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Original article

Allelopathic Effects of Pigweed (*Amaranthus viridis* L.) on Seed Germination and Seedling Growth of some Leguminous Crops

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Abstract

Laboratory and greenhouse experiments were carried out at the Faculty of Agricultural Sciences, University of Gezira, Sudan in season 2014/15. Laboratory experiments were conducted to study the allelopathic effects of aqueous extract of aboveground parts of pigweed (*Amaranthus viridis* L.) on seed germination of common bean (*Phaseolus vulgaris* L.), cowpea (*Vigna sinensis* [L.] Walp.), pigeon pea (*Cajanus cajan* [L.] Millsp.) and alfalfa (*Medicago sativa* L.). Six concentrations (0, 20, 40, 60, 80 and 100%) of the aqueous extract of aboveground parts were prepared from the stock solution (50 g/l). Treatments, for each crop, were arranged in completely randomized design with four replicates. The seeds were examined for germination at three days after initial germination. Greenhouse experiments were conducted to study the allelopathic effects of powder of aboveground parts of pigweed on seedling growth of the same crops. Powder of aboveground parts was incorporated into the soil at rate of 0, 1, 2, 3, 4 and 5% on w/w bases in pots. Treatments, for each crop, were arranged in completely randomized design with four replicates. Experiments were terminated at 30 days after sowing and plant height, number of leaves and root length of crop seedlings were measured as well as plant fresh and dry weight. Data were collected and subjected to analysis of variance procedure. Means were separated for significance using Duncan`s Multiple Range Test at $p \leq 0.05$. The results showed that the aqueous extract of aboveground parts of pigweed significantly reduced seed germination of the tested leguminous crops and there was direct negative relationship between concentration seed germination. Also, the results showed that incorporating powder of aboveground parts into the soil significantly decreased plant height and root length of crop seedlings as well as seedling fresh and dry weight. In addition, the reduction in seedling growth was increased as the powder increased in the soil. Based on results supported by different studies, it was concluded that pigweed has allelopathic effects on seed germination and seedling growth of the leguminous crops.

Keywords: Allelochemicals, Allelopathic, Alfalfa, Amaranthus, Common Bean, Cowpea, Leguminous, Pigeon Pea, Pigweed.

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INTRODUCTION

Pigweed (*Amaranthus viridis* L.), belonging to the family Amaranthaceae, is commonly known with different names such as Amaranth, Slender amaranth and Chinese spinach. It is an annual herb, erect with height of 40 - 100 cm, little branched, slightly pigmented with thick stems and rather fleshy and it only multiplies by seeds (Lorenzi and Matos, 2008; Teutonico and Knorr, 2011; Pulipati et al., 2014). Pigweed is a common wild vegetable and weed of cultivation and it is distributed in the warmer parts of the world (Hussain et al., 2003, Tabrizi and Yarnia, 2011). It is one of the most important weed species in numerous agricultural areas, being the third widespread dicotyledonous weed species in the world (Namdari et al., 2012). Pigweed has the C4 photosynthetic pathway, which confers an ability to grow rapidly at high temperatures and high light levels, to tolerate drought, and to compete aggressively with warm-season crops for light, moisture, and nutrient (Horak and Loughin, 2000; Shrestha and Swanton, 2007).

Research indicates that leaves and debris leaves, roots, pollen, flowers and stem of *Amaranthus* spp. have detrimental effects on germination and growth of different crop species (Hussain et al., 2003; Tabrizi and Yarnia, 2011). The plant possesses certain allelopathic potential, both inhibitory and stimulatory. Allelopathy is defined as both beneficial and deleterious biochemical interaction between plant and weeds, and/or plants and microorganisms through the production of chemical compounds that escape into the environment and subsequently influence the growth and development neighboring plants. Allelochemicals are present in all types of tissues and released into the rhizosphere by a variety of mechanisms, including decomposition of residues, volatilization and root exudation (Sangeetha and Baskar, 2015). The allelochemicals can reduce cell division or auxin that induces the growth of shoot and roots (Gholami *et al.,* 2011). Allelochemicals such as phenolic compounds inhibit root and shoot length (Hussain and Reigosa, 2011). Growth inhibition caused by these allelochemicals may probably be due to its interference with the plant growth processes. Allelochemicals released to the environment can either inhibit shoot/root growth, nutrient uptake, or may attack a naturally occurring symbiotic relationship thereby destroying the plant's source of a nutrient (Gholami *et al.,* 2011).

