to allow the last seed germination and the measurement of the shoot and root length. A seed was considered germinated when a radicle emerged. Germinated seeds were counted daily

and the total number of seeds germinated at various concentrations was recorded till the last day.

The length of the root and shoots were measured at the end of the experiment. And those roots and shoots were separated and their wet weight was noted down separately and kept in a hot air oven at 70°C temperature for 48 hours and their dry weight was taken.

Statistical Analysis

The results were quantified as Germination percentage, Relative germination ratio, Germination inhibition rate, root and shoot length growth and seedling biomass by Rho & Kil (1986).

Germination Percentage

The germination percentage was calculated by the using the following formula.

$$\text{Germination percentage (\%)} = \frac{\text{No. of seeds germinate}}{\text{Total seeds}} \times 100$$

Relative Germination Ratio

The relative germination ratio was calculated by the following equations.

$$\text{Relative germination ratio (R)} = G/G_r \times 100$$

Where, R is the relative germination ratio, G the germination ratio of tested plant, and G_r is the germination ratio of control.

Germination Inhibition Rate

For the calculation of percentage of inhibitory (or stimulatory) effect on germination and growth parameters of treatment plants to control, we used the following formula:

$$I = 100 - (E_2 \times 100/E_1)$$

Where, I is the % inhibition (or stimulation); E_1 the response of control plant, and E_2 the response of treatment plant.

Growth Parameters

The growth parameters such as shoot length and root length were recorded at 7 days using a centimeter scale. The seedling length was calculated by using formula:

Biomass

All root and shoot from each seedling were cut separately and oven dried at 70°C for 48 h to get dry biomass of root and shoot; total seedling biomass of seedling was calculated as the sum of biomass of root and shoot.

The data collected from the experiments were subjected to descriptive statistics to calculate mean, standard error of germination and growth records. Significance of the difference in root and shoot length of seedlings under different treatments were tested and compared using Analysis of Variance (ANOVA) and Tukey multiple comparison test. All statistical analyses were done using Statistical Package for Social Sciences (IBM SPSS statistics version 25).

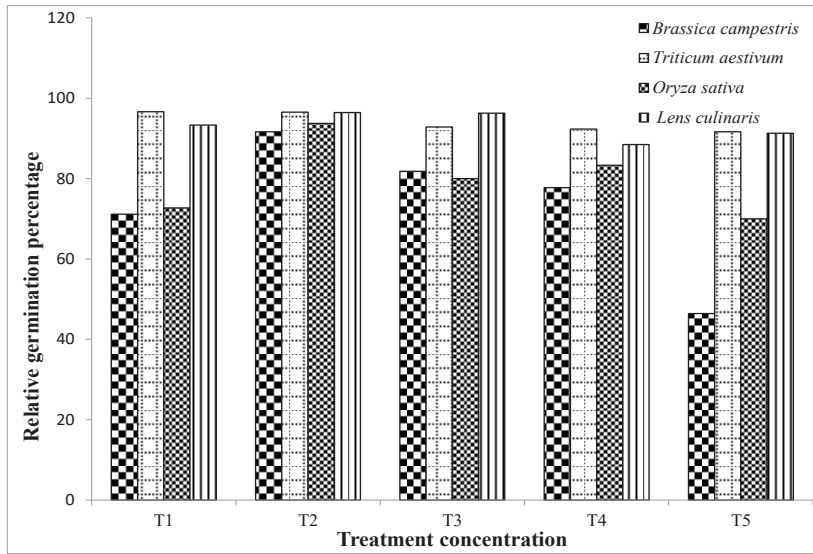
Results and Discussion

Germination

The effects of aqueous extracts of *lantana camera* leaf on seed germination of the agricultural crops as compared to the control are shown in (Table 1). The aqueous extract inhibited the germination of all species. With the increase in concentration, the inhibitory effect gradually increased. In all species, the maximum inhibitory effect was found at 10% concentration as compared to others. The results further indicated that maximum germination was observed on *Triticum aestivum* and, *Lens culinaris* and, minimum germination was noted on *Oryza sativa* and, *Brassica campestris* (Table.1) The inhibition of germination was found strong in the *Brassica campestris* (16.66 %) and, *Oryza sativa* (23.33 %). The maximum relative germination ratio was found in *Triticum aestivum* (96.66%) at 2% treatment while the minimum was (46.42%) in *Brassica campestris* at 10% treatment (Figure 1).

