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Effectiveness of Peas aqueous extracts on germination and growth of two Wheat cultivars

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Abstract

This study aims to assess the allelopathic effects of pea (Pisum sativum L.) aqueous extracts on the growth and germination of two wheat cultivars, Atlas and Aseel. The aqueous extracts, tested at concentrations of 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, and 10%, were applied in both laboratory and greenhouse conditions Randomized Complete Design (RCBD) Results show the effect of aqueous extracts of pea leaves at (1,2,3,4,5,6,7,8,9,10%) on wheat cultivars (Atlas and Aseel) in the laboratory, as a decrease was observed in germination percentage of cultivar Atlas at most of the treatments, There are also found a decrease in both plumule length and its dry weight in all treatments except for the increase at 1% concentration. Results showed a decrease in the length of the root and its dry weight in all treatments except for the increase at (1,2 and 3% concentration), and the highest rates of increase for plumule length, plumule dry weight, root length and root dry weight were (6.21, 44.06, 52.58, and 55%) respectively at 1% concentration. Results of the greenhouse showed pea leaves aqueous extracts at 3% concentration caused an increase in shoot and root length of Atlas cultivar seedlings, while it caused an increase in shoot and root dry weight in Aseel cultivar. There is a fluctuation in the chlorophyll content of the tested cultivars due to various treatments. Treatment of 1% aqueous extracts increased the leaf area of Atlas, while other treatments reduced leaf area for both cultivars.

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the essential crops, and wheat ranks first in the world in terms of cultivated area and is considered the primary source of food in the world.(Hussain and Mohammad,2024) More than a third of the world's population depends on it for their food, and it has an important and significant role in achieving food security, as it is an essential source of energy that humans need, and one of the most important characteristics that made it essential in human food is the good balance between proteins, as it covers about 20% of calories And protein in human food, and wheat ranks first in the world in terms of cultivated area (Taher and Hussain, 2021).

The allelopathic phenomenon shows complexities in the biochemical interactions between plants, including microorganisms that play an important role in agricultural systems by releasing allelopathic compounds into the environment. The allelopathic phenomenon can be exploited in agricultural systems to enhance crop production through the overlap between crops and succession in agriculture (Singh et al., 2008).

The inclusion of legumes as part of the agricultural cycle in the agricultural system has a very important role, as legumes are among the plants that have allelopathic potential, as studies have shown that they contain many allelopathic compounds, and among these effective compounds (Benzoic acid, P-hydroxybenzoic acid, Vanillic, Adipic, Lactic, Succinc acid, Mallic acid, Glycolic, P-hydroxyphenyllactic.), which have an important role in the germination and growth of plants growing thereafter (Asaduzzaman and Asao, 2012) .

It was found that peas led to a decrease in the fresh and dry weight of the shoots of pea plants planted after them and that the reason for this decrease is that they contain compounds: malonic acid, p-hydroxybenzoic acid, benzoic acid, and vanillic acid (Asaduzzaman and Asao, 2012). Navarro-Sanchezo et al. (2019) also observed an increase in the yield of both watermelon and cauliflower grown on soils previously cultivated with cowpeas and beans. Hussain (2020) showed that the soil previously cultivated with legumes caused an increase in the height and dry weight of the vegetative total of cucumber varieties. This study aims to assess the allelopathic effects of pea (*Pisum sativum* L.) aqueous extracts on the growth and germination of two wheat cultivars, Atlas and Aseel.

Material and Methods

The study included laboratory and greenhouse experiments to study the allelopathic properties of aqueous extracts of pea (*Pisum sativum* L.) residues and their effect on seed germination and seedling growth of two wheat cultivars (*Triticum aestivum* L.) Atlas and Aseel, The experiments were carried out at the Department of Biology, College of Sciences, University of Mosu.

1 .Seeds and plant residues Source

Pea plant residues were collected after harvesting from some private farms. The Plant residues were separated into shoots and roots, then dried, crushed, and stored for future use.

. Two wheat cultivars, *Triticum aestivum L.* (*Atlas* and *Aseel*), were selected for the study. These cultivars, and were obtained from the International Center for Agricultural Research in the Dry Areas (ICARDA).