The phytochemical screening showed the presence of biologically active constituents belonging to the group of saponins, tannins and phenols, flavonoids, alkaloids, cardiac glycosides, steroids and triterpenoids in the extracts of *A. viridis* (Carminate et al., 2012; Malik et al., 2016). The allelopathic interactions showed that total chlorophyll content, number of developed leaves, stem length, and total plant dry matter were negatively affected by allelopathic effect caused by Amaranth aqueous extracts. Sensitivity of crops to allelochemicals is different among species and genotypes within species (Asghari and Tewari, 2007; Baličević et al., 2014.). Moreover, plants received greater concentrations of extract matured later due to delayed flowering (Amini et al., 2013).

Understanding well the mechanism of allelopathic interactions between weeds and crops will enable to come up with proper and effective management ways to prevent further infestations. Considering the economic importance of leguminous crops (Marinov-Serafimov et al., 2019), these studies were carried out to investigate the allelopathic effects of pigweed (*A. viridis*) on seed germination and seedling growth of some leguminous crops, particularly common bean (*Phaseolus vulgaris* L.), cowpea (*Vigna sinensis* [L.] Walp.), pigeon pea (*Cajanus cajan* [L.] Millsp.) and alfalfa (*Medicago sativa* L.).

Materials and Methods

Experimental site

A series of experiment was carried out at Faculty of Agricultural Sciences (FAS), University of Gezira (UofG), Sudan, comprised germination tests and pot experiments. The germination tests were conducted in the biology laboratory having an average temperature range of 25 - 30°C and the relative humidity ranging from 60 to 70 %. The pot experiments were conducted in a greenhouse of horticulture nursery under field conditions. The experimental site was located at Latitude 14° 24′ N, Longitude 33° 29′ E and 407m asl. The climate of the region is semi-desert with a mean annual precipitation of 100-250 mm/year, with the rainy season extended from June to October and the dry season from March to June. The mean annual evapotranspiration is 2400 mm/year. The mean annual minimum and maximum temperatures are 12 °C in January and 42°C in May, respectively. The soil of the area is characterized by heavy clay soil (clay 60%), with pH 8-8.5, low organic matter and nitrogen, adequate potassium and low available phosphorous (Elbasher, 2016).

Materials collection

Matured plants of pigweed plants were collected from Experimental Farm of the FAS in season 2014/15. The stems were transferred to the biology laboratory of the FAS. The aboveground parts of plants were retrieved from plants and then washed with sterilized distill water, air dried on bench for 15 days at room temperature in a dark room to avoid the direct sun light that might cause undesired reactions. The dried aboveground parts were then crushed into powder and kept in brown bottles till used. Certified commercial seeds of common bean, cowpea, pigeon pea and alfalfa, that have a germination percentage of 95-100% and purity of 100%, were obtained from the central market of Wed Medani city, Gezira stat, Sudan. The seeds were surface sterilized by sodium hypochlorite; (NaOCl) 1% (v/v), solution, for 3 min continuously agitated to reduce fungal infection. Subsequently the seeds were washed with sterilized distill water for several times and stored at room temperature until used.