Figure 1

Relative germination percentage of studied species under different treatment of leaf aqueous extract of *Lantana camara*



T_1 : Seeds grown in extracts of 2% concentration; T_2 : Seeds grown in extracts of 4% concentration; T_3 : Seeds grown in extracts of 6% concentration; T_4 : Seeds grown in extracts of 8% concentration; T_5 : Seeds grown in extracts of 10% concentration.

Table 1

Effect of aqueous extract of *Lantana camera* leaf on germination of different plant species measured after seven days.

Treatment	<i>Brassica campestris</i>	<i>Triticumaestivum</i>	<i>Oryza sativa</i>	<i>Lens culinaris</i>
Control	86.66 a*	99.53 a	73.33 a	99.67 a
T_1	40 b (-53.84)	96.66 a (-3.33)	53.33 a (-27.27)	93.33 ab (-6.66)
T_2	36.66 bc (-8.33)	93.33 a (-3.44)	50 b (-6.25)	90 ab (-3.57)
T_3	30 bc (-18.18)	86.66 a (-7.14)	40 a (-20)	86.66 ab (-3.70)
T_4	23.33 c (-22.22)	80 a (-7.69)	33.33 a (-16.66)	76.66 ab (-11.53)
T_5	16.66 c (-28.57)	73.33 a (-8.33)	23.33 b (-30)	70 b (-8.69)

Control: Seeds grown in distill water only; **T1**: Seeds grown in extracts of 2% concentration; **T2**: Seeds

grown in extracts of 4% concentration; **T3**: Seeds grown in extracts of 6% concentration; **T4**: Seeds grown in extracts of 8% concentration; **T5**: Seeds grown in extracts of 10% concentration.

*Values in the columns followed by the same letter (s) are not significantly different ($P \leq 0.05$) according to Tukey multiple comparison test. Values in the parenthesis indicate the inhibitory effects in comparison to control treatments.

Seedling Growth

The seedling growth was greatly altered with the increased concentration, decreased the growth of root and shoot length by the aqueous extract of *Lantana camara* leaf. Those, the overall growth on extract concentration were lower than in control. The root length growth of crop seedlings was more suppressed than their shoot length growth. There was a significant difference $p \leq 0.000$ between treatments in root and shoot length of all tested species (Table 2). Among crops, seedling growth of *Oryza sativa* and *Brassica campestris* were proved to be more vulnerable to allelochemicals of *Lantana camara* leaf aqueous extracts.

Table 2

Analysis of Variance (ANOVA) in root and shoot length of different plant species among different treatments of leaf aqueous extract of Lantana camera

Plant species	Parameters	F	Sig.
<i>Brassica campestris</i>	Shoot length	11.285	$p < 0.0001$
	Root length	12.942	$p < 0.0001$
<i>Triticumaestivum</i>	Shoot length	6.385	$p < 0.0001$
	Root length	26.512	$p < 0.0001$
<i>Oryza Sativa</i>	Shoot length	12.631	$p < 0.0001$
	Root length	14.311	$p < 0.0001$
<i>Lens culinaris</i>	Shoot length	47.497	$p < 0.0001$
	Root length	40.701	$p < 0.0001$

F is degree of freedom for all treatments.

Shoot Length

The aqueous extract of *Lantana camara* leaf inhibited the shoot length growth of all tested seeds. The shoot length was gradually decreased with increased concentration

of *Lantana camera* leaf extract. The inhibitory effect was more pronounced at 10% treatment followed by 8%, 6%, 4% and 2% treatments respectively. The statistical analysis shows that aqueous extracts of leaf significantly decrease the shoot length growth as compared to the control. The minimum shoot length was noted in *Oryza sativa* i.e. 2.13cm in control and almost negligible in 10% concentration. The maximum shoot length was found in *Triticum aestivum*, *Lens culinaris* and *Brassica campestris* respectively after control at 2% and while increasing the concentration of extracts, shoot length was decreased significantly (Table 3).

