
Allelopathic effect of legumes leachates on seed germination and seedling growth of maize (*Zea mays* L.)

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Abstract In Pakistan, the trend to grow forage maize in mixture with legume forages for improving yield and quality is increasing but allelopathic effects of legumes on germination of maize and seedling growth are not known. Therefore, laboratory experiments to investigate the allelopathic effects of aqueous extracts of leaves and stems of leguminous crops viz., cowpea, sesbania, mungbean and cluster bean on seed germination, seedling growth of maize were conducted in the Department of Agronomy, University of Agriculture, Faisalabad, Pakistan, during the year 2010. The plants: mungbean, cowpea, cluster bean and sesbania were separated into leaves and stems and soaked individually in water at the rate of 100g per liter of water for 24 hours and the aqueous extract of leaves and stems were obtained by filtering water through sieve. Leachate of leguminous crops showed inhibitory effects on germination and emergence, shoot and root length, fresh and dry weight of shoots and roots and total fresh weight of seedlings, MGT, GI and T₅₀. There has also been a variation in allelopathic effect of stems and leaves and even differences were significant in some parameters between stems and leaves of same legumes. Sesbania leaves and stem extracts have more pronounced effect on shoot length and root length than extracts of other legumes crops.

Key words: Aqueous leachates, germination/emergence, mung bean, cowpea, cluster bean, sesbania and maize

Introduction

Allelopathy can simply be defined as the ability of plants to inhibit or stimulate growth of the other plants in the environment by exuding chemicals. Cover crops, such as legumes suppress weeds in corn and increase grain yield (White and Worsham, 1990; Johnson *et al.*, 1993; Yenish *et al.*, 1996). Ashrafi *et al.* (2008) reported that barley (*Hordeum vulgare*) contains watersoluble allelochemicals that inhibit the germination and growth of other crops species and weeds. Rye as cover crop caused significant reduction in density and

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biomass of several weed species in corn and soybean (*Glycine max* L.) (Teasdale *et al.*, 1996; Liebl *et al.*, 1992). The effect of cover crops to suppress weeds can be attributed to their ability to release toxic substances in the environment and to create an unfavourable environment for weed germination and establishment. Michael and Carlene (2007) reported phytotoxicity of aqueous foliar leachates and ground dried residues of sunhemp (*Crotalaria juncea* L.), cowpea (*Vigna unguiculata* L.) and velvetbean (*Mucuna deeringiana*) on crop and weed germination. There is a wide variation in the ability of cultivars of trap crops to reduce the germination and growth of *S. hermonthica* by releasing allelochemicals (Alabi, 2000). Chon and Kim (2004) found that rice (*Oryza sativa* L.), barley (*Hordeum vulgare*), wheat (*Triticum aestivum* L.), and oat (*Avena sativa* L.) release toxic substances in the environment either through root exudation or decay of their plant residues. Borner (1960) also indicated that cold water leachates of barley, rye, and wheat straws as well as alcoholic leachates of roots contain phenolic compounds, toxic to plant growth, whereas Shrestha *et al.* (2002) reported that soybean yield was increased by the presence of rye and corn used as cover crops. Similarly, Swanton *et al.* (1999) also found that corn yield was increased by a rye cover crop. Irshad and Cheema (2006) showed that sorghum leachate decreased barnyard grass dry weight 31-48%. The tree *E. camaldulensis*, *Prosopis juliflora*, and *Acacia nilotica*, significantly affected seed germination and seedling growth of several crops and/or weed species (Khan *et al.*, 2004; Khan *et al.* 2007). Hedge and Miller (1990) reported that alfalfa (*Medicago sativa* L.) plants contain water-soluble substances that are autotoxic as well as allelopathic to other plants.