Laboratory experiments

These experiments were conducted in the biology laboratory to study the allelopathic effects of aqueous extract of aboveground parts of pigweed on seed germination of common bean, cowpea, pigeon pea and alfalfa. Fifty grams of powder of aboveground parts of pigweed were placed in a conical flask, sterilized distill water was added to give a volume of 1000 ml and then flasks were shaken for 24 hours at room temperature (27±3°C) by an orbital shaker (160 rpm). The extracts were drained through double layers of cheese cloth and then through 2 layers of Whatman No-2 filter paper to remove solid material. The filtrate was centrifuged at 3000 rpm for 20 min. The supernatant was collected and filtered through a 0.22 µm membrane filter paper. The stock solution was stored at 4°C until further use. Six concentrations (0, 20, 40, 60, 80 and 100%) of the aqueous extract were prepared from the stock solution (50 g/l). Seeds of common bean, cowpea, pigeon pea and alfalfa (100 seeds each) were put on Glass Fiber Filter Paper (GFFP) (Whatman GF/C) placed in a glass Petri-dish (GPD), 9 cm internal diameter (i.d). Each GPD moistened with 20 ml of aqueous extract of aboveground parts of pigweed, sealed with Parafilm, covered with black polyethylene bag and incubated at 300C in the dark. The treatments, of each crop, were arranged in completely randomized design with four replicates. The seeds were examined for germination at three days after initial germination.

Greenhouse experiments

These experiments were conducted at the greenhouse of horticulture nursery to study the allelopathic effects of powder of aboveground parts of pigweed on seedling growth of sorghum, millet, maize and wheat. Plastic pots, 10 cm i.d. and 18 cm high with drainage holes at the bottom, were filled with Gezira soil and river silt at the ratio 1:1, oven dried at 120ºC for 48 h and screened to pass a 2-mm sieve. The powder of aboveground parts of pigweed was incorporated into the soil at the rate of 0, 1, 2, 3, 4 and 5% on w/w bases. Five seeds of each crop were sown in a pot. The pots were kept weed free, irrigated and then seedlings were thinned to 3 plants per pot, 7 days after emergence. Treatments, for each crop, were arranged in completely randomized design with four replicates. At 30 days after sowing the experiments were terminated and plant height (cm), number of leaves and root length (cm) of crop seedlings were measured as well as plant fresh and dry weight (g).

Statistical analysis

Data were collected and subjected to analysis of variance procedure. Means were separated for significance using Duncan`s Multiple Range Test at $p \leq 0.05$. The statistical analysis was done using the Statistical Analysis System software (SAS) v.9.0.

Results

Laboratory experiments

The results of laboratory experiments, at 30 days after sowing, showed that the aqueous extract of underground parts of pigweed significantly reduced seed germination of the tested leguminous crops compared to the controls (Table 1).

Table 1. Allelopathic effects of aqueous extract of aboveground parts of pigweed on seed germination of some leguminous crops

Concentration of aqueous (w/v)	Seed germination (%)			
	Common bean	Cowpea	Pigeon pea	Alfalfa
0 %	98.5 a	99.3 a	98.5 a	99.0 a
20 %	89.5 b	92.0 b	92.5 b	91.3 b
40 %	81.0 c	83.8 c	82.5 c	82.5 c
60 %	70.3 d	75.0 d	77.5 d	74.5 d
80 %	58.0 e	64.5 e	67.0 e	65.0 e
100 %	51.5 f	50.0 f	47.5 f	55.5 f
SE ±	1.26	1.32	1.41	1.57
CV %	3.4	3.4	3.6	4.0

* Means in the same column followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).

The reduction in seed germination increased with concentration of aqueous seeds extract (Fig. 1). The highest seed germination was observed in the corresponding controls.

However, the highest concentration (100%) displayed lowest seed germination which was 51.5, 50.0, 47.5 and 55.5 % in common bean, cowpea, pigeon pea and alfalfa, respectively.

Greenhouse experiments

The results of the greenhouse experiments showed that incorporating seed powder of Pigweed into the soil significantly ($p \leq 0.05$) decreased seedling growth attributes of tested leguminous crops compared to control treatments (Table 2, 4, 5 and 6).

