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RESEARCH ARTICLE
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Influence of allelopathic potential of pomegranate peel extracts on germination and growth of some plant species

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ABSTRACT

The study was conducted at the College of Agricultural Engineering Science at Salahaddin University. The purpose of this study was to investigate the allelopathic effects of aqueous extracts of pomegranate peels (*Punica granatum L*), on seed germination and some growth parameters of barley and wild barley. The study was organized for completely randomized design. In this experiment, the concentration levels of 0, 5, 10, 15, and 20% were used. During the 10-day incubation phase at 22 °C, the experiment was conducted on a sterile Petri dish. The findings showed that the higher concentrations 15 and 20 % of aqueous extracts of pomegranate peels significantly reduced seed germination of Barley (*Hordeum vulgare* L.) and Wild Barley (*Hordeum spontaneum* L.). Also, according to the results pomegranate aqueous extract at all concentration levels (5%,10%, 15% and 20 %) significantly reduced germination percentage. The germination percentage was reduced from 90 % at control treatment to 20 % at concentration level 20%. Simultaneously, the inhibition of germination increases with the increase of concentrations. Other growth parameters such as plumule, radicle and seedling length were significantly inhibited by the application of aqueous extracts of pomegranate peels. Some phenolic compounds such as Galic acid, Quercetin flavonoid, Thiamine Ascorbic acid Quercetin and Oleic Acid were profiled in pomegranate peel by using Gas Chromatography-mass spectrometry. The results of this study imply that using aqueous extracts of pomegranate peels as a bio-herbicide to inhibit weed growth and seed germination can be advised. By applying these extracts, farmers can potentially reduce the reliance on chemical herbicides, promoting sustainable agricultural practices.

***Keywords*:** Allelopathy, pomegranate, Growth Bio-herbicide, Bioassay and Gas Chromatography.

INTRODUCTION

Any direct or indirect positive or negative impact a plant has on another plant due to the release of allelochemicals into the environment is known as allelopathy [1]. Allelochemicals may promote the growth of the same or different species at varying concentrations, even when they restrict the growth of some species at specific concentrations [2]. Allelopathic active compounds may provide innovative chemistry for synthesizing herbicides, insecticides, and fungicides. Reduced herbicide uses and lower pollution rates could be achieved by implementing agricultural management strategies that use allelopathic potential [3]. Previous scientific study indicates that a substantial amount of plant secondary metabolites is thought to have allelopathic effects. Certain plant natural products, including phenolics and alkaloid chemicals, are essential for certain plant functions, such as plumule growth and seed germination [4, 5]. Allelopathic substances enter the environment through a variety of pathways, including plant leaf volatiles, leaching, root exudation, and plant decomposition [6, 7]. The number of allelopathic research studies conducted on weeds and crops that used various plant component extracts, exudates, or residues to show the allelopathic interaction between weeds and crops has significantly increased [8, 9]. A new approach is aimed at promoting plant-based bio-herbicides as a substitute for chemical pesticides in crop production because of their environmentally friendly features. [10]. Several plant species and groups have been shown to exhibit allelopathic activity; these plants may be used in ecological and agricultural systems [11]. Consequently, to avoid these predicted issues, plants with the ability to produce bio-active compounds may find it helpful to use them as bioherbicides to eradicate undesirable plants. Furthermore, allelopathy may even be considered as a potential tool to decrease the presence of weeds and increase crop yields. [12,13]

The pomegranate, or *Punica granatum* L., is a member of the Punicaceae family and is indigenous to the northwest of India, extending from Iran to the Himalayas. This species serves a variety of applications; the fruits are extracted from the pulp and bark and used in pharmaceutical and industrial settings [14]. Tests for germination using commercial seeds of lettuce (*Lactuca sativa*), tomato (*Solanum lycopersicum*), and pepper (*Capsicum baccatum*) revealed that the extracts of pomegranate (*Punica granatum*), noni (*Morinda citrifolia*), and eucalyptus (*Eucalyptus* spp.) have allelopathic inhibitory

effects on germination and delayed its development [15]. the present experiment assessed the allelopathic actions of aqueous extracts of pomegranate peels (*Punica granatum L*), on seed germination and some growth parameters of plant species.

