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Allelopathic Effect of Eucalyptus (*Eucalyptus camaldulensis* Dehnh) Leaf on Seed Germination and Seedling Growth of some Poaceous 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 to study the allelopathic effects of Eucalyptus (Eucalyptus camaldulensis Dehnh) leaf on seed germination and seedling growth of some poaceous crops. Laboratory experiments were conducted to study the allelopathic effects of leaf aqueous extract of Eucalyptus on seed germination of sorghum (Sorghum bicolor [L.] Moench), millet (Pennisetum glaucum [L.] R. Br.), maize (Zea mays L.) and wheat (Triticum vulgare L.). Six concentrations (0, 20, 40, 60, 80 and 100%) of the leaf aqueous extract of Eucalyptus 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 Eucalyptus leaf powder on seedling growth of the same crops. The leaf powder of Eucalyptus was incorporated into the soil at rate of 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% on w/w bases in pots. Treatments, for each crop, were arranged in completely randomized design with four replicates. The experiments were terminated at 30 days after sowing and the plant height, number of leaves and root length of crop seedlings were measured as well as plant fresh and dry weight. Data were subjected to analysis of variance procedure. Means were separated for significance using Duncan`s Multiple Range Test at $p \leq 0.5$. The results showed that the leaf aqueous extract of Eucalyptus significantly reduced seed germination of the tested poaceous crops and there was direct negative relationship between concentration and germination. Also, the results showed that incorporating leaf powder of Eucalyptus into the soil significantly decreased plant height, number of leaves 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 leaf powder increased in the soil. Based on results supported by different studies, it was concluded that Eucalyptus has allelopathic effects on seed germination and seedling growth of the poaceous crops.*

Keywords*— *Allelopathy; Allelochemicals; Eucalyptus; Poeaeae.

I. INTRODUCTION

Eucalyptus (*Eucalyptus camaldulensis* Dehnh), belonging to the family Myrtaceae, is commonly known with different names such as long beak eucalyptus, murray red gum, red gum, river gum, river red gum and red river gum (Shayoub, *et al*., 2015). The Eucalyptus tree is a large, fast-growing evergreen that can grow to 125-160 meters (Sani, *et al*., 2014). It is indigenous to Australia and have been introduced into many countries, owing to their fast growth and their rising demand for paper and plywood (Cossalter and Pye-Smith, 2003). To fill the widening gap between the supply and demand of forest raw materials, many *Eucalyptus* species are even grown in agricultural fields with crops (Malik, 2004), owing to its fast growth, wider adaptability and high productivity (Cossalter and Pye-Smith, 2003; Gardner, 2007). Eucalyptus occupies agricultural land intended for food crops cultivation; and may negatively affect native plant species (including crops). Also, Eucalyptus can compete with crops underlying light, water and soil nutrient (Onyewotu *et al*., 1994; Pérez Bidegain *et al*., 2001) or by changing the soil pH (Kubmarawa *et al*., 2008; Mubarak *et al*., 2011). Moreover, there is continuing controversy about the ecological functions of *Eucalyptus*, it reduces the diversity of understorey species and the productivity of understory crops (Moradshahi, *et al*., 2003), because its allelochemicals have allelopathic effects on other plant species (Turnbull, 1999).

Allelopathy is refers to direct or indirect positive or negative effect of one plant on another through the release of chemical compounds into the environment (Delabays *et. al.*, 2004). These biochemicals are known as allelochemicals (Singh and Chaundhary, 2011). Allelochemicals are released from plant parts by means of leaching, root exudation, volatilization, residue decomposition and other processes in both natural and

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agricultural systems (Chou, 1990). 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). Considering the economic importance of poaceous crops, these studies were carried out to investigate the allelopathic effects of Eucalyptus (*Eucalyptus camaldulensis* Denhn) on seed germination and seedling growth of some poaceous crops, particularly sorghum (*Sorghum bicolor* [L.] Moench), millet (*Pennisetum glaucum* [L.] R. Br.), maize (*Zea mays* L.) and wheat (*Triticum vulgare* L.).