Table 3

Effect of aqueous extract of Lantana camera leaf on shoot length of different plant species measured after seven days

Treatments	<i>Brassica campestris</i>	<i>Triticum aestivum</i>	<i>Oryza sativa</i>	<i>Lens culinaris</i>
Control	7.93 ± 0.52 a*	11.4 ± 0.58 a	2.13 ± 0.17 a	10.21 ± 0.54 a
T ₁	7.73 ± 0.67 b (2.52)	10.73 ± 0.67 a (5.87)	1.87 ± 0.18 b (12.20)	9.4 ± 0.48 a (7.93)
T ₂	7.50 ± 0.69 b (5.24)	10.57 ± 0.67 a (9.47)	1.2 ± 0.12 bc (43.66)	8.33± 0.56 b (18.41)
T ₃	5.4 ± 0.43 bc (31.90)	7.5 ± 0.71 a (34.59)	0.86 ± 0.11 bc (59.62)	5.57 ± 0.58 b (45.44)
T ₄	3.07 ± 0.22 c (61.28)	7.0 ± 0.71 ab (38.59)	0.43 ± 0.03 c (79.81)	5.46 ± 0.40 b (46.52)
T ₅	2.9 ± 0.39 bc (63.43)	6.81 ± 0.87 b (40.26)	0.4 ± 0.04 c (81.22)	1.93 ± 0.27 c (81.09)

Control: Seeds grown in distill water only; **T1:** Seeds grown in extracts of 2% concentration; **T2:** Seeds grown in extracts of 4% concentration; **T3:** Seeds grown in extracts of 6% concentration; **T4:** Seeds grown in extracts of 8% concentration; **T5:** Seeds grown in extracts of 10% concentration.

*Values in the columns followed by the same letter (s) are not significantly different (P≤0.05) according to Tukey multiple comparison test. Values in the parenthesis indicate the inhibitory effects in comparison to control treatments. ± represent standard errors, n = 30.

Root Length

The root length growth was inhibited with the increased concentration of *Lantana camara* leaf aqueous extract. The inhibitory effect was prominent at 10% treatment in all tested species. The results indicated that extract caused root length reduction of all four species as compared to the control (Table 4). The highest root length inhibition was exhibited by *Oryza sativa*, followed by *Brassica campestris* with increasing the concentration of aqueous extract. The maximum root length was shown in *Triticum aestivum* (10.03cm), *Lens culinaris* (7.1cm), *Brassica campestris* (6.1cm) and, *Oryza sativa* (4.86cm) in control, whereas minimum root length growth was found in *Oryza sativa* (0.57 cm), *Brassica campestris* (0.83 cm), *Lens culinaris* (1.13 cm) and, *Triticum aestivum* (5.41 cm) in 10% concentration.

Table 4

Effect of aqueous extract of Lantana camera leaf on root length of different plant species measured after seven days

Treatments	<i>Brassica campestris</i>	<i>Triticumaestivum</i>	<i>Oryza sativa</i>	<i>Lens culinaris</i>
Control	6.1±0.47 a*	10.23±0.68 a	4.86±0.33 a	7.1±0.13 a
T ₁	3.8±0.55 b (37.70)	10.03±0.67 a (1.95)	4.06±0.32 b (16.46)	6.76±0.42 a (4.78)
T ₂	3.26±0.5 bc (46.55)	9.61±0.67 a (6.06)	1.67±0.19 bc (65.63)	3.6±0.34 b (49.29)
T ₃	2.27±0.18 c (62.78)	8.46±0.68 a (21.22)	1.5±0.15 bc (69.13)	2.7±0.34 b (61.97)
T ₄	1.27±0.09 c (79.18)	8.20±0.69 b (19.84)	0.76±0.08 c (84.36)	2.3±0.30 b (67.60)
T ₅	0.83±0.16 bc (86.39)	5.41±0.41 c (56.98)	0.57±0.54 c (88.27)	1.13±0.12 c (84.08)

Control: Seeds grown in distill water only; **T1:** Seeds grown in extracts of 2% concentration; **T2:** Seeds grown in extracts of 4% concentration; **T3:** Seeds grown in extracts of 6% concentration; **T4:** Seeds grown in extracts of 8% concentration; **T5:** Seeds grown in extracts of 10% concentration.