Materials and methods

2.1 Sample Collection

Pomegranate peels were gathered from the local farmers in Harrer which is located on the north of Erbil- Kurdistan /Iraq in the late of October 2023. The materials were divided into tiny (5 cm) pieces. After that, the samples were left for two weeks to be dried by air. The samples were air-dried and then sent to Salahaddin University-Erbil's Agriculture Lab, where they were ground into fine particles using a blender and prepared for testing.

2.2 Aqueous extract preparation

Aqueous pomegranate peel extracts were prepared by mixing 10 grams of pomegranate peel powder and 100 milliliters of distilled water to create aqueous extracts. After that, the samples were placed in a shaker machine to improve mixing, and the extract was shaken aver night After filtering the shoot extracts using filter paper, centrifuge 1000 rpm for five minutes. A micropore filter with a 0.45 µm opening was used to filter the supernatant. Once the outcome was achieved, it was stored in a refrigerator at 4°C until needed.

2.3Bioassay

Aqueous extract of pomegranate peel that had been previously preserved was divided into five concentrations (0, 5, 10, 15, and 20%) for the bioassay procedure. Distilled water was added to achieve the desired concentration, while sterilized water was used as the control.

Twenty Barley (*Hordeum vulgare* L.) and Wild Barley (*Hordeum spontaneum* L.) seeds each were put in separate 9 cm-diameter filter paper-lined petri dishes. Since these were the treatment petri plates, five milliliters of three distinct pomegranate peel extracts were added to the petri dishes containing the two seed samples. The control group of Petri plates was given 5 milliliters of distilled water alone. Each seed species (barley and wild barely) had three replications, including test and control treatments. In a growth chamber, petri dishes were incubated at 22°C. Following a week, the percentage of seed germination, shoot and root lengths (cm), shoot dry weight, and root dry weight (g)of the sprouted seedlings were noted.

2.4 Statistical analysis:

Before Conducting or performance a statistical analysis All data were examined for homogeneity and normality before being submitted to a statistical analysis of variance (ANOVA) at the 1% significance level using SPSS version 20. Tukey's test was then used to determine the mean separation.

2.5 Recorded Parameters

The data that was recorded were germination percentage %, inhibition of germination, plumule length (%) seedling length (cm), radicle growth inhibition RGI%, plumule growth inhibition PGI% and seedling growth inhibition SGI%, according these formula's below:

1. Germination percentage %=germinated seeds/total tested seeds×100
2. I.O.G.%={(C.G.P. – T.G. P)/ C.G.P 100
3. R.L.(cm)}/T.D.]*100
4. Seedling growth inhibition% =(1−Total seedling dry weight under stress /Total seedling dry weight under non stress) ×100
5. Plumule growth inhibition % (1−plumule dry weight under stress /plumule dry weight under non stress)×100
6. Radicle growth inhibition% (1−Radicle dry weight under stress/Radicle dry weight under non stress)×100

2.4 Gas chromatography–mass spectrometry analysis

Pomegranate peel alcohol extract preparation is used to prepare the extract of plant samples (pomegranate peel). 50 g of dry powder was used, and the material was extracted from the saxholate extract using 500 milliliters of 99% ethyl alcohol for a full day. The alcohol extract was then stored in sealed bottles in the refrigerator until it was needed [16]

Result and Discussions

3.1. Phenolic compound contents in pomegranate peel

The examination of GC-MS was observed by GC-MS and the mass spectrometry of the chemical composition of the plant components under study. A total of twelve substances were identified in Table(1). It was noted that several compounds showed up in large amounts, such as the compound Galic acid 43.25% and Quercetin flavonoid 10%. While some of the other compounds showed up at very low amounts such as Octadecanoic acid 0.5% and Punicalin 1.05 %. Previous studies showed that phenolic compounds significantly inhibit the growth, water relationship chlorophyll content and photosynthetic products of soybean [17]. According to studies, allelopathic properties of gallic acid, chlorogenic acid, acid, caffeic acid, ferulic, and vanillic acid have been observed to significantly affect the prevention of seed germination and the growth of some plants. Studies have shown that gallic acid's allelopathic characteristics significantly affect certain plants' growth and the inhibition of seed germination [18].

