



Phytotoxic evaluation of wasteland weed species

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Weeds are widely recognized among one of the greatest threats to plant biodiversity. They cause loss of native floral diversity and huge yield losses in agricultural system. they also possesses strong capability to replace neighbouring flora by producing phytotoxic effects, that directly affects the growth through inhibition, suppression or mortality of the plants in a community. These phytotoxic effects attributed to natural phenomenon known as allelopathy/allelopathic interactions. Allelopathy plays a significant role under both natural and managed ecosystems (Rice 1984). Allelochemicals are plant secondary metabolites normally released into the environment through volatilization, leaching, root exudation and decomposition of plant residues in the soil (Khalaj *et al.* 2013) and have positive and negative influences on the plant growth (Jalali *et al.* 2013). These chemicals play a vital role in plant-plant interactions. To link between allelochemicals and allelopathy Makoi and Ndakidemi (2012) noted in their study that most of the allelopathic effects in plants are known to result from allelochemicals released from plants.

Bhadoria (2011) produced an extended list of readily visible effects of allelochemicals on the growth and development of other plants, includes inhibition or retardation of germination rate; reduction of root and shoot length; reduced dry weight accumulation etc.

Parthenium hysterophorus, *Croton bonplandianum*, *Cassia occidentalis*, *Cassia obtusifolia*, *Calotropis procera*, and *Tephrosia purpurea* are weeds that commonly seen growing along railway tracks, roadsides, open lands, wastelands and competes with some crops. Some of these weeds replace or displace natural vegetation and cause severe loss of natural biodiversity at national and international levels and decrease crop yields as well.

The experiments were conducted during 2014 at St. John's College, Agra, Uttar Pradesh. Fresh leaves and roots of selected wasteland weeds, *viz.* *P. hysterophorus* (W1), *C. bonplandianum* (W2), *C. occidentalis* (W3), *C. obtusifolia* (W4), *C. procera* (W5) and *T. purpurea* (W6) were collected from the nearby wasteland fields. For germination test, the seeds of wheat and barley were procured from the commercial suppliers.

Leaves and root were collected from selected test plants; 100 g leaf and root were soaked in 500 mL double distilled water separately under aseptic conditions for 9 days and placed in conical flasks in a refrigerator at 8 °C. The aqueous leachates were filtered through three layers of muslin cloth/cheese cloth to remove debris. The filtrate was then re-filtered through Whatman No.1 filter paper. Two concentrations, 50% (C₁) and 100% (C₂) were prepared and directly used for bioassay.

Seeds of wheat and barley were thoroughly washed with tap water to remove dirt and dust and then rinsed with mild detergent solution for 5-7 min. Seeds were surface sterilized with 0.1% HgCl₂ for 10 min and again washed with sterilized distilled water 4-7 times. Seeds were divided into 5 replicates of 10 seeds each in Petri Dishes and were placed on filter paper in Petri Dishes and moistened with leaf and root leachates of different weed species, whereas control received distilled water. All the seed lots were allowed to germinate and germination was recorded for 10 days. Seedlings growth (radicle length, plumule length and biomass) was also recorded after 10 days of germination.

Data obtained were analyzed using the three way analysis of variance (ANOVA) technique and least significant difference (LSD=0.05) tested by the Fisher LSD method with the help of Sigma plot software.

Seed germination

Maximum inhibition on seed germination of wheat was noticed in 100% concentration of leaf

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leachates of *P. hysterophorus* (79.3%). High concentration of leaf leachates of other plants also showed remarkable reduction in seed germination percentage of wheat. The inhibition order was observed as *C. occidentalis* (77.5%) > *C. obtusifolia* (75.6%) > *C. bonplandianum* (50.5%) > *T. purpurea* (49.7%) > *C. procera* (44.3%) as compared to control (99.7%). High concentration of root leachates (100%) showed more inhibition as compared to 50% leaf leachates, while least inhibition was observed in 50% root leachates (Table 1).

