

*Original Research Article*

**Allelopathic Effects of Winter Legumes on Germination and Seedling Indicators of Various Summer Cereals**

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**Abstract**

In the present study, the putative allelopathic potential of aqueous extracts (10 % w/v) of three legumes, i.e. alfalfa, berseem and brassica spp., including distilled water treatments as check were evaluated on germination and early seedling growth indicators of rice, sorghum and millet under laboratory conditions. The treatments were compared in completely randomized design with factorial arrangements in four replications. The recorded observations clearly indicated that the germination process and seedling growth of receptor plants were negatively affected in the presence of legume extracts. The extract of Brassica had the strongest inhibitory effect on germination process in form of increased mean germination time (MGT) and lowered the coefficient of velocity of germination (CVG), germination % age and germination index (GI) as compared to other extracts. Likewise, the lowest root and shoot length, their fresh and dry weight was also noted in petri dishes with Brassica extracts. Among the receptor crops, rice was the most susceptible in term of both seedling growth and germination attributes. The interaction between test crops and legume extracts was found to be significant for most of studied parameters except germination % age, fresh and dry weight of root and shoot. The presence of allelochemicals in the studied legumes entitled them to be used in weed management programme and is also signal for designing specific production technologies able to reduce their effects on succeeding crops.

**Keywords:** Seed germination; seedlings and roots; distilled water; aqueous extracts.

**INTRODUCTION**

The potential allelochemicals present in plant materials have often been noted to have specific communication in terms of retarding or stimulating normal growth (Farooq et al., 2008). The beneficial or deleterious effects of the chemicals/ exudates and leachates have been reported for germination and subsequent growth. The natural decomposition process of crop residues induced by microbes, dispel chemicals in soil which are potentially very toxic even though the primary substances were not toxic (Panahyan-e-Kivi et al., 2010). These phytochemicals modify the diverse plant mechanisms which are often responsible for delayed and poor germination, seedling mortality, reduced growth and lower dry matter accumulation (Herro and Callaway, 2003). The expression of the above symptoms on succeeding crop leads to the investigation of release of phytotoxic compounds from the previous crop. However, the intensity of the effect is dependent on the receptor plant types in the community and the amount and type of chemicals being released. In Pakistan, rice, sorghum and millet are often grown on land previously occupied by the rabi legumes, a considerable amount of allelochemicals are added to soil from residues and dropping leaves of alfalfa, berseem and brassica spp.

The legumes are often incorporated in soil to serve as green manure; they impart certain stresses to succeeding crops by adding the allelochemicals in soils on decomposition. The germination percentage of crops is far lower than the seeding density and is most commonly induced by allelochemicals added to soil from preceding crops. The legume crops contain a number of chemicals having allelopathic potential (Randall et al., 1989). The allelopathic effect of alfalfa and trifolium species has been reported for some plants (Mattice et al., 2000; Haffman et al., 1996; Chung and Miller, 1995). Significant deviation in germination and growth of wheat and barley was recorded when seeds were supplied with alfalfa extract (Karaaltin et al., 1999). In another study, the progressive decline in germination of weed species by application of extracts of trifolium spp. and *M. sativa* has also been noted in some studies (Maighany et al., 2007, Koloren, 2007). Besides interferential mechanisms of crop plants, we have major concern on the reduced yield of next crop (Reigosa et al., 2000). The early studies have frequently reported allelopathic activity of brassica on some weeds (Norsworthy, 2003, Turk et al., 2005) and its residual effects on succeeding crop (Bialy et al., 1990). Therefore, this study was conducted for taking out the possible effect of aqueous legume extracts on germination and early growth parameters of rice, sorghum and millet.

**MATERIALS AND METHODS**

**Extract preparation and treatments**

The mature plants along with roots of alfalfa (*Medicago sativa* L.), berseem (*Trifolium alexandrum* L.) and brassica spp. (*Brassica napus* L.) were collected from experimental farm of Agronomy Department, in January, 2010. The plant material was allowed to dry in laboratory under shade for one month and was cleaned of dust. It was chopped manually to keep the straw size of 2-3 cm and soaked in distilled water for a period of 24 hours. The 10 % solution of distilled water with ground material (1:10 w/v) was prepared. The resulting brownish solution was filtered through cotton cloth followed by Whatman No. 1 filter paper and was stored in dark at room temperature in plastic bottles for future use. The bottles were well shaken before use. The healthy and uniform size seeds (10 for each) of rice, sorghum and millet were sterilized for 15 minutes with 1% sodium hypochlorite and for removing of chemicals from seed, distilled water was used. The ten seeds were evenly arranged in double layer filter paper (Whatman No.10) in sterilized petri dishes, 9 cm diameter. The four extracts treatments i.e. distilled water, alfalfa, berseem and brassica and three summer cereals, i.e. rice, sorghum and millet were compared in factorial based completely randomized design with four replicates. The extracts (6 ml) were applied to each petri dish by pipette.