Table 1 Analysis GC-MS of an extract of pomegranate peel

Retention Time	Component Name	Peak Area%

No.			
1	3.849	Furfural	4.65
2	7.48	Pyrogallol	2.25
3	9.266	Gallic acid	43.25
4	10.01	Punicalin -	1.05
5	10.692	Thiamine	3.17
6	11.742	D-Allose	3.56
7	15.928	Ascorbic acid	3.16
8	16.285	Quercetin	10
9	17.632	Oleic Acid	4.45
10	17.849	Octadecanoic acid	0.5
11	26.545	.gamma.-Sitosterol	3.52
12	26.671	Fucosterol	0.48

3.2 The effect of pomegranate aqueous extract on germination and inhibition of germination of Barley and Wild barley.

The results from Figure 2 show that germination percentage and inhibition of germination were significantly ($P < 0.01$) affected by aqueous pomegranate extracts. It was noted that the highest germination percentage was 60.66 % in barley, whereas the lowest germination percentage was 36.94 observed in wild barley. Inhibition of germination recorded maximum value was 49.42 in wild barley. The effect of pomegranate aqueous extract's biochemicals may be the cause of the tested plant species' decreased seed germination of the studied species may be due to the effect of biochemicals present in common purslane extracts (19) Previous research has documented the reaction of early growth and germinating seeds to phenolic compounds, demonstrating that these chemicals support the plant growth system and may influence plant hormones to influence seedling growth [20]. The results are in agreement with Othman [21].

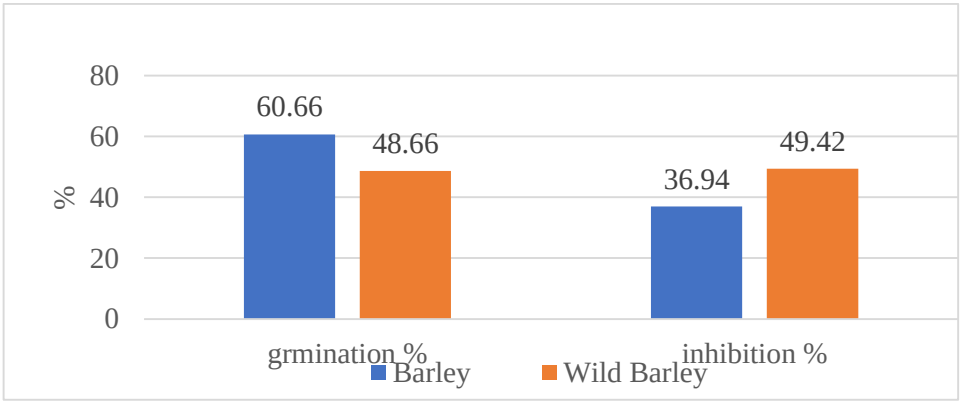

Figure 1 The effect of pomegranate aqueous extract on germination and inhibition of germination of Barley and Wild barley, Tukey's test($P \leq 0.01$) was used to calculate significant differences between means.

3.3 The effect of pomegranate aqueous extract on Radicle, plumule, and seedlings of barley and wild barley.

As shown in figure 2 radicle, plumule and seedling length were significantly ($P < 0.01$) affected by aqueous extracts of pomegranate. The results indicate that in wild barley radicle, plumule and seedling length recorded the highest values 4.52, 12.16 and 18.68 cm respectively, while for the same traits the recorded data are 4.48, 11.98 and 16.46 cm respectively. According to the findings of this study, which examined how the allelopathic potential of pomegranate aqueous affected different plant parts, different plant species are more or less susceptible to allelopathic potential depending on genetic differences [21] Pomegranate peel extract dramatically decreased the plumule, radicle and seedling length, due to their high phenolic acid content (Table 1), which inhibits photosynthesis, respiration, and root and stem growth rate, it reduced the seedling length relative to the control.

