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Comparative Allelopathic Effects of Two Weed Extracts on Seed Germination and Seedling Growth of *Vigna unguiculata* (L.) Walp. and *Abelmoschus esculentus* L.

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ABSTRACT

Pot culture experiment and Petri dish bioassay were conducted to assess the allelopathic potential of *Cyanthillium cinereum* and *Lantana camara* on seed germination and seedling growth of *Vigna unguiculata* and *Abelmoschus esculentus*. Aqueous leaf and stem extracts of weed were used for treatment. The differential inhibitory effect was observed for two weed plants on two tested crops. The experimental results revealed that in case of pot culture experiment, lowest germination percentages (40.8±0.49%, 63.6±0.60% and 83±0.78%) were recorded in *L. camara* leaf extract treated set of *A. esculentus* and maximum decrease in seedling length (0.31±0.05 cm, 4.18±0.07 cm, 6.42±0.08 cm and 13.51±0.07 cm) was observed in stem extract treated a set of *A. esculentus*. *L. camara* stem and leaf extract induced a more negative effect on seedling length in both *A. esculentus* and *V. unguiculata*. For petridish bioassay experiment, lowest germination percentages (54.60±0.40%, 59.20±0.49%, 66.20±0.74% and 69.80±0.38%) were observed in *L. camara* leaf extract treated a set of *A. esculentus*. In *V. unguiculata*, lowest germination percentage (45.8±0.49%, 75.4±0. 40%, 84.4±0.25% and 89±0.32%) was observed in stem extract treated set. Maximum suppressive effect on seedling length was recorded for stem and leaf extract of *C. cinereum* of *A. esculentus* and leaf extract of *L. camara* and stem extract of *C. cinereum* of *V. unguiculata*.

Keywords: Allelochemicals; Germination; Seedling growth

1. INTRODUCTION

Allelopathy is a biological phenomenon where one plant inhibits the growth of other plants through the production of allelochemicals[1,2]. Regarding agricultural practices, allelopathy can be considered as the interference between crops and between crops and weeds which ultimately affect the plant production[3]. The weeds are considered as unwanted, undesirable plants which compete with cultivated crop for water, nutrient and sunlight and influence growth rate and reproductive rate of cultivated crops[4]. Therefore, weeds have importance in crop production for their adverse effects on crops. Allelopathy is a type of interference where the donor plants through the release of the chemical inhibitor from living or decaying tissues exert a suppressive effect on the other plant[5]. Different parts of weeds show allelopathic effects by releasing water soluble allelochemicals which mainly affect plants at seed emergence and seedling growth levels[6]. Allelochemicals may have beneficial or detrimental effects on the target plants. Allelochemicals produced by plants as end products, by-products or metabolites and obtained from the stem, leaves, roots, flowers, inflorescence, fruits and seeds of the plants. Though, the leaves appeared to be the most consistent producers of allelochemicals[7]. These groups of chemicals may be released together and may induce toxic effects in an additive or synergistic way. Allelochemical released from various parts of plants has a direct influence (inhibitory or stimulatory effect) on seed germination and seedling growth of recipient crop plants.

Vigna unguiculata (Cowpea) is an annual herbaceous legume. The crop is mainly cultivated for seeds with extremely high protein content. This can be used as a vegetable crop, fodder and as green manure. *Abelmoschus esculentus* L is an important vegetable crop widely grown in the tropical and the subtropical regions of the world[2]. *Lantana camara* as an invasive weed and widely distributed in different parts of India. It is a rapidly growing perennial woody shrub of family Verbenaceae and regarded as one of the world's top invasive and worst weeds[8]. Further the plant is a serious weed for fourteen crop plants of several tropical and subtropical countries[9]. Allelopathic effects of *L. camara* on seed germination and seedling growth behaviour of some agricultural crops were studied[10,11]. An earlier report revealed that allelopathic plants such as *Lantana camara* inhibited or suppressed germination rate, growth and development of crops through the secretion of allelochemicals to the rhizosphere of adjacent crop plants[12]. Aqueous extracts of leaf, stem and root of *Lantana camara* exert an inhibitory effect on seed germination. Moreover, it was reported that different concentrations of *L. camara* leaf extracts induced significant inhibitory effect on seed germination of some agricultural crop such as *Oryza sativa, Triticum aestivum, Vigna sinensis* and *Abelmoschus esculentus*[13]. *Cyanthillium cinereum* (little

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ironweed) was reported as commonly available invasive alien species of Asteraceae family from West Bengal[14].