Table 1. Effect of leaf and root leachates of different weed species on seed germination of wheat

Plant	Treatment	Germination percentage (%)	
		Leaf leachates	Root leachates
W ₁	50%	42.21 ± 0.88 aA	49.17 ± 1.87 aB
	100%	20.40 ± 1.20 bB	31.41 ± 1.20 bA
	Control	99.68 ± 0.18 c	99.68 ± 0.22 c
W ₂	50%	56.11 ± 1.50 aA	65.21 ± 1.51 aB
	100%	49.21 ± 1.91 bB	58.04 ± 2.83 bA
	Control	99.68 ± 0.18 c	99.68 ± 0.22 c
W ₃	50%	44.01 ± 1.70 aA	51.40 ± 0.89 aB
	100%	22.12 ± 0.37 bB	35.21 ± 0.18 bA
	Control	99.68 ± 0.18 c	99.68 ± 0.22 c
W ₄	50%	45.45 ± 0.34 aA	54.20 ± 1.00 aB
	100%	24.12 ± 1.01 bB	35.05 ± 0.15 bA
	Control	99.68 ± 0.18 c	99.68 ± 0.22 c
W ₅	50%	65.11 ± 1.80 aA	72.58 ± 2.38 aB
	100%	55.41 ± 1.71 bB	69.27 ± 1.56 bA
	Control	99.68 ± 0.18 c	99.68 ± 0.22 c
W ₆	50%	58.21 ± 1.11 aA	64.17 ± 0.47 aB
	100%	50.01 ± 1.18 bB	61.31 ± 0.71 bA
	Control	99.68 ± 0.18 c	99.68 ± 0.22 c
LSD (P=0.05)	50%	0.98	1.04
	100%	1.02	0.88

Same trend of inhibition on seed germination of barley, where maximum inhibition was observed in 100% concentration of leaf leachates of *P. hysterophorus* (78.9%) and minimum in 50% root leachates of *C. procera* (26.9%). *C. occidentalis* and *C. obtusifolia* showed remarkable inhibition after *P. hysterophorus* in both cases *i.e.* > 75.0% at higher concentration of leaf leachates and > 70.0% at root leachates whereas control showed 100% germination. Three Way ANOVA indicated that there was a statistically significant interaction (P < 0.001) between leachates, concentrations and plants on mean germination percentage of wheat and barley, individually. It is clear from the results that all the selected weed species caused remarkable inhibition on seed germination as well as seedling growth of wheat and barley. Leaf leachates of most of the weed species especially *P. hysterophorus*, *C. bonplandianum*, *C. occidentalis*, *C. obtusifolia* and *T. purpurea* were found highly phytotoxic against both parameters in wheat and barley as compared to

root leachates. As the concentration increased their effects were also escalated. A number of previous studies support the findings of this experiment that the degree of inhibition increases with increasing extract concentration (Laosinwattana *et al.* 2007, Knox *et al.* 2011).

The degree of inhibition was higher in leaves therefore leads to more phytotoxicity damage to seed germination than roots and this is in agreement with Sarkar *et al.* (2012) findings that allelochemicals concentrations may differs between plant parts.

Seedling growth

Maximum reduction in radical length, plumule length and biomass of wheat was noticed in 100% concentration of leaf leachates of *P. hysterophorus* (2.7 cm, 3.7 cm and 0.018 g) followed by *C. occidentalis* (2.9 cm, 4.5 cm and 0.021 g) > *C. obtusifolia* (2.9 cm, 4.7 cm and 0.023 g) as compared to control (7.2 cm, 6.8 cm and 0.28 g).

Table 2. Effect of leaf and root leachates of different weed species on seed germination of barley

