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Allelopathic effect of alfalfa residues on germination and growth of barley

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Abstract : This study aimed at evaluating the allelopathic effects ofalfalfa residues on the germination and growth of the barley plant grains treated with different concentrations (6gm, 9gm, 12gm) of alfalfa residues. Results of this study showed that the percentage of germination significantly decreased when treated with different concentrations of alfalfa plant residues compared to the control. The concentration of 12gm led to the highest significant decrease. Barley seedlings germination percentage was decreased significantly with a rate that fluctuates between 26.7% - 60% during the first week of seedling emergence compared to the control. Also, the lowest significant decrease ($p < 0.05$) was recorded in the length of the foliage (12.67 cm in length in the first sample and 16.33 cm in the second sample), poxes (4.5 cm in length in the first sample and 4.67 cm in the second sample) and the whole plant (17.17 cm in length in the first sample and 21.0 cm in length in the second vessel) at a concentration of 12g compared to the control. In the case of weights, the concentration of 12g recorded the least significant difference ($p < 0.05$) in the first sample (with a weight of 0.22 g), while in the second sample the concentration of 9g recorded the lowest significant difference ($p<0.05$) for the whole plant (0.25gm weight) compared with the control, while no significant differences were recorded in the difference in the time of sampling.Further investigations are needed to determine the influence of this variations, and to identify the active compounds involved in alfalfa residues allelopathy.

Keywords : Allelopathy,Allelopathic effect; Alfalfa residues; Barley.

1. Introduction

Allelopathy is an important mechanism of plant competition by releasing the allelopatic compounds into environment to inhibit other plants growth and development [1, 2].

The allelopathic compounds are more biodegradable and less harmful to the environment than synthetic chemical herbicides, hence, they are attractive alternatives to present herbicides which have caused development of herbicide resistance in weeds[3].

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Barley (*Hordeumvulgare* L.) isa cereal crop that belong to the family Poaceae (order Poales).It is mainly used for animal feed and for brewing, although it is also considered a principal food in regions where other major cereals cannot be grown. Barley was viewed as a nutritious food and Roman gladiators were known as hordearii(barley men) because it formed part of their training diet [4].

Alfalfa (*Medicago sativa* L.) is one of the major forage legume used for soil improvement and livestock feed[5].Alfalfa plant has a great significance for agriculture in Libya and throughout the world. Several studies have shown that alfalfa is rich in pharmacologically active compounds as well as nutritional properties [6, 7].It contains water-soluble allelopathic compounds which could potentially affect the same species (autotoxic) and other plants[5, 8].

Decomposed crop residues releases allelochemicals can inhibit weed boom in farmlands, and reduce the prevalence of diseases and pests[9]. However, residues mulch can increase the content of soil organic matter and improve soil fertility. Also it shows negative effect by soil sickness [10].Plant residues or extracts and mulches of plants rich in allelochemicals can be utilized as an effective strategy for the control of parasitic weeds[11].Decomposed residues of kharif sorghum show allelopathic effect to wheat and Phalaris minor in rabi season[9].Previous study demonstrated that decomposed alfalfa roots and their associated soil produced a 51–56% reduction in blady grass seed germination [12].

Limited information exists, about the allelopathic effect of alfalfa residue on germination and growth of barely. Therefore, this research pursued a multifaceted aim as it set out to investigate (a) the effects of alfalfa plant residues on germination and growth of barley plant and (b) toknow which of the studied concentrations had the greatest impact on germination and growth of the barley plant.

Experimental:

After the samples were taken and brought to the laboratory, the plant was washed using well-running water to get rid of dirt, dust and other suspended matter. Then samples were dried by placing them in the sun for 48 hours with constant stirring to ventilate them to avoid rotting. They were cut off and placed in the "Stuart Scientific" oven at 2 ± 70 for 48 hours to ensure they were completely dry. We initially cut the dried alfalfa plant into small pieces to make grinding easier. The grinding process was carried out using a HOMMER electric grinding machine.

Soil preparation

Sandy soil was brought from the "Wadi Al-Anz" area, and the size of its particles was tested using the wet sieving method.

The ratio of the volume of soil particles

The wet sieving method was used to determine the percentage of soil particles used by using three different sizes of sieves (0.63 -250-500) mm [13]A total of 10 g of dehydrated soil sample were washed and sieves (0.63 -250-500) mL were washed under slow flow tap water. The soil sample that was in the filters was dried at 105°C in an oven until the soil sample mass had stabilized.

