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RESEARCH ARTICLE

Effect of Leaf and Root Extracts of Selected Weed Species on Seed Germination and Seedling Growth in Mung Bean

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ABSTRACT

The present investigation was attempted to focus on studies of allelopathic effect of some dominant, invasive weed species like *Alternanthera tenella* Colla., *Croton bonplandianum* Baill and *Xanthium indicum* Koen. from Baramati Tahsil, Dist. Pune, Maharashtra, India, on seed germination and seedling growth in mung bean. The allelopathic effects of leaf extracts of weeds not only affect the seed germination, but it also influences seedling growth in terms of root and shoot length, vigour index. The results on mung bean seed germination studies indicated that the lower concentration (10%) treatments of *Alternanthera* and *Xanthium* leaf and root extract were highly stimulatory for mung bean seed germination as well as root and shoot length. However, the treatments of *Croton* root and leaf extracts were highly inhibitory. The effects of leaf extract were more significant than root extract. The positive and negative influence of root and leaf extracts of selected weeds on root and shoot length might be due to specific action of secondary metabolites (allelochemicals) in them.

INTRODUCTION

The invasive weeds infesting the crop fields and other ecosystems have become a serious problem today. The agro ecosystems show association of various types of native and invasive weeds in crops and barren lands as well as waste lands. To demonstrate the allelopathic potential of selected native and invasive weeds, seed germination bioassays were conducted using the seeds of test crop like mungbean. The protection and conservation of native plant species can be achieved through studies on ecophysiology, allelopathy and biological invasion of weeds. The invasive weeds have very high abiotic stress tolerance capacity, which enables them to survive and reproduce successfully, under extreme environmental conditions like drought, salt, high temperature, nutrient deficiency, etc. Scanty research is done on isolation, identification and characterization of allelochemicals in different native and invasive weeds. Considering these facts, the present investigation was attempted to focus on allelopathic studies of some dominant, invasive weed species like *Alternanthera tenella* Colla., *Croton bonplandianum* Baill and *Xanthium indicum* Koen.

MATERIALS AND METHODS

The present investigation is based on allelopathic studies on some dominant weed species from Baramati Tahsil, which comes under semi arid region of Maharashtra state. The materials and methods used for this investigation are briefly described below

Preparation of extracts

The aqueous extracts of *Alternanthera, Croton* and *Xanthium* were prepared from freshly harvested, 100 g leaf and root samples at the time of flowering of these weeds. After crushing in 500 ml distilled water and filtered through Buchner funnel using Whatman No.1 filter paper. These extracts were stored as stock solutions (20%) in amber colored bottles, which were diluted with distilled water to make desired concentrations (5% to 20%) at the time of treatments.

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Source of seeds of test crop

The certified seeds of mungbean (*Vigna radiata* L.) var. Vaibhav were procured from the Seed Farm, College of Agriculture, Shivajinagar, Pune.

Seed germination bioassay

The healthy seeds of mung bean were used for seed germination bioassay. These seeds were surface sterilized with 0.02% aqueous $HgCl_2$ for two minutes, thoroughly washed with distilled water and 25 seeds were kept in studies using sterilized petriplates (9 cm diameter) lined with germination paper. The germination paper in petriplates was moistened with 10 ml of respective concentrations (5 to 20%) of leaf and root extracts of selected weeds. The seeds placed in petri plates moistened with 10 ml distilled water were considered as control. The experiment was arranged in triplicate by using seed germination chamber. The germination percentage, root and shoot length, vigour index, fresh and dry weight of seedlings were recorded on 7th day as per the method of Gupta *et al*. (1996).

Germination percentage

Germination percentage was determined on 7th day (Prado *et al*. 2000).

Root and shoot length

On 7th day of sowing, 10 seedlings from control and each treatment were randomly selected for measuring the root and shoot length the average values were recorded in the Table.

Vigour index (VI)

The vigour index (VI) was derived from the formula:
VI = germination percentage × seedling length (cm)
Where, seedling length is the sum of root and shoots length.

Fresh and dry weight

On 7th day of sowing, ten seedlings were randomly selected for recording fresh weight from control and each treatment. These seedlings were kept in an oven at 60 °C, till constant dry weight was obtained. The dry weight of seedlings from control and each treatment was also recorded and the average values were recorded in Table.