Plant	Treatment	Germination percentage (%)	
		Leaf leachates	Root leachates
W ₁	50%	43.14 ± 0.74 aA	54.41 ± 2.00 aB
	100%	21.1 ± 0.60 bB	34.51 ± 1.30 bA
	Control	100 ± 0.00 c	100 ± 0.00 c
W ₂	50%	56.75 ± 3.04 aA	69.47 ± 2.26 aB
	100%	51.74 ± 1.44 bB	62.17 ± 0.56 bA
	Control	100 ± 0.00 c	100 ± 0.00 c
W ₃	50%	46.20 ± 0.89 aA	56.24 ± 0.05 aB
	100%	23.22 ± 0.51 bB	39.21 ± 0.49 bA
	Control	100 ± 0.00 c	100 ± 0.00 c
W ₄	50%	48.20 ± 1.90 aA	56.2 ± 0.90 aB
	100%	25.22 ± 1.01 bB	39.80 ± 1.59 bA
	Control	100 ± 0.00 c	100 ± 0.00 c
W ₅	50%	67.08 ± 1.88 aA	73.14 ± 0.73 aB
	100%	61.14 ± 3.83 bB	70.80 ± 3.60 bA
	Control	100 ± 0.00 c	100 ± 0.00 c
W ₆	50%	59.62 ± 1.22 aA	66.24 ± 2.04 aB
	100%	50.21 ± 0.70 bB	64.74 ± 1.53 bA
	Control	100 ± 0.00 c	100 ± 0.00 c
LSD (P=0.05)	50%	1.06	0.92
	100%	0.93	1

W₁, W₂, W₃, W₄ and W₅ indicate *P. hysterophorus*, *C. bonplandianum*, *C. occidentalis*, *C. obtusifolia*, *C. procera*, and *T. purpurea*.

Each value is the average of five replicates (n=5); ± Standard deviation; CD Critical difference (Table 2)

Different letters in lower case within a column for each plant indicate a statistically significant difference in mean values of germination % of wheat (P < 0.001)

Different letters in upper case within same row for each plant indicate a statistically significant difference in mean values of germination % of wheat (P < 0.001)

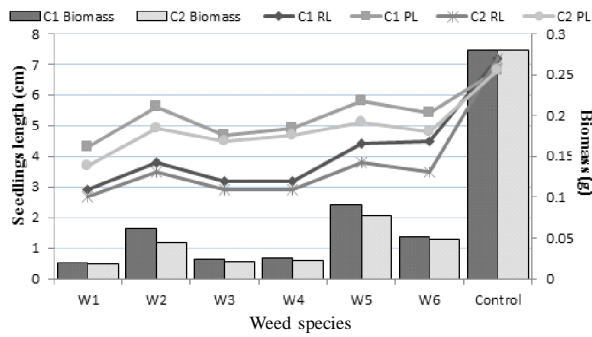
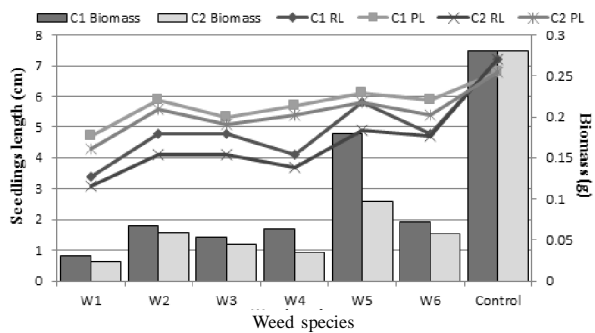


Fig. 1. Effect of leaf leachates of different weed species on seedling growth of wheat

Least inhibition on seedling growth was observed in 50% concentration of *C. procera* (4.4 cm, 5.8 cm and 0.091 g). High concentration (100%) of root leachates suppressed the growth of radicle and plumule growth, consequently reduced biomass of wheat as compared to 50% concentration (Fig. 2). Root leachates of *P. hysterophorus* showed maximum reduction in seedlings length and biomass (3.1 cm, 4.3 cm and 0.023 g) at 100% concentration, followed by *C. obtusifolia* (3.7 cm, 5.4 cm and 0.035 g). Least effect was observed in 50% concentration of *C. procera* i.e. 5.8 cm, 6.1 cm and 0.18 g.