2 .Aqueous extracts of pea plant residues Preparation

Aqueous extracts of pea plant residues (leaves) were prepared at concentrations (1, 2, 3,4,5,6,7,8,9 and 10%) For each concentration (1,2,3,4,5,6,7, 8, 9 and 10 g) of pea shoot residues were mixed with 100 ml of distilled water in an electric mixer for 10 minutes The resulting mixture was, then filtered using a Buchner funnel equipped with Whitman No.1(Abbas & Hussain, 2020) .

3 .Laboratory Experiment: Biological Test of Aqueous Extracts of Pea Plant

To study the effect of aqueous extracts of pea plant residues on seed germination and seedling growth of wheat cultivars the following procedure was followed in the laboratory.

Experimental Setup: Aqueous extracts of pea leaves at (1,2,3,4,5,6,7,8,9,10%) were added to Petri dishes with a diameter of (13.8 cm), **Seeds:** Fifteen wheat seeds were placed in each Petri dish, positioned between two filter papers. Three replicates were used for each treatment.

After seven days of cultivation, the dishes were incubated at a temperature of 25 ± 2 °C in a Gallenkhamp incubator. The germination rate was measured after 14 days of cultivation. The lengths of each Plumule and radical of the seedlings were measured, and the dry weight of the seedlings were measured after drying them in the oven at a temperature of 60 °C for 72 hours (Al-Juhaishi, 2017). Germination percentage (Gr %) was measured:

Gr% = Natural seedlings No. / Total seeds No. x 100 (Saied, 1984)

4 . Greenhouse Experiment: Irrigation with Pea Leaves Aqueous Extracts

In the greenhouse, the following procedure was applied to evaluate the effect of pea leaves aqueous extracts on wheat seedling growth,

Experimental Setup: Ten wheat seeds were planted in pots containing soil without any amendments any addition, **Irrigation:** The plants were irrigated with aqueous extracts of pea leaves at concentrations of 1% and 3% (V:V). Irrigation was carried out three times during the growing period: once immediately after sowing and twice during the plant growth cycle, after 15 days of sowing, percentage of germination was calculated. After 60 days of sowing, the plant was harvested, and then root length (cm) and the shoot height (cm) were measured. Leaf area (cm2) was calculated according to the following equation:

Leaf area (cm2) = leaf length x leaf width x 0.905 (Kemp 1966).

5. Effect of pea leaves residues in chemical traits of soil

Some chemical properties of the soil used in the experiment and taken from the farms of Mosul city were determined after drying and crushing. The analysis was carried out in the laboratories of the Department of Soil Sciences at the College of Agriculture and Forestry / University of Mosul.

The following properties were measured according to the mentioned source:

- Acidity (pH),- Electrical conductivity (EC), Nitrogen (N): Kjeldahl method according to Jones (1991), Phosphorus (P): (1982) Olsen.,- Potassium (K): (Richardes, 1954) . Chlorophyll according to kndsson 1977

6. Identification of phenolic compounds in pea plant residues

Phenolic compounds were identified using High Performance Liquid Chromatography (HPLC). Alcoholic extracts of the shoots were prepared according to the modified method of Al-Juhaishi (2017).

7. Statistical Analysis

After collecting data and various weed traits, they were statistically analyzed using a randomized complete block design (RCBD) with three replicates. Data were analyzed for the studied traits using a computer and using the program.

Results and discussion

Results of Table (1) show the effect of aqueous extracts of pea leaves at (1, 3 %) on chemical traits of soil, as a decrease was observed in N and P content in soil , this may be due to the effect of pea leaves residues increase organic material in soil can be increased in denitrification that lead to decrease N in soil , Data in hand showed increase in pH, K The changes in pH and EC further indicate that pea residues have an influence on the soil's ionic and chemical balance. These effects could have implications for soil fertility and plant growth.