Weeds are recognised to cause substantial reductions in the yield of crops. The most important effects of allelopathy on plants are reduced seed germination and seed growth[15]. Allelopathy thus plays an important role in many agroecosystems[16]. The allelopathic effects on seed germination are correlated with the types and concentrations of allelochemicals, species of recipient plants as well as environmental conditions[17]. Allelopathy as a natural and environment-friendly technique considered promising approach for weed control for sustainable agricultural practices[18]. It is evident from the data that allelochemicals present in *L. camara* might inhibit the process of seed and spore germination. Therefore the specific goals of this study were to evaluate the allelopathic effect of weeds extracts on germination and plant growth of *Vigna unguiculata* and *Abelmoschus esculentus*.

2. MATERIALS AND METHODS

2.1 Selection of the Plants

Aqueous extracts of leaves and stem parts of *Lantana camara* L. and *Cyanthillium cinereum* (L.) H. Rob were evaluated for their effects on seed germination and seedling growth of *Abelmoschus esculentus* L. and *Vigna unguiculata* L. Certified seeds of two selected crops (*Abelmoschus esculentus* L. and *Vigna unguiculata* L.) were procured from local seed distributor.

2.2 Plant Sampling and Preparation of Extracts

Leaf and stem parts of tested weeds (*L. camara* and *C. cinereum*) were collected separately from M.B.B. College campus (23° 49' 39.0108'' N; 91° 17' 56.6304'' E), Tripura, India. The plant parts were washed several times with water and after that oven-dried, grounded and sieved separately. Five percent (5%) aqueous extracts of the powered parts were prepared and stored in bottles. The bottles were shaken every 24 hours for 2 days. The extracts filtered through a muslin cloth and stored in dark bottles and properly labelled[3]. Extracts were marked as LE1= leaf extract of *Cyanthillium cinereum*, LE2=leaf extract of *Lantana camara*, SE1= stem extract of *Cyanthillium cinereum*, SE2= stem extract of *Lantana camara* L. According to Hill[19], *et al.* water is the solvent extraction medium in nature thus aqueous extracts are preferred for the present experiment.

2.3 Petri Dish Bioassay Experiment

Ten surfaces sterilised seeds of the two tested crops were placed in each sterile Petri plates (9 cm diameter) on double-layered Whatman filter paper No. 1. 5 ml of extract solution was applied to Petri plates of different treatment sets and 5 ml of distilled water was used for control set. Both treated and control sets were kept moist subsequently for germination and seedling growth by applying extract and distilled water respectively. Control set was marked as C and treated sets were marked as A (*Abelmoschus esculentus*) and B (*Vigna unguiculata*). The experiment was laid down in a complete randomised block design with 5 replicates for each set. Sets were incubated at 25°C (±2) and were regularly checked for moisture. Moisture in the sets was maintained by adding about two ml of extract or water every alternate day for 10 days. Seeds were considered germinated upon radicle emergence. Germination count, radicle and plumule length were recorded on 3rd days, 5th days, 7th days and 10th days. Growth and biomass attributes were recorded by randomly selecting 5 seedlings from each replication. Biomass attributes were estimated on the 10th day. Seedlings were dried separately for each experimental set in a hot air oven at 60 ^{0}C for 48 h and then samples were weighed[3,20].

2.4 Pot Culture Experiment

Plastic trays were filled with soil mixture (clay: sand in the ratio of 3:1). 20 Pre-soaked seeds were sown in the prepared soil at a depth of 0.5 to 1.0 cm in each tray for two tested crops and control sets. Experimental sets were irrigated with prepared aqueous extracts on every alternate day. The control sets were irrigated with water. Five replicates were prepared for each experimental set with complete randomised block design.

