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Allelopathic Effect of Clover Residues on the Germination and Growth of Barely

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ABSTRACT

A field experiment was conducted in the fields of the University of Baghdad to determine the effect of clover residues mixed with the soil in which the barley crop (Arviat variety) was planted. Residues of two varieties of clover (Red clover and Egyptian clover) were added at a rate of 3 and 6grams of plant residues. Two control treatments were also implemented with 3 and 6 grams of barley straw for comparison purposes. The results showed that the clover residues significantly reduced the germination and growth of the barley crop. However, this inhibition increased significantly when plant residues were added to the Egyptian clover variety at concentrations of 3 and 6grams, achieving inhibition in the germination of barley seeds. It achieved a clear inhibition in the growth characteristics of barley, represented by plant height, as it recorded an inhibition in the average plant height at both concentrations of (3 and 6 grams) reaching 46.27% and 26.61%, respectively. Meanwhile, the inhibition in the shoot biomass was 43.76% and 29.69%, respectively, while the inhibition in the root biomass was 50.78% and 42.81%. The results also showed an inhibition in the dry weight of the whole barley plant, consistent with what was mentioned in the results of germination rate, plant height, total green and root biomass which showed inhibition percentages of 48.18% and 37.89%, respectively for both concentrations of (3 and 6 grams) of plant residues. results indicate that as the concentration of plant residues in the clover increased, the allelopathic compounds' effect on the clover also increased.

Keywords: Allelopathy, Residues,Clover,Barly, Germination of barley

التأثير الاليلوباثي لمخلفات البرسيم في انبات ونمو الشعير

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الخلاصة

اجريت تجربة حقلية في حقول جامعة بغداد ، لتحديد تأثير مخلفات البرسيم المخلوطة مع التربة التي زرع فيها محصول الشعير صنف اريفات . فقد اضيفت مخلفات صنفي البرسيم مصري و الاحمر بمقدار 3 و6 غم مخلفات نباتية كما تم تنفيذ معاملتين 3 و6 غم بتموس لأغراض المقارنة . لقد بينت النتائج ان مخلفات البرسيم قللت معنويا انبات ونمو محصول الشعير الا ان هذا التثبيط ازداد وبشكل كبير عند اضافة المخلفات النباتية لمخلفات صنف البرسيم المصري بالتركيزين 3 و6 غم اذ حققت هذه التراكيز تثبيطا في انبات بذور الشعير كما حققت تثبيطا واضحا في صفات النمو للشعير متمثلة بطول النبات اذ سجلت تثبيطا في متوسط طول النبات عند التركيزين 6 و3 غم بلغ 46.27 و 26.61% على التوالي. في حين كان التثبيط في

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المجموع الخضري 43.76 و 29.69% على التوالي ، اما نسبة التثبيط في المجموع الجذري 50.78 و 42.81% وجاءت النتائج في تثبيط الوزن الجاف لكامل نبات الشعير متوافقة مع ما جاء في نتائج نسبة الانبات وطول النبات ومجموعي النبات الخضري والجذري اذ كانت نسبة التثبيط 48.18 و37.89% على التوالي لكلا التركيزين 6 و3 غم مخلفات نباتية.

1.Introduction

Allelopathy is an ancient phenomenon, first recognized by Molisch in 1937, as the detrimental and beneficial biochemical interactions between plant species, including microorganisms [1]. Numerous studies conducted on this phenomenon and its role in the ecosystem have confirmed that these changes are caused by the release of toxic compounds (plant toxins) called Allelochemicals, which are secondary metabolites. It has been found that leaves and roots are the main sources of allelopathic compounds, although these compounds can also be found in other parts of the plant, such as stems, flowers, fruits, rhizomes, and pollen grains [2].

"Allelopathy (antagonism) is a mechanism of chemical regulation and control in natural ecological systems, which serves as a mechanism for adaptation or suitability for the environment of living organisms. Allelopathy also plays a clear role in agricultural ecosystems, leading to a wide range of interactions between crops and different plants. These interactions often prove harmful to recipient plants while providing a selective advantage to the producing plants (the influencing ones). The low tolerance of recipient plants to allelopathic compounds produced by the donor plant can make recently introduced plants dominant in natural plant communities. This makes allelopathic influence an important mechanism for plant dominance [3].