Table (1) effect of aqueous extracts of pea leaves in chemical traits of soil

Sample	N %	P (ppm)	K (ppm)	PH	EC
Control	0.21	4. 657	13	7.9	1.6
1	0.096	2. 68	12	8.8	1.1
3	0.064	1. 671	29	8.2	1.2

Results of Table (2) show the effect of aqueous extracts of pea leaves at (1,2,3,4,5,6,7,8,9,10%) on wheat cultivars (Atlas, Aseel) in the laboratory, as a decrease was observed in germination percentage of cultivar Atlas at most of the treatments, this may be due to the effect of allelopathic compounds released from pea leaves aqueous extracts, which were isolated and diagnosed by HPLC included (P-Hydroxy benzoic acid, Benzoic acid, Chlorogenic acid, Quercetin) that effect on α-amylase that important for seed germination. Or may be due to the affecting allelopathic compounds in hormone balance inside the seed and higher hormone Abscisic acid level, which led to inhibition of embryo metabolic activity, which made seeds in an artificial dormancy (Bogatek and Gniazdowska, 2007). Data in hand showed a decreas decrease in both plumule length and its dry weight in all treatments except for the increase at 1%, Results showed a decrease in the length of the root And its dry weight in all treatments except for the increase at (1,2,3 %), and the highest rates of increase for (plumule length, plumule dry weight, root length, root dry weight) were at 1% (6.21, 44.06, 52.58, and 55%) respectively, and this could be because allelopathic compounds have a stimulatory effect at low concentrations (Hussain and Abbas, 2021) .

Table (2) Effect of pea aqueous extract On wheat growth

Varieties	Con.%	Germination percentage %	Plumule length (cm)	Radical Length (cm)	Dry Wight of Plumule(mg)	Dry Wight of Radical (mg)
Atlas	0.0	100a	8.85b	11.8d	116	120d
	1	100a	9.40a	17.00a	177a	186a
	2	98b	8.70bc	15.50b	162c	169b
	3	95c	8.42d	14.70c	164b	143c
	4	88e	7.42f	7.12g	144e	107f
	5	98b	8.20d	10.40e	146e	110e
	6	98b	8.60c	11.30d	156d	112e
	7	100a	7.80e	7.46f	130f	98g
	8	93d	6.40g	7.30g	102h	95g
	9	93d	7.90e	7.80f	110g	102f
	10	95c	6.80g	5.00h	109g	82h

Aseel	0.0	100a	10.04b	13.02c	126	99a
	1	98b	8.28d	16.73a	143b	97b
	2	100a	8.66d	14.50b	156a	94c
	3	100a	7.73e	11.43	136bc	80f
	4	95c	7.36e	12.40d	140b	82f
	5	93d	10.13b	13.30c	122d	95c
	6	96c	10.63a	12e	129c	89d
	7	98b	9.32c	10.63	139b	85e
	8	98b	7.73e	8.55g	129c	80f
	9	95c	6.40f	6.36h	118	76g
	10	93d	10.0b	11.6f	130c	89d

Table (3) shows the results of the greenhouse, as pea leaves aqueous extracts caused an increase in shoot and root length of Atlas cultivar seedlings at 3%, while in Aseel cultivar, the treatment with pea leaves aqueous extracts increase in shoot and roots dry weight because it contains the compound P-Hydroxy benzoic acid. Studies have shown that the presence of this compound in low concentrations causes an increase in the length of the shoot and root system and their dry weight in the Rapeseed plant (Ahrabi et al., 2011). These results are consistent with what Hussein (2020) concluded that the soil previously cultivated with legumes caused an increase in the height and dry weight of shoots, as well as the number of cucumber roots. As well as it was found that chickpea plant aqueous extracts increased the root length for the two varieties of wheat (Taher and Hussain, 2021).