Physical parameters: The seedling lengths (cm), fresh and dry weights of seedling were determined (mg).

Evaluation Index: Germination percentage was calculated using the standard formula of Javed[21], *et al.*

3. RESULTS

The experimental findings revealed that the seed germination percentage and seedling growth were reduced in all weed extracts treated sets in comparison to the control set of both the recipient crops. Similar findings were obtained for pot culture and Petri dish bioassay. To evaluate the allelopathic action, analysis of germination behaviour was considered a reliable index[22]. Therefore germination percentage of tested crop was considered for evaluation of the allelopathic potential of weed extract in the present investigation.

3.1 Pot Culture Experiment

In the case of Pot culture experiment, germination percentage reduced to the maximum extent for LE2A set (3rd, 5th and 10th day). Among stem extracts treated sets, the lowest germination percentage was recorded in SE2A set (3rd, 5th and 10th day). Considering crop B, for leaf extract treated set lowest germination percentage was recorded in LE2B (3rd day) and in LE1B (5th, 7th and 10th day). In LE2A set germination percentage reduced to 85.60±0.25% and in SE2A set percentage reduced to 87.40±0.40% as compared to control set (95.60±0.25%) on 10th day of observation. In crop B germination percentage of LE1B set reduced to 86.80±0.49% from control set B (97.20±0.49%). All the experimental data were shown in Figs. 1 and 2 and depicted in Table 1. Leaf and stem extracts of *L. camara* induced more reduction in germination percentage of *A. esculentus*. In the case of *V. unguiculata*, stem extract of *L. camara* exert a suppressive effect on germination percentage whereas, the leaf extract of *C. cinereum* induced slightly more reduction in germination percentage except in 3rd day of observation. The maximum decrease in seedling length from 18.19±0.11 cm in control set to 13.51±0.07 cm was observed in SE2A set. Considering leaf extract treated sets the highest reduction in seedling length was recorded in LE1A set. In the case of crop B decrease in seedling, the length was maximum