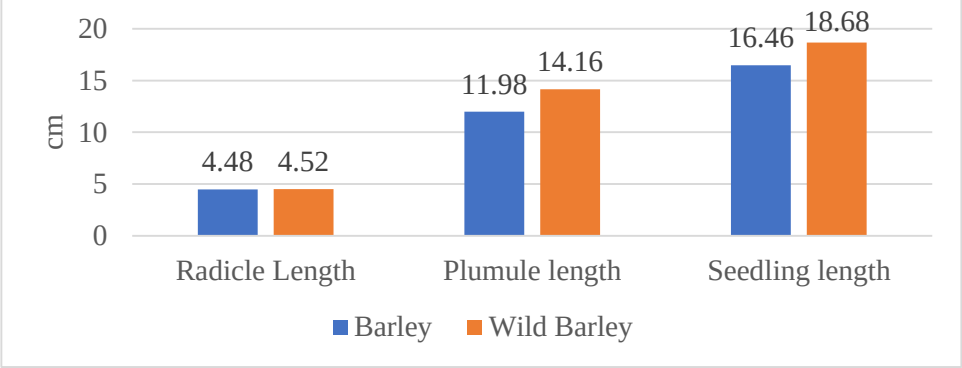

Figure 2 The effect of pomegranate aqueous extract on radicle, plumule and seedling length (cm) of barley and wild barley. Tukey's test ($P \leq 0.01$) was used to calculate significant differences between means.

3.4 The effect of pomegranate aqueous extract on Radicle, plumule and seedling growth inhibition of barley and wild barley.

RGI%, PGI% and SGI% were significantly ($P < 0.01$) affected by aqueous extracts of pomegranate (Figure 3). The highest value mentioned 68.35 % for RGI% in barley meanwhile for the same trait was 64.37 in wild barley. However, the contrary is apparent the PGI% and SGI% recorded highest values 57.25% and 59.98% respectively in wild barely.

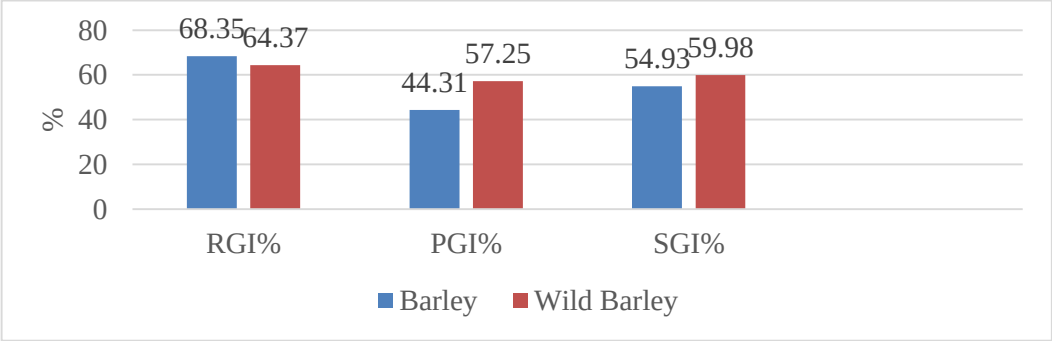

Figure 3 The effect of pomegranate aqueous extract on radicle RGI% PGI % and SGI % of barley and wild barley. Tukey's test ($P \leq 0.01$) was used to calculate significant differences between means.

3.5 The effect of pomegranate aqueous extract concentrations on germination and inhibition %.

The results from Figure 4 indicate that pomegranate aqueous extract at all concentration levels (5%,10%, 15% and 20 %) significantly reduced germination percentage. The germination percentage reduced from 90 % at control treatment to 20 % at concentration level 20%. Simultaneously, the inhibition of germination increases with the increase of concentrations. The findings showed that, in general, the germination inhibition (%) of weed seeds increased with more pomegranate peels [22]. The data on the total polyphenolic and flavonoid contents of the crude juices from pomegranates supported these results. In other words, the high concentration of total phenolic and flavonoid compounds in pomegranate peel crude juice may cause its significant herbicidal activity. In this regard, [23] noted that the primary factor limiting the germination of weed seeds may be the toxicity of pomegranate allelochemicals, such as phenolic compounds.