Table (3) "Effect of Pea Aqueous Extract on Wheat Cultivars Growth in Greenhouse

Varieties	Con.%	Germination percentage %	Shoot length (cm)	Root Length (cm)	Shoot Dry Wight (g)	Root Dry Wight (g)
Atlas	0.0	100a	34.6b	37.6c	2.79b	2.56a
	1	100a	31.5c	40.2b	2.65c	1.6c
	3	90b	39.9a	51a	3.13a	2.12b
Variety effect		96.6b	35.33b	42.93b	2.85b	2.09a
Aseel	0.0	100a	46.2a	52a	4.06c	1.13c
	1	100a	41.7c	36c	5.23a	1.60b
	3	95b	43.2b	41.8b	4.51b	1.62a
Variety effect		98.33a	43.7a	43,26a	4.6a	1.45b

Results showed in Figure (1) a variation in the chlorophyll content of the tested cultivars between increase and decrease by the effect of aqueous extracts of pea leaves. The Atlas cultivar gave the highest chlorophyll content at 3% accompanied by an increase in the dry weight of the plant. Observing an increase in dry weight with the same treatment, the reason for this discrepancy in effect could be the difference in the concentration of allelopathic compounds and their chemical nature (Abdul-Jabbar and Saeed, 2019). It may also be due to the genetic variance of the tested varieties. A difference was observed in the effect of allelopathic compounds in different cultivars (Hussain et al., 2018). As for the difference in the total chlorophyll content of the tested wheat varieties, it may be due to the effect of allelopathic compounds released on the absorption of elements from the soil (ion Mg), and its transmission within the plant, or hinder the effectiveness of the enzymes associated with its construction. The reason may also be due to a

decrease in the process of building chlorophyll and an increase in the catabolism process (Hussain et al., 2023).

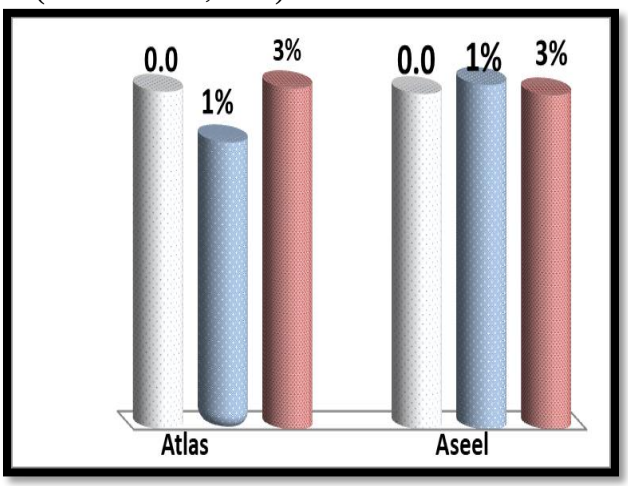

Figure (1) Effect of pea aqueous extract in Total Chlorophyll

Figure (2) shows a decrease in the leaf area of all treatments except for the increase in the leaf area of Atlas at 1%. reason for the inhibitory effect may be that it contains high concentrations of Chlorogenic acid and P-Hydroxy benzoic acid Table (4), which are known to hinder the germination and growth of the recipient plant and adverse effect on the rate of cell division or the IAA hormone responsible for cell division, whereas the allelopathic effect depends on the concentration of allelopathic compounds, soil nature, target plant species, and environmental factors (Hussain *et al*., 2023).

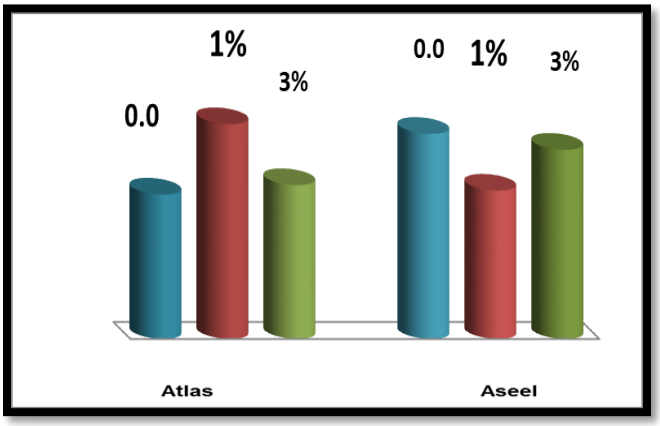

Figure (2) Effect of pea aqueous extract in leaf area (cm^2)

The results Table (4) showed an increase in the yield traits (spike length, number of grains/spike, and weight of 100 grains) for the two varieties and at all concentrations. The highest rate of increase was reached at the concentration of 3% for the Atlas variety (89.18, more than 100, 69.36%) for the three traits respectively. The reason can be due to effect of phenolic compound that diagnosis by HPLC ((P-Hydroxy benzoic acid, Benzoic acid, Chlorogenic acid, and Quercetin) can be effect on chlorophyll and increase the material in seed (Ibrahim *et al*., 2024).