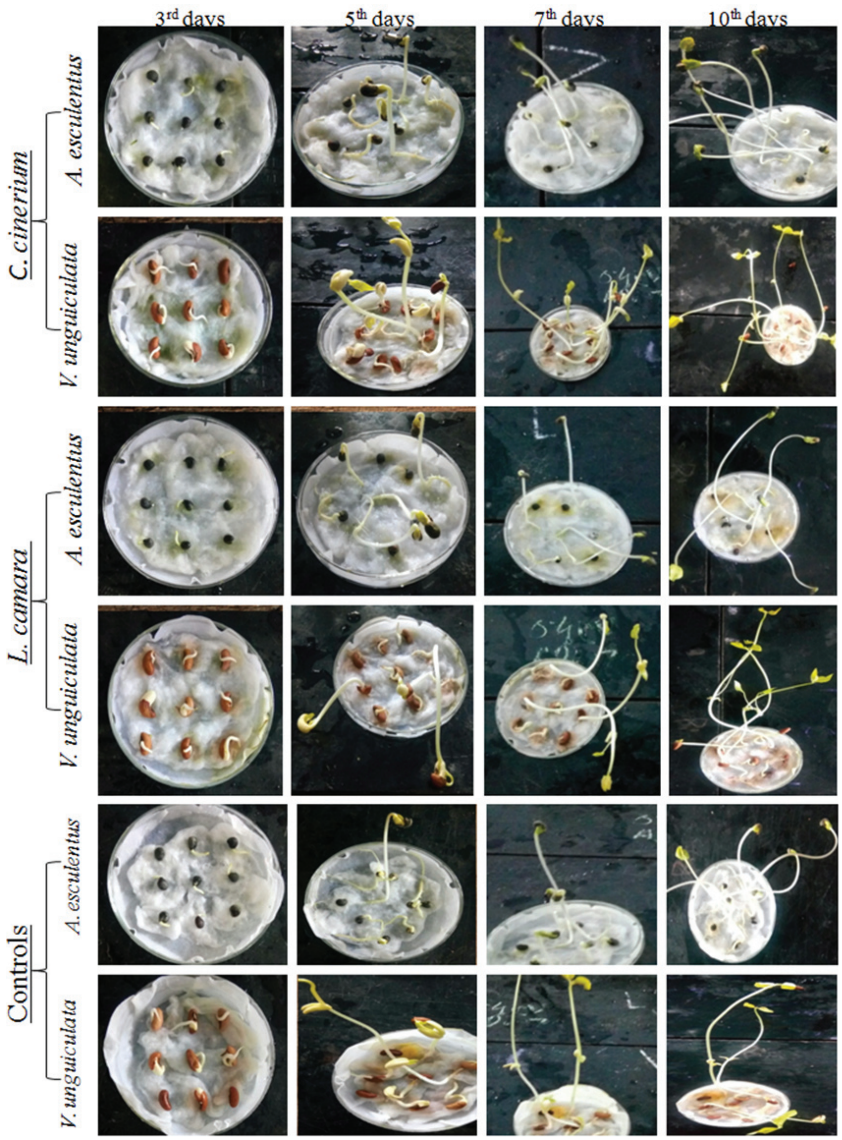

Figure 1. Effect of weed extracts of *Lantana camara* and *Cyanthillium cinereum* on germination of *Abelmoschus esculentus* and *Vigna unguiculata* of Petri dish bioassay.

in SE2B. Thus, *L. camara* stem and leaf extract induced a more negative effect on seedling length in both *A. esculentus* and *V. unguiculata*.

3.2 Petri Dish Bioassay Experiment

For Petri dish bioassay experiment, the lowest germination percentage (54.6±0.40%, 59.2±0.49%, 66.2±0.74% and 69.8±0.37%) was observed in LE2A set 3rd, 5th, 7th and 10th day, respectively, and depicted in Table 1. Among stem extracts treated sets, the lowest germination percentage was recorded in SE2A set. In *V. unguiculata*, the lowest germination percentages from 3rd day to 10th day were 56.4±0.40%, 72.80±0.37%, 76.40±0.25% and 79.80±0.49% was observed in stem extract-treated set (SE2B). Leaf and stem extracts of *L. camara* were more effective in inhibiting seed germination of *A. esculentus*. For *V. unguiculata*, stem extract of *L. camara* negatively affects germination percentage 79.80%±0.49 in respect to control set (97.00±0.63%). The seedling length was decreased (12.88±0.10 cm) most in SE1A set. Among leaf extract treated sets maximum reduction in seedling length (13.08±0.08cm) was observed in LE1A set. However, the maximum decrease in seedling length was observed in SE1B for crop B (13.82±0.06 cm). In case of crop B reduction in seedling length among leaf extract treated sets was more in *L. camara* extract treated set (13.08±0.08cm). The result indicated that stem and leaf extract of *C. cinereum* induced more reduction in seedling length of *A. esculentus*. Whereas in *V. unguiculata*, leaf extract of *L. camara* and stem extract of *C. cinereum* induced more suppressive effect on seedling length as shown in Table 1.

3.3 Biomass Attributes

Considering biomass attributes it was evident from experimental results that both fresh weight and dry weight of seedlings from treated sets were less than that of control sets of both the tested crops (Fig. 3). Moreover, in pot culture, experiment fresh weight and dry weight were minimum in SE2A (crop A) set and SE2B set (crop B). But in Petri dish bioassay experiment fresh and dry weight of seedlings were minimum in SE1A (crop A) and SE1B (crop B).