Field Capacity Calculation:

The field capacity of the soil used in agriculture is estimated by irrigating it with water until it is saturated and allows the excess water to descend "filtering" from the pot. The difference was taken between the weight of the soil which is saturated with water and the weight of the soil when it is dry. The difference represents the amount of water needed for the pot.

Preparing the pots and planting the seeds in the soil:

In this experiment, I used pots with a capacity of 3 kg, with a diameter of 16.5 cm from the top, a diameter of 13 cm from the bottom, and a depth of 15 cm. Then the pots were divided into

two groups, each group containing 12 pots, and each group containing 12 pots was divided into three groups, each group including 3 replicates, and the fourth witness.

The alfalfa plant wastes were added to the used pots on 4/16/2017 with a weight of 2-3-4 per kilo of soil, mixed well with the soil, watered the pots with water, and covered with nylon. After that, the bedding was planted on 4/23-2017 by 10 turns for each pot, which was reduced to 5 turns at a depth of 2 cm, after ten days, the germination rate was recorded 10 days after the emergence of the bed.

Germination rate

The percentage of germination was calculated as follows:

$$\text{Germination ratio} = \frac{\text{germination seed number}}{\text{seed total number}} \times 100$$

Statistical Analysis:

Each experiment was performed in triplicate, and the data are expressed as mean ± standard deviation. Quantitative data were statistically analyzed using one-way analysis of variance (ANOVA). Significant differences between the obtained data were determined using Duncan's multiple range tests, with $p < 0.05$ implying statistical significance. All statistical analyses were performed using SPSS version 25.0 from SPSS Inc. (Chicago, IL, USA).

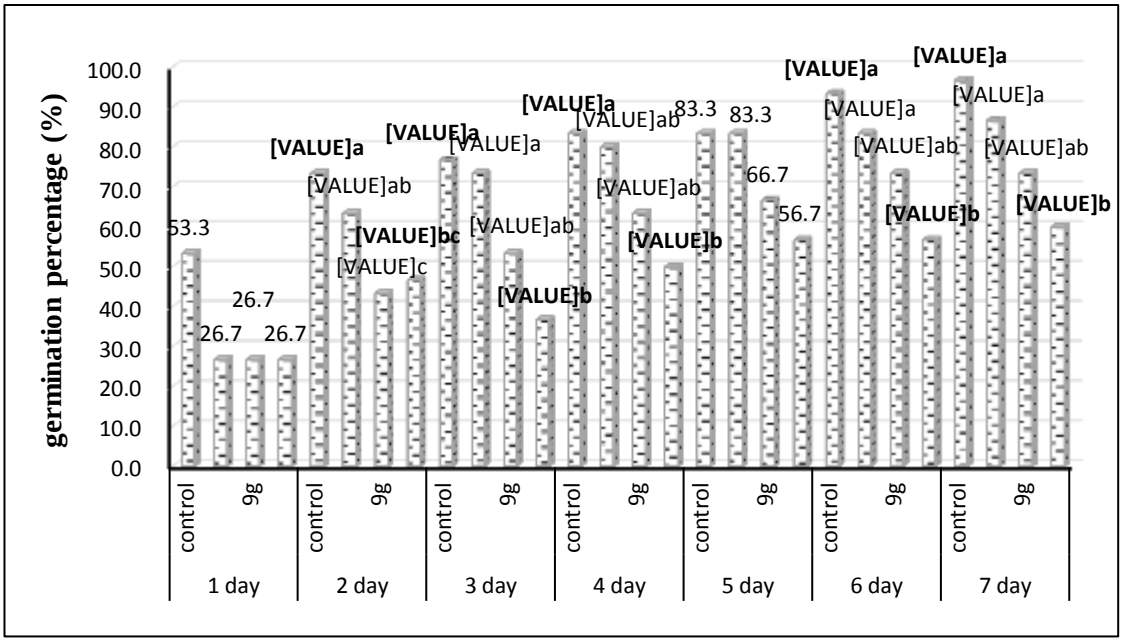

Figure 1: The effect of alfalfa plant residues on the percentage of germination of barley seeds. Bars indicate the standard error of the three replicates. Values with the same letter were not significantly different when means were separated by a Duncan's test (p<0.05)

Results and Discussion

Germination of the seed

As shown in Figure (1) there are significant differences ($p < 0.05$) in the mean germination percentage with different concentrations of alfalfa plant residues on all days with exception of first and fifth day, where no significant differences were recorded compared to the control (Concentration of 0 gm), in contrast the concentration (6gm) gave the highest percentage of germination ranging between 26.7% - 86.7%.