Different plant parts varied in their allelopathic effects on the germination and seedling growth of crops plants. Some plants parts have more inhibitory effect than other (Burhan and Shaukat, 1999; Tanveer *et al.*, 2008). Rahimi *et al.* (2006) evaluated the allelopathic responses of *P. psyllium* upon germination and growth stages of redroot pigweed (*Amaranthus retroflexus*), wild barley (*Hordeum leporinum*), black mustard (*Brassica nigra*) and Lambsquarters (*Chenopodium album* L.) and results showed that aqueous shoot leachate, shoot residue and simultaneous growth of *P. psyllium* displayed the most consistent negative effects on germination and dry matter of target plants. Shoot residue significantly decreased height and dry matter of all target species except for wild barley.

In Pakistan, trend of growing forage maize in mixture with forage legumes is increasing. Most of previous studies have indicated that allelochemicals of crops have the ability to suppress weeds seed germination and affect crop yields, but information about the allelochemical effects of

summer legume crops on seed germination, seedling emergence and growth of maize crop are lacking. The present study therefore, was conducted to assess the allelopathic potential of leaves and stem of some summer legume crops on seed germination, seedling emergence and growth of maize.

Materials and methods

Laboratory based experiments to evaluate the allelopathic effects of both leaves and stems leachates of four legumes i.e. mung bean, cowpea, cluster bean and sesbania on seed germination, emergence and seedling growth of maize, were conducted in Department of Agronomy, University of Agriculture, Faisalabad during 2010. Field grown plants of all legumes at pod development stage were taken and dried at room temperature. After drying, leaves and stems were separated and small pieces were made with help of scissors. Dried pieces of leaves and stems were immersed in tap water separately in the ratio of 1:10 (w/v) at room temperature for 24 hours. The leachates of leaves and stems were obtained by filtering water through sieve (0.4mm). Distilled water as (control) was also included for comparison.

Experiment: 1

In this experiment, ten seeds of maize were placed evenly on filter paper (Whatman No.10) in petri plates. Leaves and stem leachate of legumes measuring 4ml was added to petri plates according to treatments. Petri plates were placed at room temperature ranging from 25 to 30 °C. During this study petri plates were observed daily and water or leachate was applied if needed.

Experiment: 2

In this experiment, ten seeds of maize were sown in sand filled plastic pots (16cm in diameter). Leaves and stem leachate of legumes was added to plastic pots according to treatment. Pots were placed at room temperature (25-30 °C) for 15 days. During this study pots were observed daily and water or leachate was applied if needed. After 15 days, seedling were removed and washed with water. The root and shoot length was measured with measuring tape.

Procedure for Recording the Observations

Seed Germination

The seeds were considered as germinated when the radicle was of 2 mm long.

The germinated seeds were counted after 24 hours regularly for fourteen days and germinated seeds were discarded every day.

Time to Start Germination/Emergence

Time to start germination/emergence was recorded as when first seed started to germinate in petri plates or emerge out from soil in plastic pots.

Germination Percentage

Germination %age was calculated by the formula given below for each treatment in each replication:

$$\text{Germination \%age} = \frac{\text{Germinated Seeds}}{\text{Total seeds}} \times 100$$

Mean Germination Time (MGT)

Mean emergence time (MGT) was calculated by using the equation given by Ellis and Roberts (1981).

Time to 50% Germination (T₅₀)

Time taken to 50 % emergence of seedlings (T₅₀) was calculated according to the formulae of Coolbear *et al.* (1984) and modified by Farooq *et al.* (2005).

Germination Index (%)

The germination index (GI) was calculated as described in the Association of Official Seed Analysts (AOSA, 1983).

Total Weight (g)

After washing seedlings, they were dried for a while. The seeds were removed and whole plant was weighed by using electric weighing balance.

Shoot and Root Length

Shoot were separated from roots by using scissors and then shoot length and root length was measured by using scale.

Fresh Weight of Shoots and Roots (g)

Roots and Shoots were weighed separately by using electric weighing balance.

Dry Weight of Shoots and Roots (g)

Roots and shoots were dried separately in an oven at 80 °C to a constant weight and the weight was determined by using electric balance.

Statistical analysis

The data collected were analyzed statistically by using the Fisher's analysis of variance technique by using MSTAT-C statistical computer package and Least Significant Difference Test at 5% probability was used to compare the treatment means (Steel *et al.*, 1997).