C1 and C2= 50% and 100% concentrations; RL= radicle length; PL = plumule length

Fig. 2. Effect of root leachates of different weed species on seedling growth of wheat

Inhibitory effects of 100% concentration of leaf leachates on radical length, plumule length and biomass of barley plant. Maximum reduction in seedlings length and biomass (2.2 cm, 3.4 cm and 0.023 g) was observed in *P. hysterophorus* leaf leachates at 100% concentration closely followed by *C. occidentalis* (3.1 cm, 3.5 cm and 0.025 g), when compared with control (8.7 cm, 9.2 cm and 0.34 g). half concentration of leaf leachates of all weed species caused 50% reduction in seedlings length except *C. procera* (Fig. 3), 100% root leachates of *P. hysterophorus* and *C. occidentalis* caused maximum

reduction in seedlings lengths and biomass i.e. 3.8 cm, 4.2 cm and 0.031 g in *P. hysterophorus* leachates and 3.8 cm, 4.8 cm and 0.034 g in *C. occidentalis* leachates, respectively. half concentration (50%) of root leachates showed minimum effects on seedlings growth as compared to 100% concentration. Leachates of *C. procera* and *T. purpurea* showed least inhibitory effects on seedlings growth at 50% concentrations.

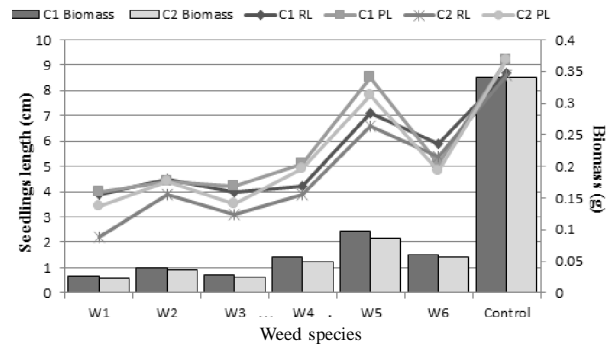


Fig. 3. Effect of leaf leachates of different weed species on seedling growth of barley

Aqueous leaf leachates of all the selected weed species retarded the seedling growth and reduced dry matter production (Fig. 4). It is in the line with the findings of Belz *et al.* 2007, Msafiri *et al.* 2013, reported adverse effects of foliar leachates of *P. hysterophorus* induces to retardation or complete inhibition of growth. It is also confirmed with the Zhang *et al.* (2009) findings that different plants have different inhibitory potential therefore they exhibit differential allelopathic potential against different species.

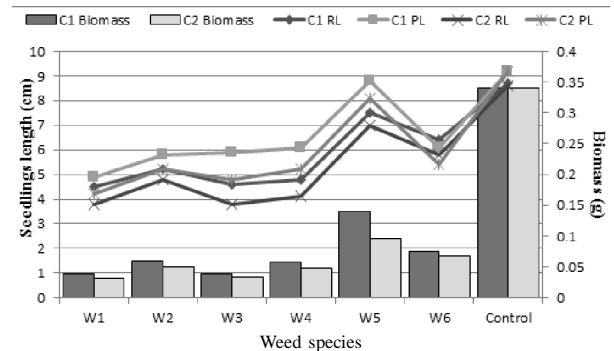


Fig. 4. Effect of root leachates of different weed species on seedling growth of barley

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SUMMARY

To characterize the phytotoxic potential, a laboratory experiment was conducted by considering some prominent wasteland weeds like *Parthenium hysterophorus*, *Croton bonplandianum*, *Cassia occidentalis*, *Cassia obtusifolia*, *Calotropis procera*, and *Tephrosia purpurea*. Aqueous leachates of leaf and root of different concentrations (50 and 100%) were tested against seed germination and seedlings growth of wheat and barley, as test plants. High concentration (100%) of leaf leachates showed remarkable inhibition on seed germination, radicle and plumule length and decreased biomass respectively, closely followed by the 50% concentration of leaf leachates. Maximum inhibition (> 70.00%) was recorded in 100% concentration of *P. hysterophorus*, *C. occidentalis* and *C. obtusifolia* leaf leachates as compared to root leachates (< 65.00%) except *P. hysterophorus* and similar trend of inhibition in radicle, plumule length and biomass was recorded. Root leachates showed least inhibitory effects at 50% concentration whereas 100% root leachates were comparatively more effective than 50% leaf leachates. A statistically significant interaction between leachates, concentrations and plants ($P < 0.001$) was observed; hence a significant inhibition was seen at higher concentration of leaf leachates. Inhibition potential of considered weeds clearly indicates the presence of phytotoxic chemicals in their tissues that supports them in establishment, competing and replacement of surrounding flora.

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