Allelopathic compounds exist in plant tissues and are released in sufficient quantities under favorable conditions to affect neighboring plants. These allelopathic chemicals can be either plant toxins or self-toxins that affect the same plants that release them or other plants nearby or following them in cultivation. Studies have shown that these released compounds are of a phenolic nature and can be released from the plant, whether alive or dead [4].

Through a comprehensive review of the research studies conducted in the field of allelopathy, their multiplication several times, as it continues to be studied by physiologists, botanists, soil scientists, and natural product chemists. The continuous emergence of additional information regarding the mechanisms of allelopathic compounds in terms of selectivity, secretion, continuity, and genetic regulation poses a continuous challenge for plant scientists to develop modern strategies that enhance the protection of biodiversity [5].

Studies have indicated the presence of several crops that have shown high allelopathic activity against other crops that accompany or follow them in the field or agricultural cycle. This is achieved through the release of allelopathic compounds into the environment through leaching, root exudates, and decomposition of plant residues by microorganisms, as well as through volatilization [6].

These compounds have inhibitory and stimulatory effects on plants and microorganisms, affecting many biological processes. It should be noted that the allelopathic effects of these compounds depend on their nature and concentration. Some compounds have caused inhibitory effects on seed germination and growth, while others have caused stimulatory impact [7].

Knowing about the existence of this phenomenon in field crops has highlighted the possibility of utilizing it in the biological control of various agricultural pests and increasing production, as plant residues decompose in the soil due to microorganisms. This results in the accumulation of released antagonistic compounds in the soil, which can be directly absorbed by neighboring or associated plants or undergo chemical or biological transformations that alter

the characteristics and nature of the soil, negatively or positively affecting the planted crop in the soil [8], [9].
However, in the field of pest control, intensive efforts have been focused on the possibility of using different allelopathic crops in weeds management and devising strategies for this purpose to reduce reliance on harmful chemical pesticides for the environment and health, as well as the possibility of resistance development and the emergence of pesticide-resistant strains [10].

To achieve this, many researchers have started exploring varieties with high allelopathic potential as a first step in using allelopathy in weeds control, and indeed various crops with high allelopathic potential have been obtained [11]. This is often done through laboratory and greenhouse experiments, as well as sometimes through field observations that are later supported by laboratory experiments [12].
Barley is considered one of the most important economic crops in the world. It represents one of the main food sources in most regions of the world and is the staple food in some countries like Morocco, India, and China. It also serves as a primary food source for animals, including livestock. Barley is important for the nutrition of the crop that follows it in agriculture, as its root system works to reduce nitrate leaching into the soil, thus improving the quality of the available water in rocky soils [13]. Barley belongs to the grass family and is a herbaceous plant with hollow stems at the nodes. Its leaves consist of parallel veins and are surrounded by sheaths. Its roots are fibrous, and the flowers cluster around an axis, forming the ear of the barley plant. Its mature fruits are known as barley grains [14].

As for clover, it is a perennial herbaceous plant from the legume family. The height of the clover can reach over a meter if not grazed. It has a deep, branched root system and compound trifoliate leaves. The leaves are inverted oval, the inflorescence is vertical, the flowers are small, about 1.5 cm long, and the corolla is pink, blue, or purple. The fruit is an open pod that contains a large number of seeds [15]. The green matter, especially the leaves, contains 9.1% carbohydrates, 5% protein, 0.9% fat, and 2.4% mineral compounds (calcium and phosphorus salts). Clover is considered an important source of greenery and vitamins, as it contains large quantities of vitamins A and D, and small quantities of vitamins B and B1 [16].

The study aimed to determine the effect of clover residues on the germination and growth of barley, in addition to identifying the optimal concentration for influencing the germination and growth of barley.

2.Materials and Methods

Barley crop seeds *Hordeum vulgare* L. variety Arviat, and the plant residues of the clovers crop, two varieties (Egyptian clover and Red clover) were obtained from one of the agricultural fields in Baghdad.

The effect of the residues of these two varieties of clovers on the germination and growth of the barley crop was studied. The dry parts of the clover's plants were ground for both varieties using an electric mill equipped with a sieve with a diameter of 10 mm. After grinding, the residues were added to plastic pots, each containing 500 g of mixed soil, at a rate of 3 and 6 grams of residues per kilogram of soil. They were mixed well with the potting soil. The control were prepared in the same manner, except that the plant residues were replaced with an equivalent weight of (peatmoss) to ensure equal organic matter.