4. DISCUSSION

Different concentration of aqueous leaf extract of *L. camara* reported to cause a significant inhibitory effect on germination and seedling growth of *V. unguiculata*[11] that correlated with present findings. Pretreatment of seeds with different concentrations of leaf extract reduced percentage of germination and growth of the seedlings. Seeds pretreated with 100% and 50% leaf extracts of *L. camara* caused a reduction in length and dry weight of roots and shoots of radish and spinach seedlings[23]. These findings also corresponded with the present experimental results. Present findings also revealed that fresh weight and dry weight of seedlings of extract treated sets reduced as compared to control sets (Fig 3). Different concentrations of aqueous leaf extracts of *L. camara* caused a significant inhibitory effect on germination, root and shoot elongation of *V. unguiculata*[24]. A similar observation was recorded in the present investigation. The cold and hot aqueous leaf extracts of *L. camara* also induced inhibitory effect on germination percentage and seedling growth of *Phalaris minor* and *Sorghum bicolor* and thus indicated the allelopathic potentiality of extracts[25] which also supported the present findings where aqueous leaf extracts of *L. camara* reduced the germination percentage and seedling growth of tested crops.

It was also reported that *L. camara* caused an inhibitory or suppressive effect on germination, growth and development of crops by secreting allelochemicals to the rhizosphere of neighbouring crop plants[10] which supported the present findings obtained in pot culture experiment. According to Oudhia[26], the significant effect of *L. camara* leaf extract on

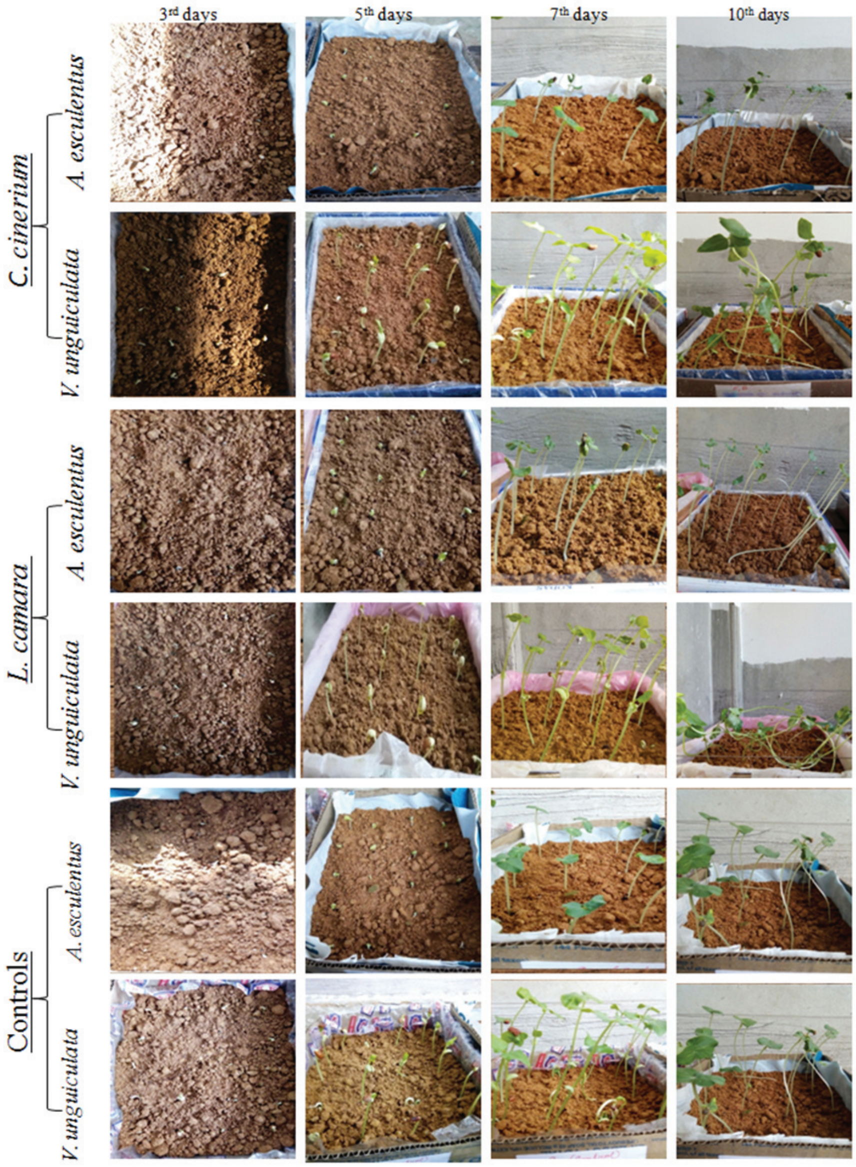

Figure 2. Effect of weed extracts of *Lantana camara* and *Cyanthillium cinereum* on germination of *Abelmoschus esculentus* and *Vigna unguiculata* of pot bioassay.