Table (4) Effect of Pea Aqueous Extract on Wheat Cultivars Growth in Greenhouse

Varieties	Con.%	Spike length cm	Grain No./Spike	Wight of 100 grain(gm)
Atlas	0.0	4.53c	9.01b	2. 35c
	1	7.01b	21.52a	3. 10b
	3	8. 57a	21.51a	3.98a
Variety effect		6.70b	17.34b	3.14ab
Aseel	0.0	4.08d	7.1c	3.68a
	1	7. 56b	22.8b	3.65a
	3	9. 51a	31.8a	3.67a
Variety effect		7.05a	20.56a	3.66a

Diagnosis and quantitative determination of phenolic compounds in plant residues by HPLC:

High-Performance Liquid Chromatography (HPLC) separates phenolic compounds by drawing curves of the absorption peaks for each compound coupled with its retention time (RT). The retention time values for the standard compounds were adopted to match them with the retention values for the compounds separated from the plant residue extracts of peas, as shown in Table (3) The retention time of the identified compounds was evaluated in the plant residues of

pea plants, and the results showed the presence of some phenolic compounds known for their allelopathic ability that were diagnosed with this technology that is (P-Hydroxy benzoic acid, Benzoic acid, Chlorogenic acid,and Quercetin).

Table (5) Distribution of identified phenolic compounds and RT values determined by HPLC in peas

No	compounds	Rt	sample
1	Hydroquinone	1.99	-
2	P-Hydroxy benzoic acid	2.44	2.35
3	Vanillin	1.65	-
4	Salicylic acid	1.72	-
5	Gallic acid	1.62	-
6	Benzoic acid	2.68	2.84
7	Ferulic acid	1.66	-
8	Cenamic acid	1.71	-
9	Chlorogenic acid	1.60	1.50
10	Quercetin	3.25	3.22