The barley seeds were planted on 25th November 2022 in pots, with 10 seeds per pot. Then, all the pots were watered with equal amounts of regular water. After seven days of germination, thinning was performed on three seedlings per pot. Then, the pots were randomly distributed within an exposed wire shade in a 4x2 factorial experimental design, using a completely randomized block design (RCBD) with four replications per treatment. The germination percentage was calculated as follows:

Germination Percentage = $\frac{\textbf{NNumber of seeds used}}{\textbf{Number of germinated seeds}} \times 100$

Throughout the experiment, the pots were watered with equal amounts of water as needed. After 35 days of planting, the plants were uprooted, and their roots were washed using running water. Then, the shoot and root groups were separately dried inside an electric oven at a temperature of 70°C for 48 hours to stabilise the weight. Subsequently, the treatments were compared with controls based on plant lengths, dry weight of the shoot, root groups, and the total plant. The inhibition effect of plant residues was determined as follows:

The inhibition ratio equation: $I=100- (E_2\times100/E_2)$

Where I= % Inhibition

E_1= Response of control plant

$E_{2=}$ Response of treatment plant

3.Results and Discussion

3.1Germination Percentage

It is clear from Figure (1) that there are significant differences in the mean germination rates of barley seeds with varying concentrations of two types of clover residues on all days. It is observed that concentrations of 3 and 6 grams of Egyptian clover residues resulted in the lowest germination rates for barley seeds, reaching 62.4% and 53.4%, respectively. On the other hand, residues of red clover ranked second with concentrations of 3 and 6 grams, recording germination rates of 76.8% and 62.2%, respectively. It is noticeable that increasing the concentration of both types of clover residues led to inhibition in the germination percentage of barley seeds, and this was evident from the first

day of seed germination. It is noted that when increasing the concentration of the residues of both types of clover, inhibited the germination rate of barley seeds, and this became clear from the first day of seed germination. It is clear from this that the decrease in the germination rate of barley seeds is directly proportional to the increase in the concentration of the clover plant residues, and this could be due to the allelopathic effect of alfalfa residues they contain. This result is consistent with [17], who explained that this effect is due to the release of some allelopathic compounds into the soil, such as phenolic acids, some alkaloids, flavonoids, etc., and these compounds are known for their effect on vital processes at the seed germination stage and seedling growth due to the effect of allelopathic compounds on most of the physiological activities of the plant when they enter the cells with water, such as affecting cell permeability, building proteins, respiration, and the process of photosynthesis.