II. MATERIAL AND METHODS

2.1. Experimental site

A series of experiment was carried out at Faculty of Agricultural Sciences (FAS), University of Gezira (UofG), Sudan, comprised germination test and pot experiments. The germination test was 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 experiment was 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).

2.2. Materials collection

Matured leaves of Eucalyptus tree were collected from Experimental Farm of the FAS in season 2014/15. The leaves were transferred to the biology laboratory of the FAS. The Leaves were washed with sterilized distilled 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 leaves were then crushed into powder and kept in brown bottles till used. Certified commercial seeds of sorghum (cv. *Tabat*), millet (cv. *Baladi*), maize (cv. *Hudeiba* I) and wheat (cv. *Imam*), that have a germination percentage of 95-100% and purity of 100%, were obtained from the central market of Wed Medani city, Gezira State, 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 till used.

2.3. Laboratory experiments

These experiments were conducted in the biology laboratory to study the allelopathic effects of aqueous extract of Eucalyptus leaf on seed germination of sorghum, millet, maize and wheat. Fifty grams of seeds powder of Jimsonweed were placed in a conical flask, sterilized distill water was added to give a volume of 1000 ml and then the 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. Seeds of sorghum, millet, maize and wheat (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 Eucalyptus leaf aqueous extract, sealed with Parafilm, covered with black polyethylene bag and incubated at 30^0C 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 for three days.

2.4. Greenhouse experiments

These experiments were conducted at the greenhouse of horticulture nursery to study the allelopathic effects of leaf powder of Eucalyptus on seedlings 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 that at the ratio 1:1, oven dried at 120 C for 48 h and screened to pass a 2-mm sieve. The leaf powder of Eucalyptus was incorporated into the soil at rate of 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% on w/w bases. Five seeds of each crop were sown in pots. 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

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randomized design with four replicates. At 30 days after sowing the experiments were terminated and plant height, number of leaves and root length of seedlings were measured as well as seedling fresh and dry weight.

2.5. Statistical analysis

Data were collected and subjected to analysis of variance procedure. Means were separated for significance using Duncan`s Multiple Range Test ($P \leq 0.05$). The statistical analysis was done using the Statistical Analysis System software v.9.0 (SAS, 2004).

III. RESULTS

3.1. Laboratory experiments

The results of laboratory experiments showed that the aqueous extract of Eucalyptus leaf significantly ($P \leq 0.05$) reduced seed germination of the tested poaceous crops compared to the controls (Table 1). The reduction in seed germination increased with concentration of aqueous leaf extract. The highest seed germination was observed in the corresponding controls. However, the highest concentration (100%) displayed lowest seed germination which was 71.3, 80.0, 83.7 and 56.0% in sorghum, millet, maize and wheat, respectively. Wheat seeds were highly affected by the aqueous extract of Eucalyptus leaf in comparison to other tested crops. Also, the results showed direct negative relationship between concentration and germination.

3.2. Greenhouse experiments

The results of the greenhouse experiments showed that incorporated leaf powder of Eucalyptus into the soil significantly ($P \leq 0.05$) decreased seedling growth attributes of tested poaceous crops in comparison to the controls (Table 2, 4, 5 and 6).

Table.1: Allelopathic effects of leaves aqueous extract of Eucalyptus on seed germination of some poaceous crops

Concentration of the aqueous extract (w/v)	Seed germination (%)			
	Sorghum	Millet	Maize	Wheat
0	99.3 a	99.0 a	96.0 a	98.7 a
20	93.3 b	98.3 a	94.0 a	92.7 a
40	90.3 b	93.3 b	92.7 a	84.7 b
60	82.7 c	87.0 c	89.0 b	77.3 b
80	74.7 d	84.0 c	86.0 bc	69.0 c
100	71.3d	80.0 d	83.7 c	56.0 d
SE±	1.4	1.2	1.1	2.4
CV%	8	2	2	5