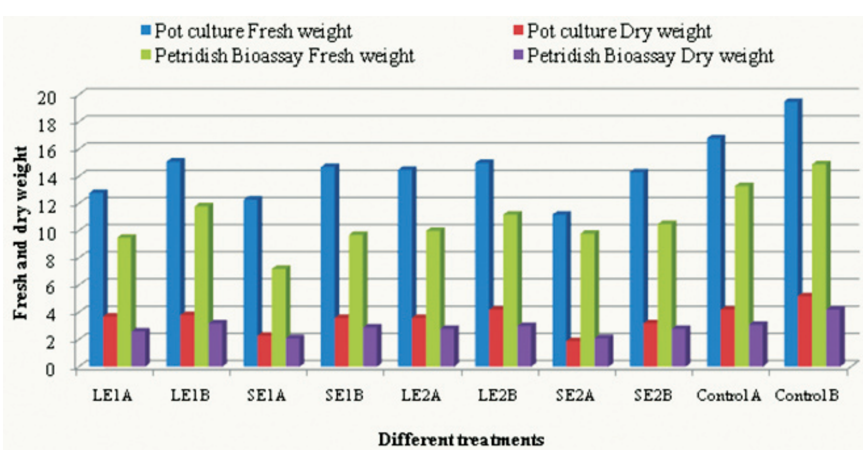

Figure 3. Fresh and dry weight of seedlings of the tested crop in treated and control sets.

germination of *Melilotus alba* was observed with the lower germination rate than the control set which supported the present findings. The negative effect on seed germination and seedling growth of tested plant observed in the present experiments might be due to that leaves, roots and fruits of *L. camara* reported to have allelochemicals such as aromatic alkaloids and phenolics that interfere with germination and growth of many species[27]. Comparison of seeds germination percentages between Petri dish and pot experiments indicated that seeds were germinated better in pot experiment as compared to Petri dishes which corresponded with present findings related to seed germination percentage of tested crops. This might be because allelochemicals were inactivated in the soil by different factors[28].

Experimental results showed the differential effect of the extracts obtained from two weed plants on germination percentage and seedling growth of two tested crops. The suppressive effects observed in germination percentage and seedling growth was dependent upon the nature of plant parts used for extract preparation and type of crop plants. The sensitivity of the different tested crop to weed extracts observed in the present investigation might be due to that allelopathic effect of donor plant mainly dependent upon corresponding competitivity and sensitivity of the receiving plant[29]. The differences observed in germination percentage and seedling growth of the tested crop in response to different weed extracts might be attributed to the presence of the different amount of phytotoxic substances in different parts[30].

The present experimental results related to biomass attributes can be supported by the earlier report where fresh weight and dry weight were significantly reduced in seedling obtained from seeds pretreated with leaf extracts and leaf leachates of *L. camara*[31].

5. CONCLUSIONS

Experimental results indicated suppressive effects of weed extracts (*L. camara* and *C. cinereum*) on seed germination percentage and seedling growth of two tested crop plants (*A. esculentus* and *V. unguiculata*). More pronounced inhibitory effects were observed in *A. esculentus*. Leaf and stem extracts of *L. camara* induced more negative effects in both pot culture and petridish bioassay except in case of seedling growth parameter in petridish bioassay where *C. cinereum* stem extract treated set induced maximum reduction. Isolation and identification of allelochemicals

Table 1. Germination percentage and seedling growth parameters of *A. esculentus* and *V. unguiculata* in different experimental sets