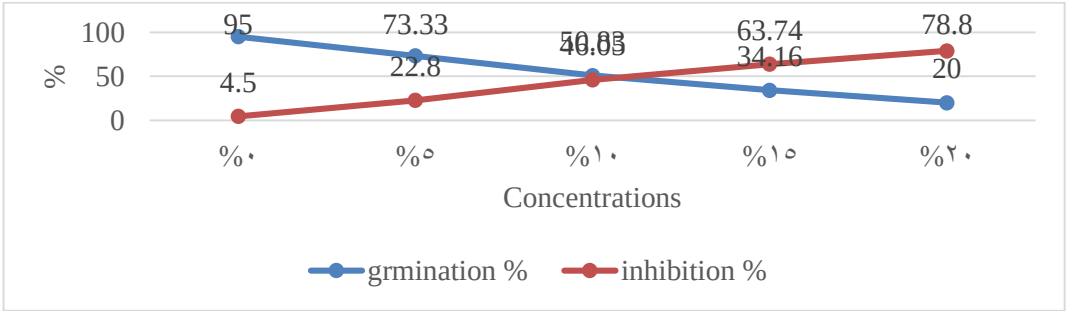

Figure 4 The effect of pomegranate aqueous extract concentrations on germination and inhibition %. Tukey's test ($P \leq 0.01$) was used to calculate significant differences between means.

3.6 The effect of pomegranate aqueous extract concentrations on radicle, plumule and seedling length of barley and wild barely.

Figure 5 indicates that all concentration levels (5%,10%, 15% and 20 %) significantly affected the radicle, plumule and seedling length. The highest number 31.82 cm observed in seedling length at control treatment then decreased to 10.2 cm at concentration 20 %. According to the findings, radicle and plumule lengths decrease with increasing concentrations, resulting in a reduced total seedling length. The effects were concentration dependent on all studied parameters [24]. The radicle, appears to be especially susceptible to the concentration; as the concentration rises, its length decreases and, at the greatest concentration, it stops completely. This would suggest that the treatment has a greater negative impact on root development than shoot [25]. Furthermore, all concentration levels, particularly higher concentration levels, impacted germination and all growth indices. [26]

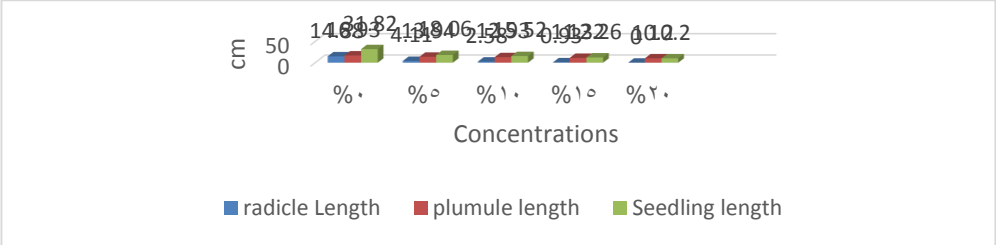

Figure 5 The effect of pomegranate aqueous extract concentrations on radicle, plumule and seedling length of barley and wild barely. %. Tukey's test ($P \leq 0.01$) was used to calculate significant differences between means.

3.7 The effect of pomegranate aqueous extract concentrations on RGI%, PGI% and SGI% of barley and wild barely.

The results in Figure 6 show the RGI%, PGI% and SGI% were %) significantly affected by the concentration levels of pomegranate aqueous extract concentrations. RGI% documented highest values 100% at concentration level 20% while this number reduced to 0 % at control treatment 0 %. For all three parameters the level of inhibition increases with the increase of concentration levels. according to Esmaeilli et al. [27]. The allelochemicals in question suppress the germination of binary seedlings. These allelochemicals included m-coumaric acid, ferulic acid, Vanillic acid, p-hydroxy benzoic acid, and p-coumaric acid.