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 plant height

At 30 days after sowing, the highest plant crop seedlings were observed in the control treatments (Table 2). The plant height of sorghum, millet, maize and wheat in the control treatments were 36.7, 30.0, 39.3 and 28.0 cm, respectively. However, increasing the concentration of leaf powder of Eucalyptus into the soil exhibited lowest plant height in all tested crops. The leaf powder when incorporated into the soil at rate of 0.5 to 3% decreased the plant height of poaceous crops in comparison to control treatments. Moreover, the reduction in the plant height was increased as leaf powder increased in the soil. The greatest reduction in plant height was observed when leaf powder was incorporated into the soil at the rate of 3%. At high concentration of leaf powder, the plant heights were significantly decreased to 7.3 cm in sorghum, 7.3 cm in millet, 20.3 cm maize and 2.6 cm in wheat seedlings.

Effects on number of leaves

At 30 days after sowing, the results showed that incorporated Eucalyptus leaf powder into the soil at rate of 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% negatively affected the leaves number of seedlings of all tested crops compared to the control treatments (Table 3). The highest leaves numbers of crop seedlings were obtained in the control treatments. The leaves number of sorghum, millet, maize and wheat in the control treatments was 6.3, 5.3, 5.3 and 5.0, respectively. Incorporating leaf powder into soil at the rate of 3.0 and 2.5% significantly ($P \leq 0.05$) reduced leaves number of seedlings of sorghum and maize in comparison to the control treatments. While, non-significant reduction in leave number of seedlings of millet was obtained as leaf powder incorporated into soil at the rate of 3% in millet and wheat.

Table.2: Allelopathic effects of incorporated leaf powder of Eucalyptus into soil on plant height of some poaceous crops

Concentration seeds powder (w/w)	Plant height (cm)			
	Sorghum	Millet	Maize	Wheat
0.0	36.7 a	30.0 a	39.3 a	28.0 a
0.5	33.0 ab	25.7 ab	35.0 ab	26.7 a
1.0	25.0 bc	21.3 bc	33.3 ab	23.3 ab
1.5	20.0 cd	19.0 c	32.3 ab	19.0 bc

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2.0	15.0 de	17.0 cd	30.3 ab	18.0 bc
2.5	9.0 e	13.3 d	26.7 bc	16.7 c
3.0	7.3 e	7.3 e	20.3 c	16.0 c
$SE_{\pm}$	2.7	1.8	3.0	2.6
$CV_{\%}$	20	16	17	15

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

Table.3: Allelopathic effects of incorporated leaf powder of Eucalyptus into soil on number of leaves of some poaceous crops

Concentration seeds powder (w/w)	Number of leaves			
	Sorghum	Millet	Maize	Wheat
0.0	6.3 a	5.3 a	5.3 a	5.0 a
0.5	6.0 ab	5.3 a	5.0 ab	5.0 a
1.0	6.0 ab	4.3 a	4.7 ab	4.7 a
1.5	4.7 ab	4.0 a	4.7 ab	4.3 a
2.0	4.0 ab	4.3 a	4.3 ab	3.7 a
2.5	3.3 ab	4.0 a	3.7 b	3.3 a
3.0	3.7 b	4.0 a	3.7 b	3.3 a
$SE_{\pm}$	0.82	0.80	0.49	0.77
$CV_{\%}$	18	20	19	18

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

Table 4. Allelopathic effects of incorporated leaf powder of Eucalyptus into soil on seedlings root length of some poaceous crops

Concentration seeds powder (w/w)	Seedlings root length (cm)			
	Sorghum	Millet	Maize	Wheat
0.0	26.7 a	20.0 a	29.0 a	20.0 a
0.5	26.0 a	20.0 a	25.0 ab	19.0 ab
1.0	21.3 ab	17.7 a	25.0 ab	17.0 ab
1.5	18.3 bc	17.3 a	22.7 ab	15.0 b
2.0	15.0 cd	14.7 a	19.3 ab	10.0 c
2.5	10.0 de	6.0 b	20.3 ab	9.0 c
3.0	5.3 e	5.7 b	20.3 b	10.3 c
$SE_{\pm}$	1.8	1.6	2.7	1.4
$CV_{\%}$	18	19	20	17

* 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 Eucalyptus leaf powder into the soil significantly reduced root length of poaceous crops (Table 4). The reduction in root lengths was increased with Eucalyptus leaf powder concentration in the soil. At 30 days after sowing, the longest root lengths of crop seedlings were observed in the control treatments and amounted to 26.7, 20.0, 29.0 and 20.0cm in sorghum, millet, maize and wheat, respectively. The root length was decreased to 5.3 cm in sorghum, 5.7 cm in millet, 20.3 cm maize and 10.3 cm in wheat seedlings when Eucalyptus leaf powder was incorporated into the soil at concentration of 3%.