RESULTS

Effect of leaf and root extracts of selected weeds on seed germination and seedling growth in mung bean

Germination percentage

The results recorded in Table 1 on seed germination in sensitive test crop like mung bean indicated that *Alternanthera* and *Xanthium* leaf extract treatments (10%) were stimulatory for mung bean seed germination. However, the higher concentration treatments of extracts (15 and 20%) caused significant reduction in seed germination percentage. Similar was the trend obtained for root extracts. The effects of leaf extracts were more inhibitory than root extracts, indicating their higher allelopathic potential. The *Croton* leaf extracts had caused higher reduction in seed germination percentage of mung bean, the seed germination was reduced to 50% due to lower concentration (10%) treatment. Further higher concentration treatments (15 to 20 %) did not allow the seed germination at all (Table 1). The root extracts at very low concentration (5%) showed slight increase in seed germination (86.66%) over control (85.50%). But at 15% concentration and above, there was 50% reduction in seed germination. The influence of leaf extracts was more negative for the seed germination of mung bean than the root extracts.

Root length

The results shown in Table 2 indicated that *Alternanthera* and *Xanthium* leaf as well as root extracts had caused increase in root length over control at lower concentrations (5 to 10%). Comparatively higher increase was noted at 10% concentration, by root extract as compared to leaf extract. The treatments of *Croton* leaf and root extracts had caused significant However, the higher concentrations (15 and 20%), caused reduction in root length. decrease in root length. But the inhibition at 15 and 20% concentrations due to leaf extracts was very high as compared to root extracts. The inhibitory effects of leaf extracts were more pronounced than root extracts of *Croton* like the observation with *Alternanthera* and *Xanthium.*

Shoot length

The statistically significant results recorded in Table 3 on shoot length in mung bean indicated that *Alternanthera* and *Xanthium* leaf and root extract treatments (10%) were highly significant at P=<0.001. The lower concentrations caused maximum increase in shoot length over control. However, the higher concentration treatments (15 and 20%) had induced significant reduction in shoot length of mung bean seedlings. As opposite to influence on root length the effects of leaf and root extracts on shoot length were more stimulatory or inhibitory. The results revealed that *Croton* leaf extracts were more influencive, which had caused very high reduction in shoot length of mung bean seedlings. The maximum inhibition was noted at 10% conc., but above this concentration, there was complete reduction in shoot length of mung bean seedlings. The treatments of root extracts had also caused decrease in shoot length with increase in concentration. The results of leaf extracts were more influencive than root extracts.

Vigor index

The results recorded in Table 4 clearly indicated that *Alternanthera* leaf extracts caused comparatively more increase in vigor index of mung bean seedlings at lower concentration (10%) than the root extracts. However, the higher concentration treatments (15 and 20%) showed very less increase in vigor index. The influence of *Xanthium* leaf and root extracts on vigor index had shown same trend like that of *Alternanthera*. The leaf and root extracts of *Croton* caused significant decrease in vigor index of mung bean seedlings, at low as well as high concentration. However, the effects of leaf extracts were more inhibitory than root extracts.

Table 1. Effect of leaf and root extracts of selected weeds on seed germination percentage of mungbean

Concentration (%)	*Alternanthera*		*Croton*		*Xanthium*	
	Leaf	Root	Leaf	Root	Leaf	Root
Control	85.50 ±3.42a	85.50 ±3.42a	85.50 ±3.42a	85.50 ±3.42a	85.50 ±3.42a	85.50 ±3.42ab
5.00	76.33 ±5.34b	74.66 ±5.23b	66.66 ±3.33b	86.66 ±6.07a	70.00 ±3.50b	82.33 ±3.29b
10.00	85.50 ±2.57a	86.66 ±2.60a	50.00 ±3.00c	76.66 ±2.30b	86.66 ±6.07a	90.33 ±2.71a
15.00	70.66 ±3.53b	70.66 ±3.53b	-	50.66 ±2.53c	46.66 ±1.40c	80.33 ±5.62b
20.00	60.66 ±1.82c	62.33 ±3.74c	-	46.66 ±1.40c	-	66.66 ±4.00c
p-value	<0.001	<0.001	0.003	<0.001	<0.001	<0.001

Table 2. Effect of leaf and root extracts of selected weeds on root length (cm) of mungbean seedlings

Concentration (%)	*Alternanthera*		*Croton*		*Xanthium*	
	Leaf	Root	Leaf	Root	Leaf	Root
Control	6.76 ±0.41a	6.76 ±0.41a	6.76 ±0.41a	6.76 ±0.41a	6.76 ±0.41a	6.76 ±0.41a
5.00	5.26 ±0.26c	6.30 ±0.38a	3.73 ±0.26b	4.06 ±0.12b	2.93 ±0.09c	4.16 ±0.12c
10.00	5.93 ±0.18b	6.70 ±0.27a	2.10 ±0.08c	3.66 ±0.22bc	5.50 ±0.28b	5.80 ±0.41b
15.00	5.13 ±0.31c	4.63 ±0.32b	--	3.33 ±0.20c	2.53 ±0.08c	3.40 ±0.20d
20.00	4.16 ±0.29d	3.53 ±0.11c	--	2.36 ±0.09d	--	3.13 ±0.16d
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

#Data are means of three replicates ±standard deviation. The significance of mean differences tested by one-way-ANOVA. Values followed by different letters differ significantly by Duncan's multiple range test at p<0.05.