On the other hand, thelowest effective inhibition was seen in concentration of 12gm which was recorded between 26.7% - 60% during the first week of the emergence of the seedlings. It is evident that the decrease in the percentage of germination is directly proportional to the concentrations of the plant extracts. This is due to the inhibitory effect of the inhibitory substances present in alfalfa plant.

Previous study demonstrated that alfalfa (*Medicago sativa* L.) residue is toxic to cucumber (Cucumissativus L.) seed germination and seedling growth. Ground alfalfa roots at 0.5% (w/w, dry weight) inhibited germination when added to the growing medium [14]. In addition Alfalfa roots at 0.5% were toxic to pregerminated cucumber seed[14].Previously, [15]observed that under laboratory conditions, alfalfa residue inhibited cotton seed germination [15].

Table (1) shows the effect of alfalfa plant residues on the feather length of the barley plant compared to the control. The statistical analysis using Duncan revealed that the lowest significant feather length ($p < 0.05$) was recorded at a concentration of 12gm with a length of 12.67 cm and the highest significant feather length ($p < 0.05$) was recorded at concentration of 9gm with a length of 17.0 cm in the first sample. While in the second sample, the highest significant difference ($p < 0.05$) was recorded at a concentration of 0gm (control) with a length of 21.33 cm, and the lowest significant difference in the feather length was recorded at the concentration of 12gm with a length of 16.33 cm. Through this study, it is evident that the greater the concentration of alfalfa plant residues in the soil, the lower the feather length. The results suggest that a higher concentration of alfalfa retards barely growth, which might be due to inhibition of cell division, as allelopathic chemicals have been found to inhibit gibberellin and indoleacetic acid function [16].

Table (1): the average lengths of feather for barley seedlings (cm), treated with different concentrations of alfalfa plant residues

Concentration	First sample	Second sample
Control	20.333 ± 0.577^{a}	21.333 ± 1.528^{a}
6g	16.000 ± 2.646^{bc}	17.667 ± 1.528^{b}
9g	17.000 ± 2.646^{ab}	18.333 ± 0.577^{b}
12g	12.667 ± 0.289^{c}	16.333 ± 0.577^{b}

All values are mean ± SD in (cm), n=3.Different letters in each column denote significant difference between means according to Duncan's Test ($P < 0.05$)

Table (2) shows the average length of barley seedlings (cm) treated with different concentrations of alfalfa plant residues

Concentration	First sample	Second sample
Control	9.667 ± 0.577^{a}	10.500 ± 2.784^{a}
6g	5.667 ± 2.309^{b}	8.000 ± 1.000^{a}
9g	7.333 ± 1.528^{ab}	8.667 ± 1.528^{a}
12g	4.500 ± 0.866^{b}	4.667 ± 0.577^{b}

All values are mean ± SD in (cm), n=3.Different letters in each column denote significant difference between means according to Duncan's Test ($P < 0.05$).

Root length

Table (2) shows the effect of alfalfa plant residues on the barley root length in comparison with the control. The statistical analysis using Duncan revealed that the lowest significant length of root ($p < 0.05$) was recorded at a concentration of 12g with a length of 4.500 cm and the highest significant length of root ($p < 0.05$) was recorded at the concentration of 9gm with a length of 5.667 cm in the first sample. Whereas in the second sample, the highest significant difference ($p < 0.05$) was recorded at a concentration of 9gm with a length of 8.667 cm, and the lowest significant difference ($p < 0.05$) for the length was recorded at a concentration of 12gm

(4.667 cm). Through this result, it is evident that high concentrations of alfalfa plant residues in the soil had a clear effect on the length of barley seedlings.

Our results was in agreement with previous study that demonstrated the effect of alfalfa residues on germination and growth of two legumes species *vignaunguiculata* and *cicerarientinum* . The study showed that the aqueous extract of the residues at the concentrations5,10,15,20,25% w:v caused significant reduction in seed germination and seedling growth of the two legumes as compared with distilled water,the less germination was recorded 32% at the concentration 25% in *cicerarientinum* seeds also the less plumule and radical length recorded as 0.23,0.10cm in *cicerarientinum* at same concentration[17].