Effects on fresh weight

The greatest fresh weights of both shoot and root of crop seedlings, at 30 days after sowing, were recorded in control treatments (Table 5). The incorporation Eucalyptus leaf powder into soil at the rate of 1% or more significantly reduced shoot fresh weight of sorghum, millet, maize and wheat in comparison to control treatments, while, the root fresh weights were significantly reduced as Eucalyptus leaf powder incorporated into soil at the rate of 2.5% or more. Moreover, the reduction in the fresh weight was increased as seed powder increased in the soil.

Effects on dry weight

The results of incorporated Eucalyptus leaf powder into the soil at rate of 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0%on seedling dry weight had same trend as seedlings fresh weight (Table 6). Incorporating 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% powder into the soil at rate of > 3% significantly reduced dry weight of sorghum, millet, maize and wheat in comparison to the control treatments.

IV. DISCUSSION

This study revealed that the aqueous extract of *E. camaldulensis* significantly reduced seed germination of the tested poaceous crops and there was a direct negative relationship between concentration and germination. Also, the study indicated that incorporating powder of leaf powder of Eucalyptus into the soil at rate of 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% (w/w) significantly reduced seedlings growth. Moreover, the reduction in seedling growth was increased as the powder increased in the soil. These findings were in agreement with observation made by Patil (2002) who studied a greenhouse experiment to determine the allelopathic effect of Eucalyptus site soil on the germination and growth of maize, wheat and sorghum. Soil samples were collected at 3, 6, 9, 12, 15 and 18 m distances from the tree row. Two kg of soil containing natural eucalyptus litter from each distance

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was placed into the polybags and then watered to bring the soil moisture to field capacity. Ten seeds of each crop were dibbled into the polybags and watered regularly to maintain uniform soil moisture. Twenty one days after sowing, shoot length and dry weight, and root length and dry weight per plant were recorded. The site soil had significant effect on all the parameters studied. The soil collected from 3 and 6 m distances significantly reduced the shoot and root dry weights per plant. The shoot length, and shoot and root dry weights of wheat was the most affected in polybag containing soil collected from 3 m distance. Maize was relatively tolerant to the allelopathic effect of the eucalyptus site soil. Also, Mohamadi and Rajaie (2009) examined the allelopathic effect of leaf leachate of *E camaldulensis* on germination, growth, morphological and physiological criteria of sorghum *(Sorghum bicolor)* and kidney-bean *(Phaseolus vulgaris).* Leaf leachate was tried at 5, 10 and 20% concentrations and sterilized distilled water used as control. Seed germination, seedlings dry matter, shoot/root length were significantly reduced by all concentrations in both species (at 0/05 level). Decrease in chlorophyll content, soluble sugar content and consequently protein content is proportional to the increase in concentration of leaf leachate in both species. Similar results were obtained by Khan *et al*., (2007) who studied the allelopathic effect of soaked, crushedand boiled in tape water *E. camaldulensis* extracts on seed germination and growth of maize var. 'Kissan'. Results showed that all the extracts significantly reduced maize seed germination, root and shoot length, as well as fresh and dry weight. Gurmu (2015) carried out a pot experiment to determine the effects of different leaves powder proportions of *E. camaldulensis* and *E. grandis* on agricultural crops (haricot bean and maize) as well as change in soil reaction. Results suggested that leaves powder of each tree species induced significant inhibitory effect on germination and seedling growth of each crops compared to the control treatments. Application of low-dose leaves powder of either tree species had low effect on both crops. This indicates that maize and haricot bean should not be planted very close to *Eucalyptus* trees and crops seed rate should be increased to get the maximum germination.