**Procedure for data collection**

The germination data was recorded on 24 hours intervals up to 10 days by counting the germinated seeds. The seeds were considered as germinating upon the appearance of 2 mm radicle as described by Association of Official Seed Analysts. The following formulas for mean germination time (MGT), coefficient of velocity of germination (CVG) and germination index (GI) were used.

$$CVG = \frac{G_1+G_2+G_3+-----+G_n}{(1 \times G_1) + (2 \times G) + ----- (N \times G_N)} \quad (\text{Maguire, 1962})$$

where G, is number of germinated seeds

$$MGT = \frac{\sum Dn}{\sum n} \quad (\text{Ellis and Roberts, 1981})$$

Where n is the number of seeds germinated on day D and D is the number of days counted from the start of germination.

$$G.I = \frac{\text{No. of germinated seeds}}{\text{Days of first count}} + \dots + \frac{\text{No. of germinated seeds}}{\text{Days of final count}} \quad (\text{AOSA, 1983})$$

The root and shoot parameters were recorded at 10<sup>th</sup> day of sowing. The root and shoot length was measured and seedlings were separated into root and shoot for fresh weight and dry weight. The dry weight was determined by oven drying at 72 °C up to a point when no further reduction in weight was observed. The statistical analysis (ANOVA) was performed by computer base programme Statistix 9.0 and significance differences among treatments means were evaluated at 0.05 probability level by using LSD test (Steel and Torrie, 1997).

**RESULTS AND DISCUSSION**

**Germination indicators**

The germination % age, CVG and germination index (GI) of tested crops were significantly reduced by extracts application as compared to control. The presence of extracts potentially reduced the seed germination and extracts from brassica proved to be the most retardant. The seeds allowed to germinate with extracts required more periods for mean germination time (MGT). The maximum value for MGT was observed for petri dishes provided with brassica extract followed by alfalfa for all studied crops. It is quite visible from the data presented in Table 1 that there was no significant variation among the tested crops for germination % age. The rice was the most sensitive crop in case of CVG, MGT and GI while the sorghum and millet produced statistically similar value for the said traits. When looking at the interaction factor, we realized that brassica extracts, no doubt, reduced the CVG, GI and improved the value of MGT for all receptor plants but rice seemed to be the relatively most sensitive. Millet and sorghum showed a statistically similar behavior for germination data especially when seeds were allowed to germinate in brassica extract. The presence of brassica extracts limits germinability of cereal seeds and therefore, reduced the GI and CVG over the normal germination. The retardation of germination with extracts related to presence of allelochemicals and relative sensitivity of tested crops. The secondary hydrolysis products of glucosinolates in brassica plant may be the major cause for obtained results (Vera et al., 1987). These results had also been confirmed by Miri (2011) and Tawaha and Turk (2003) where a degree of germination % age was reported against various extracts. It is therefore suggested that on decomposition, the given legumes will expel the allelochemicals to soil and special measures should be adapted to obtain the desired germination level in field previously sown with such legumes.

**Table 1:** Effect of aqueous legume extracts on germination data of rice, sorghum and millet

Extracts	Germination (%)				CVG (seed day <sup>-1</sup> )			
	Rice	Sorghum	Millet	Means Lsd = 3.3090	Rice	Sorghum	Millet	Means Lsd = 0.0077
Control	100.00 ns	100.00	100.00	100.00 a	0.22 abcd Lsd = 0.0134	0.23 a	0.22 abc	0.22 a
Alfalfa	82.50	85.00	85.00	84.17 c	0.20 ef	0.21 de	0.22 ab	0.21 b
Berseem	95.00	95.00	95.00	95.00 b	0.21 cde	0.22 ab	0.21 bcd	0.21 ab
Brassica	80.00	80.00	80.00	80.00 d	0.16 g	0.20 ef	0.18 f	0.18 c
Means	89.38 ns	90.00	90.00		0.20 b Lsd = 0.0066	0.21 a	0.21 a	
Extracts	Mean Germination Time (days)				Germination index (G.I)			
	Rice	Sorghum	Millet	Means Lsd = 0.2443	Rice	Sorghum	Millet	Means Lsd = 0.1051
Control	4.60 cde Lsd = 0.4231	4.38 e	4.44 de	4.48 c	2.28 ab Lsd = 0.1820	2.39 a	2.38 a	2.35 a
Alfalfa	5.18 b	4.80 bcde	4.67 cde	4.88 b	1.71 d	1.97 c	2.19 b	1.96 c
Berseem	4.84 bcd	4.48 de	5.00 bc	4.77 b	2.13 bc	2.22 ab	2.23 ab	2.19 b
Brassica	6.06 a	5.16 b	4.97 bc	5.40 a	1.42 e	1.70 d	1.60 de	1.58 d
Means	5.17 a Lsd = 0.2115	4.70 b	4.77 b		1.88 b Lsd = 0.0910	2.07 a	2.10 a	

The means in columns not sharing a common letter are significantly different at 0.05% probability level