Experiment: I Allelopathic Effect of Legumes Leachates on Maize Germination and Growth

Time to start germination (TSG) [days]

The data presented in (Table 1) showed significant effect of legumes leachates on time to start germination in maize. The legumes leachate treatments of both leaves and stem significantly took more time to germinate than control treatment. The maximum numbers of days (6.00) were recorded in treatment 4 (T₄) where cowpea stem leachate was applied. However, it did not differ significantly in T₂, T₅, T₆, T₇, T₈ and T₉. Mung bean leaves have minimum effect on time to start germination.

Data regarding the effect of legume leachates on time to start emergence (pot experiment) in maize is presented in Table 1. All treatment having legumes leachate either from stems or leaves significantly took more time to emerge than control. The mung bean leaves have minimum affect on time to start emergence. In pot experiment, maximum time (8.25 days) to start emergence of maize was recorded in treatment T₄ (cow pea stem leachate) and minimum time (2.75 days) to start emergence was recorded in control treatment. Khan *et al.* (1999) and Khan *et al.* (2007) have also reported that leaf leachate delayed the germination of maize.

Table 1. Allelopathic Effect of Legumes Extracts on Maize Germination and Emergence

Treatments	Time to start germination		G %		MGT (Days)		T50 (Days)		GI	
	Petri E.	PotE .	Petri E.	PotE.	Petri E.	Pot E.	Petri E.	Pot E.	Petri E.	Pot E.
T ₁ Control (distilled water)	1.75 d	2.75 c	100.00 a	100.0 a	3.68 c	5.22 c	0.46 c	1.37 d	7.80 a	6.25 a
T ₂ Mung bean stem extract	5.0 abc	7.5 a	80.0 bc	55.00 cd	6.06 b	8.78a b	0.64 c	6.68 ab	5.32 b	1.74 cd
T ₃ Mung bean leaves extract	4.50 c	5.2 b	62.5 de	82.5 ab	7.47 a	7.72 b	5.00 a	5.50 abc	5.35 b	3.57 b
T ₄ Cow pea stem extract	6.0 a	8.2 a	62.5 de	45.0 d	6.25b	8.95a b	1.29bc	4.00 bcd	5.05 bc	1.24 d
T ₅ Cow pea leaves extract	4.75 bc	7.0 ab	57.50 e	80.0 b	6.82a b	8.80a b	1.87bc	2.87 cd	4.31 bc	2.65 bc
T ₆ Sesbania stem extract	5.5ab c	7.2 ab	70.0 cde	65.0 bc	6.12 b	9.77a b	1.85bc	7.00 ab	4.48 bc	1.83 cd
T ₇ Sesbania leaves extract	5.5ab c	7.2 ab	97.5 a	52.50 cd	7.37 a	9.02a b	2.02bc	6.43 ab	5.08 b	1.76 cd
T ₈ Cluster bean stem extract	5.5ab c	7.0 ab	95.0 a	67.5 bc	6.72a b	9.54 a	3.31ab	6.01 abc	5.26 b	2.20 cd
T ₉ Cluster bean leaves extract	5.75a b	7.50 a	75.0 cd	55.0c d	6.81a b	9.77 a	3.11ab	7.75 a	3.25 c	1.52 cd
LSD	1.12	2.09	16.9	19.94	1.005	1.42	2.21	3.48	1.92	1.18

Germination/emergence percentage

Some legume leachates significantly decreased the germination percentage of maize as compared to control treatments (Table 1). The lowest germination (57.50%) was recorded in T₃ where cowpea leaf-leachate was applied and the maximum germination (100 %) was recorded in control treatment (T₁). The differences among control, sesbania leaves and cluster bean stem leachate were not significant. The sesbania leaf-leachate have least effect on germination percentage. Cowpea leaf-leachate have maximum inhibitory effect on germination percentage of maize.