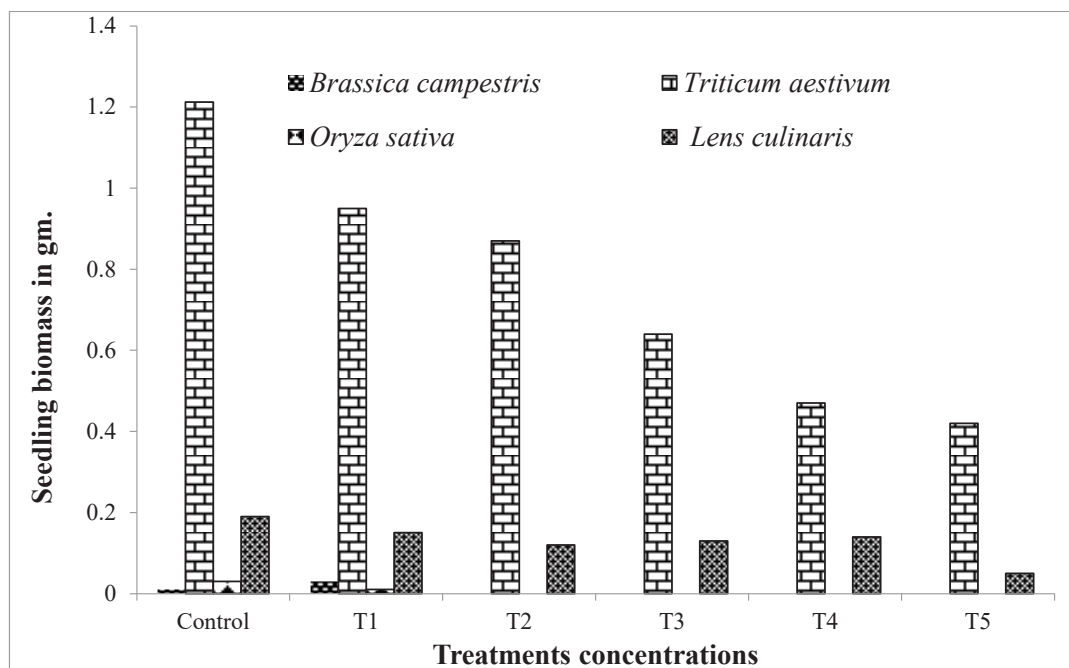
*Values in the columns followed by the same letter (s) are not significantly different ($P \leq 0.05$) according to Tukey multiple comparison test. Values in the parenthesis indicate the inhibitory effects in comparison to control treatments. \pm represent standard errors, n = 30.

Biomass

The seedling biomass gradually decreased as the concentration of *lantana camara* extract increased. Among the tested species, *Triticum aestivum* seedlings biomass was the highest and followed by *Lens culinaris* (Figure 2). The minimum biomass value of 0.01 g was recorded in *Brassica campestris* at control. The biomass of *Oryza sativa* and *Brassica campestris* as concentration increased was completely negligible. The value couldn't be measured because the mass was beyond the limit of our balance (0.001g).

Figure 2

Seedling biomass in g. of the tested species under different treatment of leaf aqueous extract of *Lantana camara*.



Control: Seeds grown in distill water only; **T1:** Seeds grown in extracts of 2% concentration; **T2:** Seeds grown in extracts of 4% concentration; **T3:** Seeds grown in extracts of 6% concentration; **T4:** Seeds grown in extracts of 8% concentration; **T5:** Seeds grown in extracts of 10% concentration.