**Fresh and dry weight of root and shoot**

The crops expressed the diversity of response and all seems to be susceptible to applied extracts in term of both fresh and dry weight of root and shoot and maximum growth inhibition was recorded for brassica extract followed by alfalfa (Table 2). The significant variations among studied crops were also exhibited and it actually showed their relative tolerance or susceptibility for extracts. However, rice was the most sensitive and sorghum and millet produced statistically similar behaviour for shoot and root attributes. The interactive effects between extracts and crops for root and shoot traits were non-significant except for root and shoot length. Although the interaction in this study was significant for most of germination attributes like CVG, MGT and GI (Table 1) but the effectiveness declined with the advancement of growth as indicated in Table 2 for root and shoot mass. When looking on behaviour of target crops, we realized that rice root and shoot growth was the most sensitive to stress imposed by allelochemicals (Table 2). The allelopathic activity of alfalfa has also been argued by Abdul-Rahman and Habib, (1989) for bladygrass.

The seeds treated with brassica extracts also showed the lowest seedling height and root length. Alfalfa extract was the second most important retardant for shoot length but for root length, it did not cause a statistically higher

value over berseem extract (Table 2). Although all tested crops showed a degree of inhibition of shoot and root elongation in presence of extracts but the severity of inhibition was the highest in rice. The retardation of germination and seedlings growth of rice induced by extracts, even though not effective for some observations in sorghum and millet showed its comparative sensitivity to allelochemicals. The retardation of root and shoot growth suggested that their potential must be explored to serve the purpose of pre or post emergence weed control. The inhibition of root and shoot growth by brassica extracts had already been cited by Jafarihyazdi and Javidfar (2011) in sunflower. The reduction of shoot and root elongation caused by allelochemicals extracts might be the result of modification of mitochondrial respiration mechanism which reduces the ATP production which proved to be detrimental for ATP based cell functions (Gniazdowska and Bogatek 2005).

In conclusion, the aqueous extracts of legumes had inhibitory effects on germination and seedling growth of receptor cereals. The inhibitory potential of legume can be described as brassica < alfalfa < berseem. From the cereal side, rice was most susceptible to extracts; both sorghum and millet had similar response mechanisms. The future investigation must be focused for isolation and identification of allelochemicals in the studied crops and their possible use in crop production.

**Table 2:** Effect of aqueous legume extracts on shoot and root growth of rice, sorghum and millet

Extracts	Fresh weight of shoot (g)				Dry weight of shoot (g)			
	Rice	Sorghum	Millet	Means Lsd = 0.0400	Rice	Sorghum	Millet	Means Lsd = 0.0403
Control	0.67 ns	0.91	0.92	0.83 a	0.25 ns	0.44	0.46	0.38 a
Alfalfa	0.53	0.78	0.81	0.71 c	0.25	0.39	0.38	0.34 b
Berseem	0.56	0.88	0.82	0.75 b	0.26	0.42	0.42	0.37 ab
Brassica	0.50	0.72	0.75	0.66 d	0.20	0.35	0.34	0.30 c
Means Lsd = 0.0346	0.56 b	0.82 a	0.82 a		0.24 b Lsd = 0.0349	0.40 a	0.40 a	
Extracts	Fresh weight of root (g)				Dry weight of root (g)			
	Rice	Sorghum	Millet	Means Lsd = 0.0383	Rice	Sorghum	Millet	Means Lsd = 0.0239
Control	0.62 ns	0.84	0.82	0.76 a	0.26 ns	0.36	0.37	0.33 a
Alfalfa	0.44	0.71	0.70	0.62 c	0.16	0.28	0.32	0.25 b
Berseem	0.56	0.81	0.78	0.71 b	0.23	0.35	0.34	0.31 a
Brassica	0.35	0.61	0.59	0.52 d	0.16	0.25	0.27	0.23 c
Means Lsd = 0.0332	0.49 b	0.74 a	0.72 a		0.20 b Lsd = 0.0207	0.31 a	0.33 a	
Extracts	Shoot length (cm)				Root length (cm)			
	Rice	Sorghum	Millet	Means Lsd = 0.7910	Rice	Sorghum	Millet	Means Lsd = 0.6606
Control	13.96 d Lsd = 1.3701	21.00 a	20.96 a	18.64 a	8.63 b Lsd = 1.1442	12.31 a	12.09 a	11.01 a
Alfalfa	9.50 e	15.35 c	14.26 cd	13.04 c	7.48 cde	8.48 bc	8.67 b	8.21 b
Berseem	13.46 d	18.68 b	19.20 b	17.11 b	7.32 de	8.38 bcd	8.16 bed	7.95 b
Brassica	6.89 f	14.54 cd	14.70 cd	12.05 d	5.67 f	9.15 b	6.78 ef	7.20 c
Means Lsd = 0.6851	10.95 b	17.39 a	17.28 a		7.28 c Lsd = 0.5721	9.58 a	8.93 b	

The means in columns not sharing a common letter are significantly different at 0.05 % probability level

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