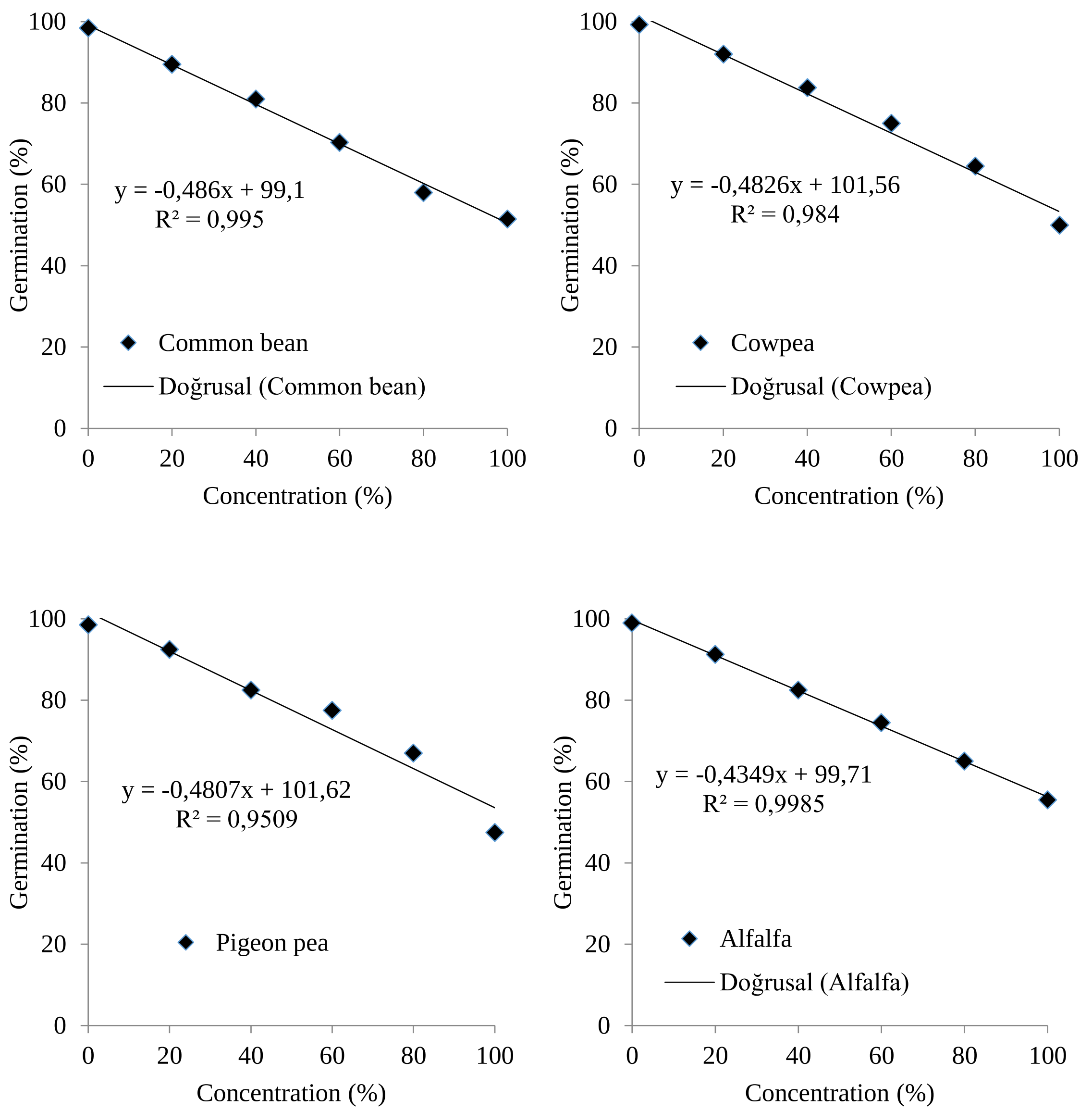

Figure 1. Allelopathic effects of aqueous extract of aboveground parts of pigweed on seed germination of some leguminous crops