Similarly, in pot experiment, emergence (%) of seedlings was reduced significantly by almost all legume leachates (Table 1). The minimum emergence (45%) was found in T₄ where cow pea stem leachate was applied. Maximum value of emergence (100.0%) was recorded in control treatment and it differs significantly from mung bean leaf-leachate treatment. Significant reduction in seedling emergence has also been reported by Suman *et al.* (2002). They found that chickpea seeds leachates inhibit seed emergence % of sugarcane crop. The observations indicate a release of inhibitory substances

from legumes plant both from leaves and stems that inhibited emergence percentage.

Time to 50% germination (T_{50}) [days]

The data presented in Table 1 showed significant effects of legume leachates on time to 50% emergence of maize. Both stems and leaf-leachates of legumes took more time to attain T_{50} than control treatment. Maximum time (5.00 days) to complete T_{50} was recorded in T_3 where mungbean leaf-leachate was used and it did not differ significantly from clusterbean leaves and stem leachates treatment. Minimum numbers of days (0.62 days) were recorded in control treatments (T_1) to complete T_{50} .

In pot experiment, maximum time (7.75 days) to complete T_{50} was recorded in treatment T_9 where clusterbean leaf-leachate was applied (Table 1). Minimum numbers of days (1.37 days) were observed in the control treatment (T_1). However, differences among T_1 , T_4 and T_5 were not significant. These results are in accordance with those of Chon and Kim (2004), who reported that allelopathy may vary among plant parts. Sang *et al.* (2004) also reported that allelochemicals present in plant increase the time to 50% germination as compared to control in alfalfa plant.

Mean germination time (MGT) [days]

Mean germination time of maize crop was also affected by the stems and leaf-leachates of legumes (Table-1). All leachate- treatments have high MGT as compared to control in pot experiment. Maximum MGT (7.47days) was recorded in T_3 where mungbean leaf-leachate was applied. The lowest value of MGT (3.68 days) was recorded in control treatment (T_1). In pot experiment (Table1), MGT was also influenced significantly. All those treatment where leachate of legumes were applied, MGT was significantly more than the control, being maximum in clusterbean leaf-leachate and sesbania stem leachate (9.77 days). The treatments: T_2 , T_4 , T_5 , T_6 , T_7 , T_8 and T_9 have statistically similar mean germination time. Similar results were reported by Michael and Carlene (2007).

Germination Index (GI)

All legume leachate treatments significantly reduced the germination index (Table 1). Maximum value of GI (7.80) was noted in control treatment (T_1) and minimum value of GI (3.25) was recorded in treatment where cluster

bean leaf-leachate (T₉) was applied and it did not differ significantly from T₄, T₅ and T₆.

In pot experiment GI was also reduced by all leachate treatments as compared to control (Table 1). Maximum value of GI (6.25) was recorded in control treatment and minimum value of GI (1.24) was found in T₄ where cowpea stem leachate was applied. These results are supported by the findings of Shahid (2010).

Experiment: II Allelopathic effect of legumes leachates on maize seedling and growth

Shoot length of seedlings (cm)

The leachates from stems and leaves of legumes caused a significant reduction in shoot length of maize (Fig. 1). The leaf-leachates of sesbania showed more inhibitory effect on shoot length of maize seedlings as compared to all other leachates of legumes. Maximum shoot length 8.55 cm was recorded in control treatment (T₁) and minimum value (2.32 cm) was recorded in T₇ where sesbania leaves was applied. These results are supported by the findings of Shukla *et al.* (2003). They also reported a reduction in seedling height in plants receiving allelochemicals.