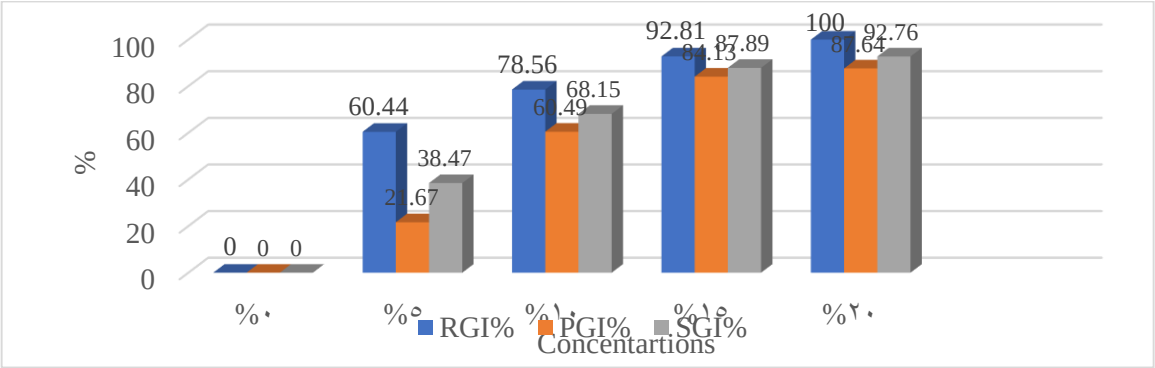

Figure 6 The effect of pomegranate aqueous extract concentrations on RGI%, PGI% and SGI% of barley and wild barely. %. Tukey's test ($P \leq 0.01$) calculated significant differences between means.

3.8 The interaction effect of species and aqueous extract concentrations on germination and some seedling growth parameters.

Table 1 elucidates the combination between plant species and different concentrations of pomegranate aqueous extracts significantly ($P \leq 0.01$) affected on all studied parameters. The germination% noted highest value 95% in barley and wild barely at 0% control treatment while the minimum value 16.66% was recorded in wild barley at concentration 20 %. The highest value 82.26% observed in wild barely at 20 % concentration while the lowest value 4.00 % was with barley at control treatment for inhibition parameter. Radicle length, plumule length and seedling length indicated highest values 15.60cm 18.87cm and 34.47 cm respectively in wild barely at control treatment and the lowest values were 0.00 cm, 9.74cm and 9.74e for the same parameters in barley. The maximum values 100.00%, 88.63% and 92.97% documented in RGI%, PGI% and SGI% respectively at 20 % concentration in wild barely meanwhile the minimum values were 0 % for the same parameters with both plant species. Allelochemicals found in pomegranate peel extracts may be the source of the decrease in seed germination and growth characteristics. These compounds may have detrimental effects on physiological processes

and cell division. Furthermore, biochemicals may be the cause of variations in the permeability of the cell membranes of the studied plant and weed species during the process of seed germination [28]. These findings are in line with those of Sharma and Satsangi [29], who found that extracts with lower concentrations on *Amaranthus viridis* and *Parthenium hysterophorus* had greater beneficial effects than extracts with higher concentrations (50–100%) of sunflower aqueous shoot extracts.

Table 1 The interaction effect of species and aqueous extract concentrations on germination and some seedling growth parameters.