Effects on plant height

At 30 days after sowing, the highest plant crop seedlings were observed in the control treatments (Table 2). The plant height of common bean, cowpea, pigeon pea and alfalfa in the control treatments was 286.3, 150.0, 200.0 and 249.5 mm, respectively. Incorporating powder of aboveground parts of pigweed into the soil at rate of 1 % significantly decreased the plant height of leguminous crops in comparison to control treatments. Moreover, the reduction in the plant height was increased as powder increased in the soil. The greatest reduction in plant height was observed when powder was added to the soil at the rate of 5 %. The plant height was decreased to 209.3 mm in common bean, 95.5 mm in cowpea, 142.0 mm pigeon pea and 86.3 mm in alfalfa seedlings ($p \leq 0.05$).

Table 2. Allelopathic effects of incorporated powder of aboveground parts of pigweed into the soil on plant height of some leguminous crops

Concentration of the powder (w/w)	Plant height (mm)			
	Common bean	Cowpea	Pigeon pea	Alfalfa
0 %	286.3 a	150.0 a	200.0 a	249.5 a
1 %	276.3 b	139.5 b	181.5 b	221.3 b
2 %	268.5 c	128.5 c	168.3 c	188.8 c
3 %	251.5 d	116.8 d	155.3 d	159.0 d
4 %	229.3 e	110.0 e	150.8 d	128.8 e
5 %	209.3 f	95.5 g	142.0 e	86.3 f
SE ±	2.25	1.7	1.76	2.47
CV %	1.8	2.8	2.1	2.87

* Means in the same column followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).

Effects on number of leaves

At 30 days after sowing, the results showed that incorporating powder of aboveground parts of pigweed into the soil at rate of 1, 2, 3, 4 and 5 % did not significantly ($p \leq 0.05$) affected the leaves number of seedlings of the leguminous crops tested compared to control treatments (Table 3).

Table 3. Allelopathic effects of incorporated powder of aboveground parts of pigweed into the soil on number of leaves of some leguminous crops

Concentration of the powder (w/w)	Number of leaves			
	Common bean	Cowpea	Pigeon pea	Alfalfa
0 %	6.5 a	10.8 a	8.8 a	7.5 a
1 %	6.5 a	10.3 a	8.3 a	7.3 a
2 %	6.3 a	10.3 a	8.3 a	7.3 a
3 %	6.3 a	10.3 a	8.0 a	7.3 a
4 %	6.3 a	10.0 a	8.0 a	7.0 a
5 %	5.8 a	9.8 a	8.0 a	6.8 a
SE ±	0.46	0.64	0.69	0.58
CV %	14.6	12.6	16.8	16.1

* Means in the same column followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).

Effects on root length

Incorporation of the powder of aboveground parts of pigweed into the soil significantly reduced root length of leguminous crops (Table 4). The reduction in root lengths was increased with concentration of the powder of aboveground parts of pigweed into the soil.

Table 4. Allelopathic effects of incorporated powder of aboveground parts of pigweed into the soil on root length of some leguminous crops

Concentration of the powder (w/w)	Seedlings root length (mm)			
	Common bean	Cowpea	Pigeon pea	Alfalfa
0 %	168.8 a	82.8 a	98.5 a	81.5 a
1 %	166.5 a	75.8 b	95.5 a	76.3 b
2 %	163.5 b	71.5 c	87.3 b	71.8 c
3 %	159.0 c	63.3 d	79.5 c	67.0 d
4 %	151.5 d	61.0 d	74.5 d	64.3 d
5 %	146.3 e	60.8 d	68.3 e	58.0 e
SE $_\pm$	0.98	1.27	1.37	1.04
CV $_\%$	1.2	3.7	3.27	3.0

* Means in the same column followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

At 30 days after sowing, the longest root lengths of crop seedlings were observed in the control treatments and amounted to 168.8, 82.8, 98.5 and 81.5 mm in common bean, cowpea, pigeon pea and alfalfa, respectively. Incorporating the powder of aboveground parts of pigweed into the soil at rate of 1 % significantly decreased the plant height of leguminous crops in comparison to control treatments. In addition, the reduction in the root length was increased with further increase of pigweed powder in the soil. The greatest reduction in root length was observed when seed powder was added to the soil at the rate of 5 %. The corresponding root length was decreased to 51.0 mm in common bean, 30.5 mm in cowpea, 81.3 mm pigeon pea and 59.8 mm in alfalfa seedlings ($p \leq 0.05$).