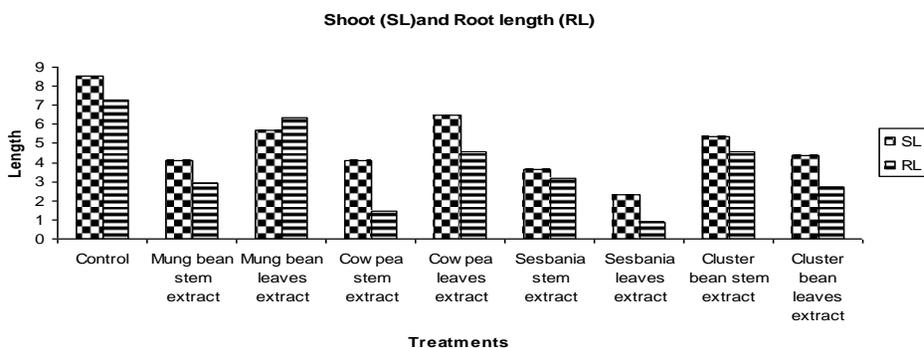


Fig. 1. Allelopathic effect of stems and leaves extract of different legumes on Shoot (SL) and Root (a) length of maize seedlings

Root length (cm)

The leachates from stems and leaves of legume crops also caused a significant reduction in root length as compared with the seedlings raised in distilled water (Fig. 1). The leaf-leachates of sesbania showed more inhibitory effect on root length of maize seedling as compared to all other leachates of

legumes. Maximum root length (7.27 cm) was recorded in control treatment (T₁) and minimum length of roots (0.88 cm) was recorded in T₇ where sesbania leaf-leachate was applied. Treatments T₃, T₅ and T₈ were statistically similar to one other. These results are in agreement with the findings of Chung and Miller (1995) they reported that water leachates of allelopathic plants significantly reduced the growth of roots.

Fresh weight of Shoots

Data regarding the effect of legume crop leachates on fresh weight of shoots of maize seedlings (Fig. 2) showed that all leachates either leaves or stems reduced the fresh weight of maize seedlings as compared to control treatment. Maximum fresh weight (0.54g) per seedling was recorded in control treatment and minimum (0.10g) per seedling was recorded in treatment T₈ where cluster bean stem leachate was applied. Decrease in fresh weight of shoot by allelochemicals has also been reported by Nelson (1996).

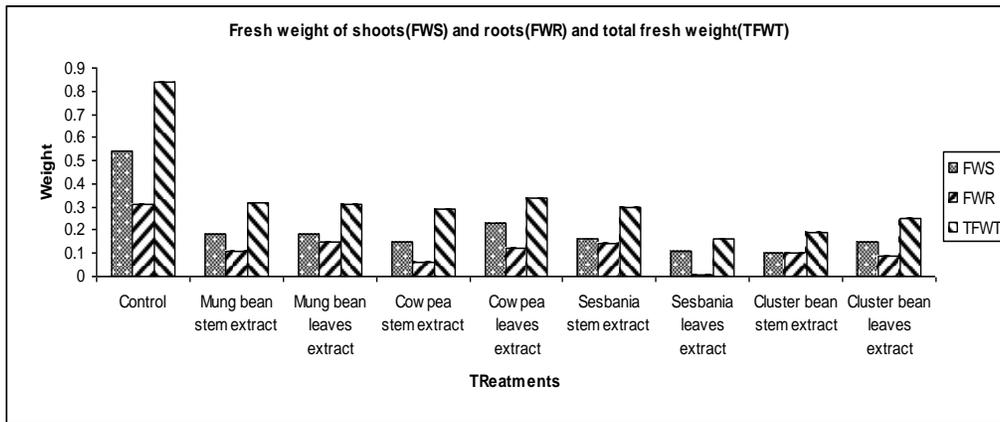


Fig. 2. Allelopathic effect of stems and leaves extracts of different legumes on fresh weight of shoots (FWS), fresh weight of Root (FWR) and total fresh weight (TFWT) of maize seedlings

Fresh weight of Roots (g)

Fresh weight of roots of seedling was also affected significantly by leachates of leguminous crops as compared to control treatment (Fig. 2). All legume leachate either from stems or root significantly reduced the fresh weight of roots. Sesbania leaf-leachate remain at par with cowpea leaf-leachate produced minimum fresh weight (0.005g) of roots. The differences among mung bean stem leachate; cowpea stem leachate, cowpea leaf-leachate; cluster bean stem leachate and cluster bean leaf-leachate were not significant.