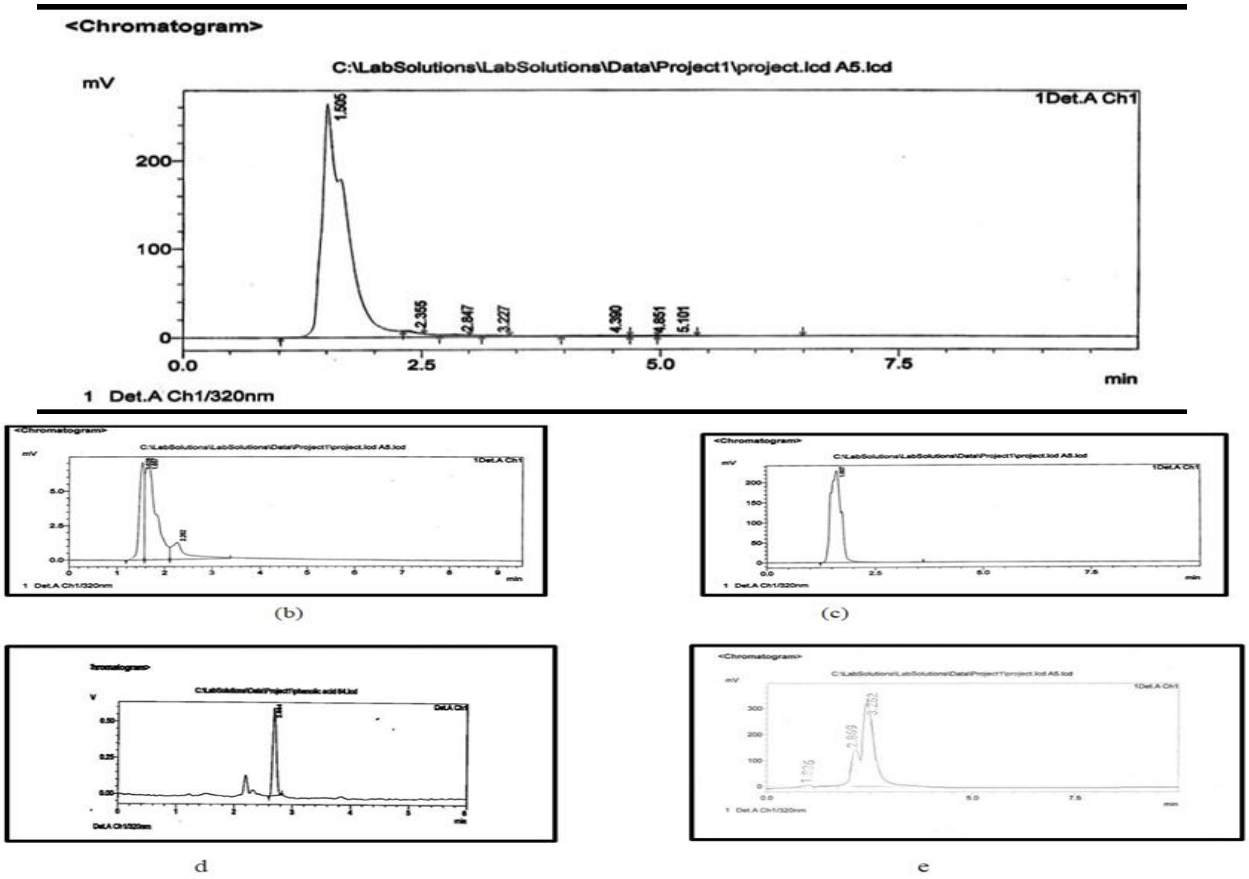

Figure (3) Absorbance peaks of HPLC-separated standard samples of pea extract (a) (b) Hydroquinone and (c) Chlorogenic acid (d) Benzoic acid (e) Querestin.

The identification and quantification of these phenolic compounds in peas provide valuable insight into their chemical composition. The findings suggest that peas contain a diverse range of bioactive phenolic acids and flavonoids, which contribute to their health benefits. The variability in retention times and the compounds detected underscores the complexity of the pea's phytochemical profile.

Quantitative determination of phenolic compounds isolated from pea plant residues

Based on HPLC, the concentration of phenolic compounds was calculated and estimated quantitatively by comparing the area % of the standard substance with the area % of plant residue samples. The results of Table (4) indicated that there were differences in the concentration of chemical compounds isolated from plant residues, and the highest concentration of the compound Chlorogenic acid was in the shoot reached 99 µg/g, as determined by HPLC, while the lowest concentration was for the compound Quercetin.

Table (4): Estimation of the amount of phenolic acids (µg/g) identified using HPLC of the alcoholic extract of pea plant residues

No	Compound	%
1	Hydroquinone	**-**
2	P-Hydroxy benzoic acid	**0.46**
3	Vanillin	**-**
4	Salicylic acid	**-**
5	Gallic acid	
6	Benzoic acid	**0.126**
7	Ferulic acid	**-**
8	Cenamic acid	**-**
9	Chlorogenic acid	**99**
10	Quercetin	**0.061**

CONCLUSION:

This research study the allelopathic impact of Pea (*Pisum sativum* L.) aqueous extracts on the growth and germination of two Wheat cultivars. Results of the greenhouse experiment showed pea leaves aqueous extracts at 3% concentration increase in shoot and root length of Atlas cultivar seedlings, while in the Aseel cultivar, its treatment increase in shoot and root dry weight, and showed a variation in the chlorophyll content of the tested cultivars between increase and decrease High-performance liquid Chromatography (HPLC) data the results showed that presence of some phenolic compounds known for their allelopathic ability that were diagnosed with this technology that is (P-Hydroxy benzoic acid, Benzoic acid, Chlorogenic acid, Quercetin). and better yield components such as spike length and grain number. The extract's bioactive phenolic compounds, such as **Chlorogenic acid**, likely contribute to these effects. The findings recommend further exploration of pea aqueous extract as a natural growth enhancer for wheat, particularly at higher concentrations, while also highlighting its potential as a sustainable and effective agricultural input.

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