Discussion

The study demonstrated that leaf aqueous extracts of *Lantana camera* exhibited significant inhibitory effects on seed germination and seedling growth of all the receptor crops compared to the control treatment. Ahmed *et al.*, (2007) also reported that leaf aqueous extract of *Lantana camera* resulted inhibitory effects on germination and growth behavior of some agricultural crops. The findings were in agreement with the (Talhi *et al.*, 2020) who reported that *Lantanacamera* release certain secondary metabolites as phenolic acids (Narwal, 1994) or/and phytotoxic chemicals, called as allelochemicals. Such allelochemicals hampers the seed germination and seedling growth due to the suppression of cellular membrane developments and increase in the production of reactive oxygen forms (Gindri *et al.*, 2020). According to Kong *et al.*, (2006) Lantadene A and lantadene B were isolated from aqueous extracts of *Lantana camara* leaves as allelochemicals. Phenolics are the most common and widely distributed water soluble allelochemicals (Jain *et al.*, 1989). The escape of these chemicals into the environment occurs through various mechanisms such as leachation, volatilization and microbial decay of dead and fallen parts, as well as root exudation (Rice, 1984). Presence of such allelochemicals in the plant extracts may prevent the growth of seed embryo or caused its death (Abugreet *et al.*, 2011). These chemicals were reported to have had allelopathic potential on various crops (Abhaet *et al.*, 2012). The roots and shoots elongation of the studied species is reduced when treated with increasing concentrations of extracts compared to the control. This reduction might be due to the allelopathic effects of the phytochemicals which are mainly polyphenols and alkaloids (Ambikaet *et al.*, 2003) and the phytotoxicity of the aqueous extract due to the interaction of these compounds (Talhi *et al.*, 2020).

The leaf extracts inhibited root growth of *Triticum aestivum*, *Brassica campestris*, *Lens culinaris*, and *Oryza sativa* with increasing extract concentrations and hence, it is concentration dependent (Table 2). From the homogeneity test it was found that the shoot length of *Triticumaestivum* and *Lens culinaris* at 2% concentration were not significantly different whereas *Brassica campestris* and *Oryza sativa* were significantly different from that of control; in case of root length at the same concentration (2%) from the control observed same result. This results indicats that the effects of leaf extracts on root and shoot growth was species specific *i.e.* *Triticumaestivum* and *Lens culinaris* pronounced less effect than the rest of the

species. Plants containing allelochemicals can affect germination and seedling growth of other plants in a concentration dependent manner and the effects of these chemicals are selective and can vary with different plant species (Hossain & Alam, 2010). In all crops species, shoot growth was comparatively less affected by the leaf extracts than root growth. More sensitive and stronger responses of roots of the crops to *Lantana camara* leaf extracts might be due to close contact of the root with the extract solution (Tefera, 2002).

In present study there were strong inhibition in seed germination of the *Brassica campestris* and *Oryza sativa* in 2% concentration and above this. The seeds appeared to be the most sensitive among the test species to inhibitory effect of leaf aqueous extract of *Lantana camara*. Since mustard and rice are important crops, invasion by *Lantana camara* into agricultural land may have adverse effect on their production. In contrast, when the seeds of maize and finger millet treated with *Lantana camara* leaf extracts exerted a positive action on germination and growth of roots and shoots (Tadele, 2014). The results, thus, indicate the possibility to cultivate maize and finger millet in agricultural lands invaded by *Lantana camara* after its removal or growth of these crops close to *Lantana camara* thickets. However, this result reveals that negative effect when treated with aqueous leaf extract of *Lantana camara* on tested crop species like *Triticum aestivum*, *Brassica campestris*, *Lens culinaris* and *Oryza sativa*. Gentle & Duggin (1997) reported that *Lantana camara* had significant reduction effect on biomass and it was associated with inhibited growth of the seedlings (Tripathi *et al.*, 2000). There is variation on biomass with increase in concentration.

Conclusion and Implication

The aqueous leaf extracts of the *Lantana camara* has critical inhibitory effect on germination and seedling growth of *Brassica campestris* and *Oryza sativa* than *Triticum aestivum* and *Lens culinaris*. Similarly the effect was strong on root elongation than shoot elongation. The allelochemicals effect on germination and seedling growth is concentration-dependent and, can vary with different plant species. Though laboratory bioassays are important to signal out the allelopathic effects, it requires analyzing the significance of these results under field conditions. This is because factors such as environmental conditions and plant interactions can influence the expression of allelopathic effects in natural settings, which may not be fully captured in laboratory

experiments. Therefore, it is important to assess the relevance of laboratory findings in field conditions to gain a better understanding of the potential impact of allelopathy on plant communities. We suggest that further investigations are required to better understand the underlying causes and actual mechanisms responsible for the differential effects of leaf allelochemicals on crop plants.

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