Table 3. Effect of leaf and root extracts of selected weeds on shoot length (cm) of mungbean seedlings

Concentration (%)	*Alternanthera*		*Croton*		*Xanthium*	
	Leaf	Root	Leaf	Root	Leaf	Root
Control	9.33 ±0.89a	9.33 ±0.89ab	9.33 ±0.89a	9.33 ±0.89a	9.33 ±0.89a	9.33 ±0.89abc
5.00	9.23 ±0.92a	9.50 ±0.64ab	5.83 ±0.48b	6.90 ±0.66b	9.16 ±0.62a	9.40 ±0.81ab
10.00	9.40 ±0.75a	10.33 ±0.70a	3.56 ±0.33c	6.20 ±0.62b	9.76 ±0.80a	10.60 ±1.06a
15.00	5.96 ±0.37b	8.70 ±0.71b	--	5.16 ±0.41c	5.87 ±0.55b	9.06 ±0.72bc
20.00	5.43 ±0.32b	8.30 ±0.78b	--	4.13 ±0.27d	--	7.90 ±0.49c
p-value	<0.001	<0.001	0.002	<0.001	0.002	0.021

#Data are means of three replicates ±standard deviation. The significance of mean differences tested by one-way-ANOVA. Values followed by different letters differ significantly by Duncan's multiple range test at $p<0.05$.

Table 4. Effect of leaf and root extracts of selected weeds on vigor index of mungbean seedlings

Concentration (%)	*Alternanthera*		*Croton*		*Xanthium*	
	Leaf	Root	Leaf	Root	Leaf	Root
Control	1127.50ab ±67.65	1127.50a ±67.65	1127.50a ±67.65	1127.50ab ±67.65	1127.50a ±67.65	1127.50a ±67.65
5.00	1068.76bc ±42.75	959.90b ±57.59	950.46b ±28.51	1033.02bc ±72.31	1023.10b ±51.16	1061.27a ±63.68
10.00	1234.06a ±86.38	1142.25a ±79.96	469.80c ±23.49	1225.03a ±73.50	1132.37a ±33.97	1157.54a ±34.73
15.00	1049.05bc ±31.47	1014.97b ±40.60	--	1009.84c ±50.49	649.02c ±45.43	1052.40a ±73.67
20.00	977.62c ±48.88	923.03b ±46.15	--	969.04c ±29.07	--	897.50b ±44.88
p-value	0.004	0.002	0.001	0.001	<0.001	0.001

Table 5. Effect of leaf and root extracts of selected weeds on dry biomass (g)/ three seedlings in mungbean

Concentration (%)	*Alternanthera*		*Croton*		*Xanthium*	
	Leaf	Root	Leaf	Root	Leaf	Root
Control	0.326 ±0.020ab	0.326 ±0.020a	0.326 ±0.020	0.326 ±0.020b	0.326 ±0.020a	0.326 ±0.020a
5.00	0.332 ±0.015ab	0.203 ±0.010b	0.357 ±0.020	0.357 ±0.015a	0.221 ±0.010b	0.304 ±0.025ab
10.00	0.352 ±0.025a	0.304 ±0.025a	0.332 ±0.025	0.329 ±0.020ab	0.261 ±0.010ab	0.319 ±0.010a
15.00	0.319 ±0.010b	0.194 ±0.005b	--	0.312 ±0.010bc	0.198 ±0.015c	0.283 ±0.015bc
20.00	0.236 ±0.015c	0.111 ±0.006c	--	0.296 ±0.015c	--	0.264 ±0.015c
p-value	<0.001	<0.001	0.635	0.007	<0.001	0.007

Data are means of three replicates ±standard deviation. The significance of mean differences tested by one-way-ANOVA. Values followed by different letters differ significantly by Duncan's multiple range test at $p<0.05$.

Dry biomass

The results illustrated in Table 5 revealed that the treatments of *Alternanthera* leaf extracts at lower concentrations had caused substantial increase in the seedling dry weight of mung bean and maximum values were recorded at 10% concentration. The leaf extract was more effective than root extract for increasing the dry biomass. However, the higher concentration treatments had resulted into reduction of seedling dry weight of mung bean. In *Xanthium* the root extracts had caused more increase in dry biomass as compared to the treatments of leaf extracts. The lower concentration treatments of *Croton* root extracts (5%) had induced slight increase in dry weight of mung bean seedlings. But all the higher concentration treatments showed opposite trend.