Effects on fresh weight

The greatest fresh weights of crop seedlings, at 30 days after sowing, were recorded in the control treatments (Table 5).

Table 5. Allelopathic effects of incorporated powder of aboveground parts of pigweed into the soil on fresh weight of some leguminous crops

Concentration of the powder (w/w)	Seedlings fresh weight (mg)			
	Common bean	Cowpea	Pigeon pea	Alfalfa
0 %	133.3 a	87.8 a	117.0 a	77.5 a
1 %	120.8 b	72.0 b	101.8 b	74.8 a b
2 %	108.8 c	65.8 c	96.0 c	72.5 b
3 %	83.8 d	53.0 d	85.8 d	67.0 c
4 %	62.0 e	41.0 e	83.5 d e	66.0 c
5 %	51.0 f	31.0 f	80.3 e	60.8 d
SE $_\pm$	3.25	1.11	1.35	1.13
CV $_\%$	7.0	3.8	2.9	3.2

* Means in the same column followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).

Incorporating powder of aboveground parts of pigweed in soil at the rate of 1% or more significantly reduced fresh weight of common bean, cowpea and pigeon pea in comparison to control treatments. While, significant reduction in fresh weight of alfalfa seedlings were obtained when seed powder incorporated in soil at the rate of 2% or more compared to control treatment. Moreover, the reduction in the fresh weight was increased as powder increased in the soil and the most significant effect was observed at the rate of 5%. The incorporation of powder of aboveground parts of pigweed into the soil at rate of 5% resulted in seedling fresh weights amounted to 51.0, 31.0, 80.3 and 60.8mg in common bean, cowpea, pigeon pea and alfalfa, respectively.

Effects on dry weight

The results of incorporated powder of aboveground parts of pigweed into the soil at rate of 1, 2, 3, 4 and 5 % on seedling dry weight had same trend as seedlings fresh weight (Table 6).

Table 6. Allelopathic effects of incorporated powder of aboveground parts of pigweed into the soil on dry weight of some leguminous crops

Concentration of the powder (w/w)	Seedlings dry weight (mg)			
	Common bean	Cowpea	Pigeon pea	Alfalfa
0 %	27.0 a	69.5 a	50.0 a	41.0 a
1 %	19.3 b	62.8 b	45.3 b	31.3 b
2 %	16.3 c	51.8 c	36.8 c	26.0 c
3 %	11.5 d	40.0 d	32.8 d	22.0 d
4 %	9.0 e	31.8 e	20.8 e	16.3 e
5 %	6.3 f	21.5 f	16.8 f	11.0 e
SE $_{\pm}$	0.66	1.35	1.29	1.23
CV $_{\%}$	8.9	5.8	7.7	9.2

* Means in the same column followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).

Incorporating pigweed powder in soil at the rate of 1 % significantly reduced fresh weight of common bean, cowpea, pigeon pea, and alfalfa in comparison to control treatments. The dry weight was decreased to 6.3 mg in common bean, 21.5 mg in cowpea, 16.8 mg pigeon pea and 11.0 mg in alfalfa seedlings ($p \leq 0.05$).