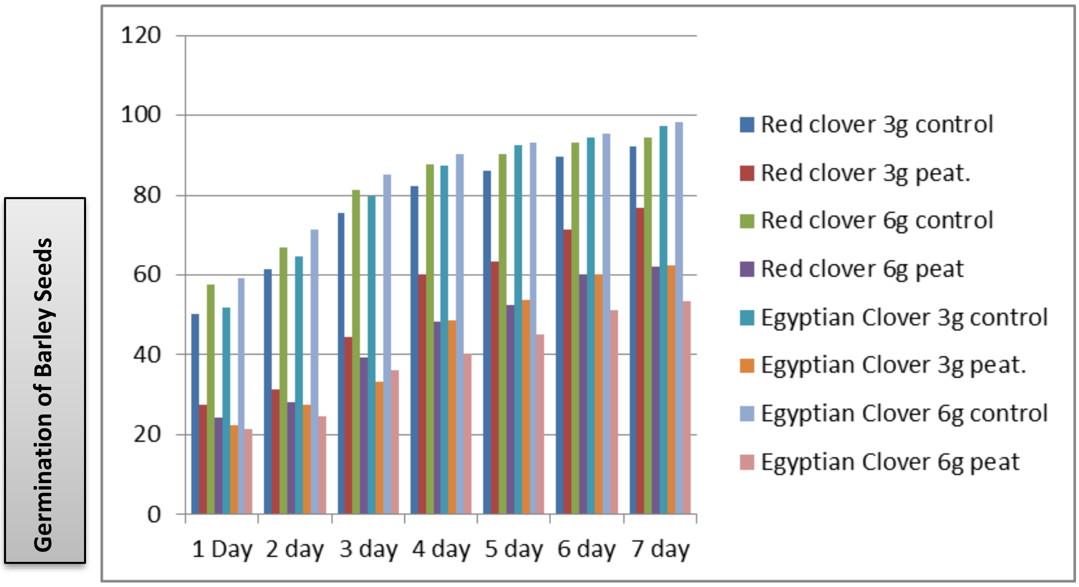

Figure 1: The Effect of clover residues on the germination of barley seeds

3.2 The Effect of Clover Residues

The results illustrated in Table (1) clearly show the significant effect of clover residues on the height of barley plants. Clover residues, especially at high concentrations, significantly reduced the plant length compared to the controls. The height decreased when adding 6 grams of Red and Egyptian clover residues; it reached 10.38 and 12.63 cm, respectively, at a rate of 46.27 and 26.61% compared to the controls, in which the plant length reached 18.95 and 19.32 cm. The results also showed that the interference between clover residues and their concentrations had a significant effect on the plant height characteristic of barley, as the treatment of adding residues of the Egyptian variety with a concentration of 6 grams of residues recorded the lowest average plant length of 10.38 cm. This combined effect of residues and their concentrations led to a reduction in plant height. From this result, it is clear that clover residues contain reduced allelopathic substances that work to disrupt cell division and elongation [18]. Some phenolic acids inhibit the germination and growth of other plants through allelopathy, as the presence of active compounds such as phenols, glycosides, tannins, and terpenes work to inhibit cell division and elongation plant height [19].

Table 1: Effect of clover residues on plant height (cm)

Cultivars Residues	concentrations of plant residues				Average
	3g Patmos/Kg soil	3g Residues/Kg soil	6g Patmos/Kg soil	6g Residues/Kg soil	
Red clover	**16.87**	**14.63**	**18.95**	**12.21**	**15.67**
Egyptian clover	**17.21**	**12.63**	**19.32**	**10.38**	**14.88**
Average	**17.04**	**13.64**	**19.13**	**11.29**	
L.S.D. 0.05	**Cultivars Residues 0.34**	**concentrations of plant residues 0.47**		**Cultivars Residues × concentrations of plant residues 0.57**	

4.Average Dry Weight in the Entire Plant.

4.1 Total shoot dry weight (mg).

The results in Table 2 showed significant differences between the different treatments of clover residues concentrations and their effect on the vegetative group of barley plants. The inhibition ratio increased when adding Egyptian clover residues at a concentration of 6 grams of residues, reaching 43.76%, In contrast adding 6 grams of residues of the red clover variety, it reached 37.45%. As for the inhibition in the vegetative group of the treatment of adding 3 grams of residues, the inhibition rates were 29.69 % and 23.26% for Egyptian clover residues and red clover residues, respectively. This indicates that the presence of plant residues of clover plays the main role in the growth of barley crops, as the green yield decreased when adding plant residues of clover at concentrations of 3 and 6g for both varieties. It is also worth mentioning the clear superiority of Egyptian clover variety residues and both concentrations in inhibiting the green yield of barley plants. The significant interaction between the residues of the varieties and their concentrations, indicates the synergistic or cumulative effect of plant residues and their concentrations. The increased concentration of plant residues for the two varieties of clover and has reflected an increase in the inhibition rate in the total green mass of barley plants. The reason for these decreases is attributed to the inhibition in plant height (Table 1) when the concentration of plant residues increases, which has resulted in a reduction in the overall green mass of the plant [20]. Additionally, the decrease can be attributed to the presence of allelopathic compounds that have interfered with various growth mechanisms and inhibited the process of photosynthesis, resulting in the inhibition of the green mass of the barley plant [21].