DISCUSSION

Seed germination

In the present investigation, the lower concentration treatments of *Alternanthera* and *Xanthium* leaf as well as root extracts except *Croton* had caused increase in seed germination percentage of mung bean, while the effects of higher concentrations were less stimulatory as compared to control (Table 1). The different types of allelochemicals e.g. flavonoids, bitter essential oils, saponins, etc. present in *Alternanthera, Croton* and *Xanthium* leaf as well as root extracts, at very low concentration, might have stimulated the seed germination percentage by acting positively and synergistically on the process. The secondary metabolites present in the leaf and root extracts might have caused stimulation in enzymatic activities, responsible for degrading the stored food materials in the germinating seeds. As a result of this seed germination might have increased at lower concentration. In the seeds treated with leaf and root extracts, the process of mobilization of reserve food towards the developing embryo might have enhanced, resulting into improved germination and seedling growth. However at higher concentrations of leaf and root extracts, the secondary metabolites (allelochemicals) present in them might have acted negatively, by inhibiting the enzymes and even mobilization of reserve food materials, resulting into reduction in percent seed germination and root as well as shoot length of seedlings. The favourable or adverse impact of allelochemicals on the processes like seed germination is concentration (dose) dependant, which is explained by An *et al*. (2005) as "Hormesis". According to him, there is stimulation of growth at lower concentration and inhibition at higher concentrations of leaf and root extracts. Various allelopathy researchers had reported positive as well as negative influence of aqueous extract/leachates and residues of donor plants and their allelochemicals on seed germination in different treated plants. The observations of the present investigation are similar to that of the findings of Bhalerao *et al*. (2000), Hossain *et al*. (2002), Dongre *et al*. (2004), Gupta *et al*. (2005), Mandal *et al*. (2005), Misra (2006), and Ghayal and Dhumal (2007a,b). They noted significant increase in seed germination of black gram, green gram, red gram, sorghum, rice, wheat, bajara, radish and mustard due to lower concentration treatments of leaf extracts and leachates of *Ageratum, Anagallis, Parthenium, Phyllanthus, Chromolaena, Aspidium, Terminalia, Populus* and *Cassia*. Abdulla *et al*. (2005) have also noted that lower concentrations of *Melia, Morus, Albizia* and *Mangifera* leaf and bark leachates significantly stimulated seed germination of mustard, while the higher concentrations showed inhibitory effect. The stimulation of seed germination at lower concentration and inhibition at higher concentration in mung bean might be due to the presence of allelochemicals like flavonoids, triterpenoids and steroids in the leaves and roots of selected invasive weeds. Orr *et al*. (2005) recorded the potential allelopathic influence of two invasive weed species like *Lolium* and *Elaeganus* on native species of *Acer, Populus* and *Platanus.*

They found reduction and delay in seedling emergence, root and leaf biomass in treated plants. They explained that it was due to the action of allelochemicals. Oyun (2006) had also reported inhibition of seed germination and seedling growth in maize due to inhibition of water absorption in germinating seeds and that of nutrient uptake in the growing seedlings, when treated with leaf leachates of *Acacia* and *Gliricidia*. Swain *et al*. (2005) had also reported that the leachates of root, stem and leaves of *Physalis* inhibited seed germination of *Parthenium* at higher concentrations. They have claimed that phenolics were responsible for reduction in seed germination and seedling growth. According to Todaria *et al*. (2005) the tannins, phenols and other secondary metabolites were responsible for inhibition of seed germination in treated plants. Travlos and Paspatis (2008), Aziz *et al*. (2008) and Tanveer *et al*. (2008) also observed similar inhibition of germination of their respective test crops. Einhellig *et al*. (1986), Reigosa *et al*. (1999) claimed that the allelochemicals present in the extracts, leachates or residues are responsible for the changes in water relations, membrane permeability and enzymatic activities of protein and carbohydrate metabolism during seed germination process, which determine the success of seed germination. It may be the reasons for the stimulation of seed germination in mung bean when, treated with leaf and root extracts of *Alternanthera*, *Croton* and *Xanthium*.