Table (3): The average of the entire length of the barley plant (cm), treatment with different concentrations of alfalfa plant residues

Concentration	First sample	Second sample
Control	29.667 ± 0.577^{a}	31.500 ± 1.323^{a}
6g	21.667 ± 4.933^{b}	25.667 ± 0.577^{b}
9g	24.333 ± 3.786^{ab}	25.000 ± 1.000^{b}
12g	17.167 ± 0.764^{c}	21.000 ± 1.000^{c}

All values are mean ± SD in (cm), n=3.Different letters in each column denote significant difference between means according to Duncan's Test (P <0.05)

The length of a whole plant

Table (3) shows the effect of alfalfa plant residues on the entire length of the barley plant compared to the control. The statistical analysis using Duncan revealed that the lowest significant height of the whole barley plant (p <0.05) was recorded at a concentration of 12g with a length of 17.167 cm and the highest significant height of the plant (p <0.05) was recorded. At a concentration of 9g with a length of 24.333 cm in the first sample. While in the second sample, the highest significant difference (p <0.05) was recorded at a concentration of 6g with a length of 25.667 cm, and the lowest significant difference was recorded for the length of the whole barley plant (p <0.05) was recorded in the concentration of 12g with a length of 21,000 cm.

From this result, it is clear that alfalfa contains inhibitory substances that disrupt cell division and elongation. However,phytotoxic compounds may disrupt many biological processes, such as inhibiting cell division and elongation, and may also interfere with the activities of enzymes and growth hormones [18]. This effect could be attributed to the presence of active compounds such as phenolic acids that work to inhibit cell division and elongation, thus reducing the length of the shoot and root system.

Phenolic acids are of great importance in allelopathy[19-21]. Phenolic acids such ascaffeic acid, trans-cinnamic acid, hydrocinnamic acid, coumarin, ferulic acid, m-coumaric acid, o-coumaric acid and p-coumaric acid are the main groups of phytotoxic substances associated with alfalfa allelopathy[21-23].

A previous study showed that inhibition in seed germination and growth of the two legumes growing in the soil containing alfalfa residues added at the ratio 10%w:w and incubated for (0,1,2) weeks as compared with control (without residues) ,the less germination recorded as 65% in Cicer growing in the soil containing residues zero incubation[17].

Table (4) shows the average weights of the whole plant (g), treatment with different concentrations of alfalfa plant residues

Concentration	First sample	Second sample
Control	0.473 ± 0.006^{a}	0.539 ± 0.022^{a}
6g	0.277 ± 0.049^{b}	0.331 ± 0.026^{b}
9g	0.442 ± 0.127^{a}	0.247 ± 0.108^{b}
12g	0.224 ± 0.064^{b}	0.259 ± 0.115^{b}

All values are mean ± SD in (cm), n=3.Different letters in each column denote significant difference between means according to Duncan's Test (P <0.05)

Table (5): the mean and average difference between the two samples treated with different concentrations of alfalfa plant residues

	First sample	**Second sample**	Mean Difference±SE	*t*	Sig. (2-tailed)
	Mean±SD	Mean±SD			
hypocotyl	16.500±3.282	18.417±2.151	-1.917±1.133	-1.692	0.105
Radicle	6.792±2.388	7.958±2.632	-1.167±1.026	-1.137	0.268
Whole plant	23.208±5.433	25.792±4.008	-2.583±1.949	-1.325	0.199
Weights	0.354±0.128	0.344±0.140	0.010±0.055	0.184	0.856

All values are mean ± SD, n=12.*t*, Student's *t*-test

Wet weight of the barley

From Table (4), it is clear that there are significant differences in the average fresh weight of the whole barley plant according to the different concentrations of the plant residue used compared to the control. The statistical analysis using Duncan revealed that the lowest significant weight of the whole barley plant (p <0.05) was recorded at a concentration of 12g with a weight of 0.224 g in the first sample. While in the second sample, the lowest significant difference was recorded for the weight of the whole barley plant (p <0.05) at a concentration of 9g with a weight of 0.247 g, compared to the control. From this result, it is clear that alfalfa contains inhibitory substances that disrupt cell division and elongation. This is due to the decrease that occurred in the height of the plant when increasing the concentration of plant residue, which was consequently reflected in the fresh weight of the whole plant. Also, may be due to the presence of allelopathic materials that interfered with the various growth mechanisms and inhibited the photosynthesis process, leading to a decrease in the wet weight.

It is noticed from Table (5) that there were no significant differences between the two samples of barley plants treated with different concentrations of alfalfa plant residues.

Conclusion

This study proved that alfalfa plant residues had a clear effect on germination percentage, desiccation and feather length, as well as on the wet weight of the barley plant. This explains that the higher the concentration of alfalfa plant residues in agricultural soil, the greater the effect of the physical and chemical properties of allelopathic substances of alfalfa. The producers must understand the influence of alfalfa residues on allelopathy and must consider the positive benefits that allelopathy would have on weed suppression along with the negative impacts that allelopathy might have on barley seedlings.

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