Species* Concentrations		germination %	inhibition %	radicle Length	plumule length	Seedling length	RGI%	PGI%	seedling growth inhibition
Barley	0%	95.00^{a}	4.00^{e}	14.16^{b}	15.00^{ab}	29.16^{b}	0.00^{h}	0.00^{h}	0.00^{h}
	5%	83.33^{b}	12.28^{e}	4.23^{c}	12.96^{bc}	17.20^{cd}	70.27^{f}	3.81^{g}	33.15^{g}
	10%	58.33^{c}	38.59^{d}	3.10^{c}	12.54^{bc}	15.64^{cd}	77.11^{e}	51.75^{e}	62.94^{e}
	15%	43.33^{d}	54.38^{c}	0.94^{ef}	9.65^{bc}	10.59^{e}	94.37^{b}	79.36^{c}	85.99^{c}
	20%	23.33^{ef}	75.43^{ab}	0.00^{f}	9.74^{c}	9.74^{e}	100.00^{a}	86.66^{b}	92.55^{a}
Wild barley	0%	95.00^{a}	5.00^{e}	15.60^{a}	18.87^{a}	34.47^{a}	0.00^{h}	0.00^{h}	0.00^{h}
	5%	63.33c	33.33^{d}	4.00^{c}	14.92^{ab}	18.92^{c}	50.62^{g}	39.53^{f}	43.79^{f}
	10%	43.33^{d}	53.50^{c}	2.06^{cd}	13.33^{bc}	15.40^{cd}	80.01^{d}	69.23^{d}	73.35^{d}
	15%	25.00^{e}	73.100^{b}	0.933^{ef}	13.00^{bc}	13.93^{de}	91.25^{c}	88.89^{a}	89.79^{b}
	20%	16.66^{f}	82.16^{a}	0.00^{f}	10.66^{bc}	10.66^{e}	100.00^{a}	88.63^{a}	92.97^{a}

Conclusion

Based on this research, it is determined that pomegranate Peel aqueous extracts often slow down the germination and growth of the barley and wild barley seeds that were studied. Furthermore, 20% pomegranate extracts significantly reduced the germination percentage to (0%) of the plant species. The presence of allelochemical compounds may all raise the allelopathic potentiality. Because bioherbicides are more environmentally friendly than chemical herbicides, they may be a new method of weed control and an additional tool in plant development.

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تأثير القدرة الأليلوباثية لمستخلصات قشور الرمان على إنبات ونمو بعض الأنواع النباتية

سامان عبد رسول

قسم المحاصيل الحقلية والنباتات الطبية، كلية علوم الهندسة الزراعية، جامعة صلاح الدين ،أربيل، إقليم كوردستان ،العراق.

الخلاصة

أجريت الدراسة في كلية علوم الهندسة الزراعية بجامعة صلاح الدين. وكان الهدف منها معرفة التأثيرات الأليلوباثية للمستخلصات المائية لقشور الرمان *(Punica granatum L)*على الأنبات والنمو المحصول الشعير والشعير البري. وقد نظمت الدراسة بتصميم عشوائي كامل. تم استخدام مستويات تركيز 0 ،5 ، 10 ، 15 و 20٪. لمدة 10 أيام تحت 22 درجة مئوية، حيث تم وضع 20 بذره فى اطباق البترى . وأظهرت النتائج أن التراكيز الأعلى 15 و 20٪ من المستخلصات المائية لقشور الرمان اثرت بشكل ملحوظ من إنبات بذور الشعير *(Hordeum vulgare L.)* والشعير البري *(Hordeum spontaneum L.)*.كما أظهرت النتائج أن جميع تراكيز مستخلص الرمان المائي (5%، 10%، 15% و20%) أدى إلى انخفاض معنوى في نسبة الإنبات. حيث انخفضت نسبة الإنبات من 90% عند معاملة كونترول إلى 20% عند مستوى التركيز 20%. ولوحظة ازدياد التاثير التثبيتى مع زيادة التركيزات. تم تثبيط صفات النمو الأخرى مثل طول الريشة والجذير وطول الشتلة بشكل كبير عن طريق تطبيق المستخلصات المائية لقشور الرمان. كما تم تحديد بعض المركبات الفينولية مثل حمض الجاليك وفلافونويد كيرسيتين وحمض الأسكوربيك كيرسيتين وحمض الأوليك في قشور الرمان باستخدام كروماتوغرافيا الغاز–مطيافية الكتلة. تشير نتائج هذه الدراسة إلى أنه يمكن التوصية باستخدام المستخلصات المائية لقشور الرمان كمبيد حيوي للأعشاب لمنع نمو الأعشاب وإنبات البذور. ومن خلال استخدام هذه المستخلصات، يمكن للمزارعين تقليل الاعتماد على مبيدات الأعشاب الكيميائية، وتعزيز الممارسات الزراعية المستدامة

الكلمات المفتاحية: الأليلوباثي، الرمان، مبيدات الأعشاب الحيوية للنمو، التحليل الحيوي والكروماتوغرافيا الغازية.