Table.5: Allelopathic effects of incorporated leaf powder of Eucalyptus into soil on seedlings fresh weight of some poaceous crops

Concentration seeds powder (w/w)	Seedlings fresh weight (g)							
	Sorghum		Millet		Maize		Wheat	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
0.0	3.7 a	1.70 a	3.8 a	0.48 a	6.9 a	3.21 a	0.50 a	0.40 a
0.5	3.2 ab	1.70 a	3.1 b	0.48 a	6.0 b	3.20 a	0.46 ab	0.40 a
1.0	2.7 bc	1.50 ab	2.5 c	0.47 a	5.1 c	3.00 a	0.43 bc	0.38 a
1.5	2.2 cd	1.50 ab	1.6 de	0.43 b	4.2 d	2.70 a	0.38 cd	0.38 a
2.0	1.7 de	1.30 b	1.2 e	0.40 bc	3.3 e	1.50 b	0.34 de	0.30 ab
2.5	1.2 e	0.70 c	0.7 e	0.40 bc	2.4 f	0.75 c	0.30 e	0.25 b
3.0	0.4 f	0.16 d	0.7 e	0.37 c	1.4 g	0.73 c	0.24 f	0.20 b
SE±	0.17	0.10	0.17	0.01	0.29	0.16	0.02	0.03
CV%	14	15	16	4	12	13	7.7	16

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

Table.6: Allelopathic effects of incorporated leaf powder of Eucalyptus into soil on seedlings dry weight of some poaceous crops

Concentration seeds powder (w/w)	Seedlings dry weight (g)							
	Sorghum		Millet		Maize		Wheat	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
0.0	0.8 a	0.81 a	0.32 a	0.24 a	1.3 a	1.31 a	0.30 a	0.20 a
0.5	0.6 b	0.80 a	0.30 ab	0.23 ab	1.2 a	1.30 a	0.30 a	0.20 a
1.0	0.4 c	0.60 b	0.28 abc	0.20 bc	0.9 b	1.20 a	0.20 b	0.20 a
1.5	0.3 d	0.60 b	0.26 bc	0.20 bc	0.7 bc	1.20 a	0.20 b	0.20 a
2.0	0.2 e	0.60 b	0.26 bc	0.20 bc	0.5 cd	1.00 a	0.19 b	0.10 b
2.5	0.2 e	0.30 c	0.24 c	0.19 c	0.4 d	0.60 b	0.17 b	0.10 b
3.0	0.2 e	0.12 d	0.24 c	0.18 c	0.4 d	0.39 b	0.17 b	0.10 b

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SE±	0.01	0.05	0.01	0.01	0.09	0.11	0.01	0.02
CV%	6	16	9	10	20	19	12	19

* 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 results of this study were also in agreement with observation made by Ziaebrahimi *et al.*, (2007) who evaluate the allelopathic effect of Eucalyptus (leaves extract) on germination and growth of three wheat cultivars and reported the decreased germination percentage, leaf and root lengths, dry and wet weights of both roots and shoots. Khan *et al*., (2009) studied the allelopathic influence of aqueous extracts of *E. camaldulensis* on the germination (%) and seedling growths (fresh and dry weight) of wheat have been determined. It was noted that aqueous extracts at a concentration of 10, 15 and 20% had inhibitory effect on wheat germination and effect was found significantly higher than control treatment. Fresh and dry weight of seedling was also reduced significantly over control. The inhibitory effects were increased as the extract concentration increased. These findings indicate that wheat sown in fields which had leaf litter of *E. camaldulensis*. will be adversely affected regarding germination, growth and ultimately resulting in lower yields of wheat.

V. CONCLUSION

- The aqueous extract of Eucalyptus leaf significantly reduced seed germination of the poaceous crops; sorghum, millet, maize and wheat. There was a direct negative relationship between concentration germination.
- Incorporating leaf powder of Eucalyptus into the soil at rate of 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% significantly decreased plant height, number of leaves 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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