Shoot and root length

Many allelopathy researchers have reported positive and negative effects of aqueous extracts, leachates and residues of different plants on root and shoot length of treated plants, which were completely dependant on concentrations used for treatments. In the present investigation stimulatory effects on shoot and root length were recorded due to lower concentration treatments, while it was less stimulatory or inhibitory at higher concentrations (Tables 2 and 3). These results corroborates with the findings of Channal *et al*. (2002) and Navaz *et al*. (2003). They recorded the stimulatory influence of lower concentrations of aqueous leaf leachates/extracts of various forest tree species and weeds including *Eupatorium* and *Cassia* on root and shoot length of rice, wheat, maize, cowpea, pigeon pea, mung bean, chickpea, soybean, radish and mustard. The negative impact of higher concentrations of extracts and leachates of different weeds on various crops had been well documented by several workers like Mallik and Pellissier (2000), Djanaguiraman *et al*. (2002), Duary (2002), Patil *et al*. (2002), Ali *et al*. (2005), Punjani (2005), Travlos and Paspatis (2008), Aziz *et al*. (2008) and Tanveer *et al*. (2008). They have reported significant inhibition of root and shoot length in *Picea*, wheat, maize, sorghum, rice, blackgram, greengram, soybean, cowpea, cotton and sunflower due to higher concentration treatments of *Vaccinium, Eupatorium, Sesamum, Prosopis, Eucalyptus, Acacia, Grewia, Populus, Casuarina, Amaranthus* and *Parthenium*. Similar explanations might be true for the inhibition of seedling growth in mung bean due to higher concentration treatments of invasive weeds' extracts. As reported above, the phenolic compounds present in the invasive weeds might have caused inhibition of the shoot and root growth in mung bean, because higher concentrations of phenols were detected in the leaves and roots of these invasive weeds. The stimulatory as well as inhibitory effects caused by the lower and higher concentration treatments of *Alternanthera, Croton* and *Xanthium* leaf as well as root extracts on root and shoot length in mung bean showed the similar trend as described by the above workers. The inhibition or stimulation may be due to various types of allelochemicals such as terpenoids, flavonoids and steroids as well as high phenolic compounds present in the extracts of *Alternanthera, Croton* and *Xanthium.*

Vigor index (VI)

The vigor index is a vital parameter to determine the efficiency of any germinating seed to utilize and mobilize the reserved food material. The results recorded on vigor index (Table 4) revealed that the lower concentration treatments of *Alternanthera, Croton* and *Xanthium* leaf as well as root extracts had caused increase in vigor index of mung bean. The results of treatments of higher concentrations were at par with control. Several workers like Duary *et al*. (2002), Channal *et al*. (2002), Ghayal *et al*. (2007a,b) had noted increase in vigor index of radish, mustard, black gram, green gram, pigeon pea and rice seedlings due to lower concentration treatments of leaf extracts/leachates of *Cassia* and other plant species. The results of the present investigation corroborate with the findings of the above workers.

Dry biomass of seedlings

The results on dry biomass of treated seedlings in mung bean indicated increase due to application of lower concentration treatments of invasive weed's extracts, while the reverse trend was noted with higher concentration treatments (Table 5). The research workers like Tripathi *et al*. (2000), Channal *et al*. (2002), Navaz *et al*. (2003), Kaushal *et al*. (2003), Singh *et al*. (2003), Al-Wakeel *et al*. (2007) have reported increase in dry biomass of pea, chilli, fingermillet, cowpea, mung bean, pigeon pea, sorghum and rice seedlings due to lower concentration treatments of leaf leachates of different donar plants like *Acacia, Eupatorium, Zizyphus, Grewia, Populus, Dalbergia, Syzygium*, *Tamarindus, Eucalyptus, Tectona, Samanea* and *Azadirachta.* They have claimed that it was due to positive effect of allelochemicals. Similar reason may be true for the increased biomass in mung bean. However, Mallik and Pellissier (2000), Duary *et al*. (2002) and Djanaguiraman *et al*. (2002), Lodha (2004) and Ali *et al*. (2005) have reported considerable inhibitory effects on dry weight in wheat, rice, black gram, mung bean, cowpea and *Picea* due to higher concentrations of aqueous leaf extracts and decomposing litter of sunflower, *Eucalyptus, Amaranthus, Parthenium, Sphaeranthus,* sesame and *Vaccinium* respectively. The level of inhibition increased with increase in concentration of the extracts for all the crops tested. In the present investigation, similar results were obtained regarding the increase or decrease in the dry weight of mung bean seedlings due to the lower and higher concentration treatment of leaf and root extracts of *Alternanthera, Croton* and *Xanthium*. This concentration dependant increase or decrease in dry biomass may be attributed to the explanations given by An *et al*. (2005), which indicated that the favorable or adverse impact of the allelochemicals present in extracts, might have caused positive action at cellular, organ and organism level, leading to enhanced biomass at lower concentration.

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