Pot culture								
Experimental sets	**Germination percentage/days**				**Seedling length (cm)**			
	3rd	**5th**	**7th**	**10th**	**3rd**	**5th**	**7th**	**10th**
LE1A	56.20±0.49	66.00±0.55	91.60±0.60	89.20±0.58	3.99±0.06	6.98±0.08	11.96±0.09	14.99±0.13
LE1B	55.20±0.37	65.80±0.80	84.20±0.37	86.80±0.49	5.01±0.11	11.05±0.09	16.46±0.09	19.57±0.079
SE1A	64.20±0.20	76.80±0.74	83.20±0.58	88.60±0.25	0.60±0.06	5.42±0.07	8.33±0.07	14.12±0.07
SE1B	68.60±0.40	80.80±0.49	85.80±0.49	88.80±0.49	1.12±0.07	7.32±0.06	9.54±0.05	16.30±0.24
LE2A	40.80±0.49	63.60±0.60	83.00±0.78	85.60±0.25	4.43±0.09	8.05±0.10	11.96±0.05	15.43±0.10
LE2B	45.20±0.20	70.80±0.49	85.20±0.37	87.60±0.25	5.54±0.07	10.25±0.06	14.34±0.07	19.17±0.08
SE2A	42.80±0.49	66.60±0.25	84.60±0.25	87.40±0.40	0.31±0.05	4.18±0.07	6.42±0.08	13.51±0.06
SE2B	45.80±0.49	75.40±0.40	84.40±0.25	89.00±0.32	0.64±0.05	6.71±0.05	9.43±0.06	15.28±0.09
Control A	67.60±0.40	88.60±0.25	91.20±0.49	95.60±0.25	7.77±0.20	10.10±0.06	14.11±0.07	18.19±0.11
Control B	69.20±0.49	85.00±0.45	94.60±0.245	97.20±0.49	9.24±0.21	15.01±0.08	19.87±0.08	23.29±0.11
Petridish bioassay								
Experimental sets	**Germination percentage/days**				**Seedling length (cm)**			
	3rd	**5th**	**7th**	**10th**	**3rd**	**5th**	**7th**	**10th**
LE1A	65.60±0.68	76.00±0.45	81.20±0.49	88.20±0.20	3.55±0.07	4.62±0.05	10.39±0.07	13.08±0.07
LE1B	75.80±0.49	79.40±0.25	82.80±0.20	86.40±0.40	4.09±0.04	6.34±0.05	12.17±0.06	16.31±0.04
SE1A	66.60±0.60	79.60±0.25	84.00±0.45	87.80±0.49	1.25±0.05	4.42±0.06	7.73±0.08	12.88±0.10
SE1B	61.60±0.40	85.00±0.45	88.60±0.25	89.60±0.25	3.24±0.04	4.79±0.085	10.21±0.06	13.82±0.06
LE2A	54.60±0.40	59.20±0.49	66.20±0.74	69.80±0.37	5.17±0.05	5.83+0.04	11.32±0.06	14.64±0.09
LE2B	66.80±0.49	81.20±0.49	84.60±0.60	89.80±0.49	3.73±0.06	6.25±0.05	11.91±0.04	15.02±0.14
SE2A	55.80±0.58	66.40±0.68	72.20±0.49	77.40±0.25	2.36±0.06	5.34±0.05	9.37±0.07	14.11±0.04
SE2B	56.40±0.40	72.80±0.37	76.40±0.25	79.80±0.49	3.56±0.05	7.00±0.06	11.95±0.10	14.89±0.07
Control A	78.40±0.40	85.40±0.25	90.20±0.20	93.80±0.49	3.96±0.05	8.15±0.05	14.42±0.05	17.81±0.04
Control B	71.20±0.49	88.60±0.25	93.40±0.40	97.00±0.63	5.98±0.05	8.85±0.11	12.88±0.12	20.29±0.24

released by the selected plants may help in determining the distinctive role of specific chemical on crop plants. Comprehensive field trials of tested crops against the selected weed extracts considering other growth parameters and yield attributes are required to be evaluated. There also remains scope for assessing the plant defence mechanism of crop plant against such allelochemicals released by the weed plants.

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