Discussion

The results of these studies revealed that the aqueous extract of aboveground parts of pigweed significantly reduced seed germination of the tested leguminous crops and there was a direct relationship between concentration and reduction in germination. Also, the study indicated that incorporating powder of aboveground parts of pigweed into the soil at rate of 1, 2, 3, 4 and 5% (w/w) significantly reduced seedlings growth. Moreover, the reduction in seedling growth was increased as the powder increased in the soil. This finding was in agreement with observation made by de Souza et al., (2011) who stated that Amaranthus spp. are plants with proven allelopathic potential. The release of allelochemicals from Amaranthus spp. in the environment can influence seed germination, photosynthesis rate, reduce growth and, consequently, the productivity of various plant species. Among the Amaranthus spp. with allelopathic potential is A. viridis**.**

Bakhshayeshan-Agam et al. (2015) studied the allelopathic effects of redroot pigweed (Amaranthus retroflexus L.) on germination and growth of four important crop species including cucumber (Cucumis sativus L.), alfalfa, common bean and wheat. The authors found that the effect of different concentrations of redroot pigweed leachate on seed germination and seedlings growth parameters of tested plants was significant, but not same in all studied species. Common bean and alfalfa were more sensitive in seed germination stage in comparison to wheat and cucumber. Namdari et al. (2012) evaluated the allelopathic effects of redroot pigweed root exudates on seedling growth of common bean cultivars. Results indicated that the effects of common bean cultivar, redroot pigweed seed density and growth time were significant on common bean root and shoot length. The redroot pigweed growing time of 6 days and densities of 16 and 24 plants per beaker had the greatest inhibitory effect on common bean root and shoot length. Redroot pigweed allelopathic effect on common bean may be through release of allelochemicals and thereby affecting the common bean growth and establishment.

These results are in accordance with previous studies reported by Velu et al. (1990) who observed that extracts of roots, aerial part and whole plant of various pigweed species reduced the germination and growth of seedlings of green mung bean (*Vigna radiate* L.) and black mung bean (*Vigna mungo* L.). The aqueous leached components of the different parts of spiny Amaranthus (*Amaranthus spinosus* L.) inhibited the germination and the growth of seedlings of pigeon pea and guar (*Cyamopsis tetragonoloba* [L.] Taub.). The maximum inhibition was caused by leached components of the leaves and the minimum by leached components from the roots (Suma, 1998). Leaf and aerial part extracts of redroot pigweed promoted greater inhibition in the germination, vigor and weight of alfalfa seedlings than root extracts (Chung et al., 1994; Chung and Miller, 1995).

Similar result was obtained by Amini et al. (2013) who carried out a pot experiment in order to evaluate the allelopathic effects caused by smooth amaranth (*Amaranthus hybridus* L.) dry shoot extracts on some morpho-physiological characteristics and phenology of three different types of dry beans. The results revealed that total chlorophyll content, number of developed leaves, stem length, and total plant dry matter were negatively affected by allelopathic effect caused by smooth amaranth aqueous extracts. Plants received greater concentrations of extract matured later due to delayed flowering, but differential reactions were observed depending on the dry bean type. Considerable losses of grain yield were recorded at 15 and 20% extract concentrations.

Thus, *Amaranthus* spp. are plants with proven allelopathic potential, which require more studies related to the effects of their allelochemicals over cultivated plants and other weed plants. Isolation and identification of allelochemical compounds from these plants could provide means to minimize their negative effects over the cultures and potentially could provide structural models for the development of bioherbicides or semisynthetic herbicides **(**de Souza et al., 2011)**.**

Conclusion

- The aqueous extract of aboveground parts of pigweed significantly reduced seed germination of the leguminous crops; common bean, cowpea, pigeon pea and alfalfa. There was a direct relationship between concentration and reduction in germination.
- Incorporating powder of aboveground parts of pigweed into the soil at rate of 1, 2, 3, 4 and 5% significantly decreased plant height and root length of crop seedlings as well as plant fresh and dry weight. In addition, the reduction in seedling growth was increased as seed powder increased in the soil.
- More studies related to the effects of pigweed allelochemicals over cultivated plants and other weed plants are required.
- Isolation and identification of allelochemical compounds from this plant could provide means to minimize their negative effects over the cultures and potentially could provide structural models for the development of bio-herbicides.

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