Maximum fresh weight (0.31 g) of roots was recorded in control. Alabi (2000) have also reported that cowpea leachate reduced the fresh weight of root crops and weeds.

Total fresh weight of seedlings (g)

The data presented in Fig. 2 showed that the total fresh weight of seedlings was influenced significantly by the treatment under study. Weight of seedlings of maize grown in different leachates was significantly reduced compared to control treatment. Maximum fresh weight (0.84 g) of seedling of maize was recorded in control treatment and minimum (0.16 g) was recorded in treatment T₇ where sesbania leaf-leachate was applied. The differences among the T₇, T₈ and T₉ were not significant. Hedge and Miller (1990) have also reported a reduction in fresh weight per plant of alfalfa and fresh weight per plant of maize.

Dry weight of Shoots (g)

Data presented in (Fig-3) showed that allelopathic effect of legumes leachates on dry weight of shoots of maize seedlings was significant. All leachate either from stem or leaves significantly reduced the dry weight of maize seedling. Minimum dry weight of shoot (0.03g) was recorded in T₈ where cluster bean stem leachate was applied and maximum (0.41g) dry weight of shoots was recorded in control treatment. The differences among the leachate treatment were not significant except cowpea leaf-leachate, which have significantly higher dry weight of shoots than all other leachate treatments. Significant reduction in dry weight of barnyard grass has been reported by applying leachate of maize by Irshad and Cheema (2006). Ashrafi *et al.* (2008) also reported that leachate from sunflower reduced the dry weight of wild barley seedlings.

Dry weight of Roots (g)

Data presented in Fig. 3 showed significant differences among the treatments regarding the dry weight of roots of maize. The control treatment (T₁) gave significant higher dry weight of roots than stems and leaf-leachate treatment of legumes. The minimum dry weight (0.002g) was recorded in T₇ where sesbania leaf-leachate was applied. Reduction in dry weight of roots of crops due to allelopathic effect of crops has also been reported by Rahimi *et al.* (2006). Jabeen and Ahmad (2009) also reported that allelochemicals from weeds reduced the fresh and dry weight of roots of maize crop.

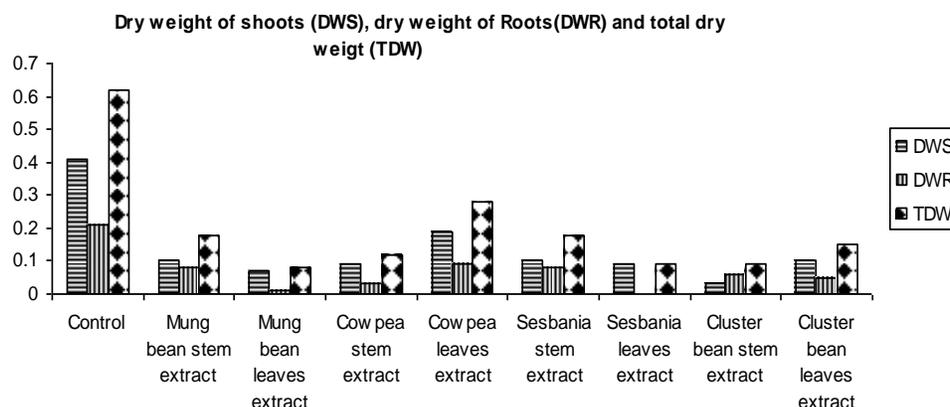


Fig. 3. Allelopathic effect of stems and leaves extracts of different legumes on dry weight of shoots (DWS), dry weight of Root (DWR) and total dry weight (TDW) of maize seedlings

Total dry weight of seedlings (g)

The total dry weight of maize seedling was reduced by all legume extracts as compared to control treatment as shown in Fig. 3. Maximum dry weight of maize seedlings was recorded in control treatment (T1) and mung bean leaves extract reduced the dry weight of maize seedlings. All other extracts treatment significantly reduced the dry weight of maize seedlings as compared control.

Conclusion

It can be concluded from the results that leachate of all leaves and stems of all legumes crops that suppressed germination and growth of maize seedlings.

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