Table 2: Effect of clover residues on shoots of barley plant (mg)

Cultivars Residues	concentrations of plant residues				Average
	3g Patmos/Kg soil	3g Residues/Kg soil	6g Patmos/Kg soil	6g Residues/Kg soil	
Red clover	249.50	191.45	288.00	180.12	227.26
Egyptian clover	249.28	175.25	287.45	161.65	218.38
Average	249.39	183.35	287.72	170.88	
L.S.D. 0.05	Cultivars Residues 11.22	concentrations of plant residues 16.14		Cultivars Residues × concentrations of plant residues 22.28	

4.2 Total root dry weight

The findings indicate that the addition of plant residues significantly affects the total root with the addition coefficients of the two varieties of Egyptian clover and Red clover. For both concentrations, the addition of 3 grams of the residues of the Egyptian clover and Red clover varieties resulted in a lower average of the total root, reaching 237.35 mg and a suppression rate (33.58%) compared to the control treatment (3 grams of peatmoss). Meanwhile, the suppression rate in the total root in add 6 grams of residues reached 43.27%. Regarding the concentrations of plant residues for the two varieties of clover, Egyptian clover residues showed a clear superiority over red clover residues in inhibiting root growth for both concentrations, The inhibition rates reached 42.81% and 24.52% in the case of the addition of 3 grams of the plant residues of the Egyptian clover variety and red clover variety respectively. As for the inhibition rate with the addition of 6 grams of Egyptian clover residues, it reached 50.78%, followed by 35.76% with the red clover variety. As for the interference, which was symbolic, when recording the transaction of adding 3 grams of plant residues to the Egyptian clover variety, the lowest average for the total root was 202.57 mg. This synergistic effect between the residues of the two clover varieties and their concentrations caused a reduction in the total root, and this result is consistent with what was reported [22].

Table 3 :Effect of clover residues on the root system of barley plant (mg)

Cultivars Residues	concentrations of plant residues				
	3g Patmos/Kg soil	**3g Residues/Kg soil**	**6g Patmos/Kg soil**	**6g Residues/Kg soil**	**Average**
Red clover	**360.55**	**272.13**	**485.75**	**312.12**	**357.63**
Egyptian clover	**354.25**	**202.57**	**487.18**	**239.75**	**320.93**
Average	**357.4**	**237.35**	**486.46**	**275.93**	
L.S.D. 0.05	**Cultivars Residues 12.29**	**concentrations of plant residues 20.28**		**Cultivars Residues × concentrations of plant residues 30.51**	

4.3 The Dry Weight of The Entire Plant (mg)

According to the results in Table 4, the addition of residues from both clover varieties to the soil led to the inhibition of the dry weight of the entire plant. The results showed that the addition of Egyptian clover residues at a concentration of 6 grams caused a reduction in the dry weight rate of barley plants, which decreased from 603.53 mg to 377.82 mg, with a reduction percentage of 48.18%. In comparison, the reduction percentage was 37.89% at a concentration of 3 grams. Adding red clover residues at a concentration of 6 grams decreased the dry weight of barley plants from 773.75 mg to 492.24 mg and had an inhibition rate of 36.38%, while it was 24% at a concentration of 3 grams. These results indicate the sensitivity of barley crop to clover residues, followed by the impact of red clover residues. This effect was a result of the influence of clover plant residues on germination rate (Figure 1), plant height (Table 1), and total green mass. (Table 2) and the total root (Table 3). The dry weight of the plant is one of the characteristics most affected by all biological processes within the plant, such as respiration, photosynthesis, and others. Any effect on these processes appear in one way or another on the dry weight of the entire plant [23] , [24].

Table 4: Effect of clovers residues on the whole barley plant (mg)

Cultivars Residues	concentrations of plant residues				
	3g Patmos/Kg soil	**3g Residues/Kg soil**	**6g Patmos/Kg soil**	**6g Residues/Kg soil**	**Average**
Red clover	**610.05**	**463.58**	**773.75**	**492.24**	**584.90**
Egyptian clover	**603.53**	**377.82**	**774.63**	**401.40**	**539.34**
Average	**606.79**	**420.70**	**774.19**	**446.82**	
L.S.D. 0.05	**Cultivars Residues 20.58**	**concentrations of plant residues 29.61**		**Cultivars Residues × concentrations of plant residues 41.58**	

5.Conclusion

In this research, it was found that mixing clover residues with soil significantly reduced the germination and growth of barley crops, and this effect increased with the concentration of plant residues, indicating an increase in the allelopathic effect of clover residues. This necessitates the identification of the chemical composition of clover residues responsible for these impacts in the future.

6. Conflict of Interest: No conflict of interests is declared.

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