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Determination of Allelopathic and Antimicrobial Effects of Four Different Plant Species

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Abstract

The investigation of allelopathic and antimicrobial effects of four different plant extracts is the aim of this study. The aqueous extract of plants (*Lepidium draba* L., *Acroptilon repens* (L.) DC., *Thymus kotchyanus* Boiss. & Hohen. var. *kotchyanus*, *Inula peacockiana* (Aitch.&Hemsl.) Koravin,) were applied to seedlings of cultivated (*Pisum sativum* L. and *Hordeum vulgare* L.) and weed (*Amaranthus retroflexus* L. and *Portulaca oleraceae* L.). Seedlings were irrigated with aqueous extract (1 %) for 15 days to observe the physiological responses. The morphological changes, chlorophyll levels and phytohormone concentrations were determined. The *Lepidium draba* and *Inula peacockiana* extracts affected development of all of the target seedlings and caused lesion on the leaves. *Acroptilon repens* and *Thymus kotschyanus* extracts caused a decrease in gibberellic acid (GA) whereas increase in absisic acid (ABA) levels. For investigation of antimicrobial activities, hexane, methanol and water extracts were prepared. Extracts were applied on *Bacillus cereus*, *Staphylococ aureus*, *Pseudomonas syringae* and *Escherichia coli for* antibacterial and *Rhizoctonia solani*, *Fusarium oxysporum* and *Aspergillus niger for* antifungal effects. *Inula peacockiana* and *Acroptilon repens* extracts had a great antimicrobial activity. While all plant extracts inhibited *Rhizoctonia solani*, *Fusarium oxysporum* growth and spore development of *Aspergillus niger*. The antifungal activity of extracts were better than antibacterial activity. As a conclusion, these plants have allelopathic potential for natural weed control and antimicrobial potential for medicinal uses.

Key words: Alelopatyhy, Alellochemicals, Antimicrobial, Weed Control

1. Introduction

Scientists have tried to learn about the mysterious life of plant interactions and find alternative solutions instead of synthetic substances harmful to nature. Additionally, they have wondered the communication of plants

with each other and with other organisms in their environment. This communication phenomena is called allelopathy. Allelopathy; is known as a term consisting of the Greek words Allelo and Patos, which means one to harm the other. Although it has a short definition as a term, the subject of allelopathy has spread to a wide area of studies recently. The concept of allelopathy, especially associated with secondary metabolites, is now included in the work of many pharmacologists, herbologists, ecologists and physiologists. In order to understand the concept and mechanism of allelopathy, the effects and causes of allelochemical substances in plants should be well known. There are many studies reporting that allelochemicals affect on germination and shoot and root length (Li et al., 2020), also cell division, ion and water uptake, phytohormone metabolism, respiration, photosynthesis, enzyme functions and even gene expression (Singh and Thapar, 2003). In addition to their direct phytotoxic effects, allelochemicals are known to affect development indirectly by inhibiting the synthesis and/or accumulation of compounds in sugar and triacylglycerol pathway (Belz and Hurle, 2004). Also extract application can trigger the photosynthesis pathway (Chen et al., 2021)

Allelochemicals cause many physiological changes in plants resulting with similar and predictable responses. These responses vary according to structure and dose of the allelochemical, genetic potential of the target plant and metabolic pathway (Bao et al., 2019; Inderjit and Mukerji, 2006). Allelochemicals such as cinnamic acid, vanillic acid, coumarin and sorgolonon affect chlorophyll function by changing the efficiency of photosystems. For this reason, researchers attribute the decrease in chlorophyll caused by allelochemicals is related to blocking of the non-lethal chlorophyll biosynthesis pathway or the stimulation of the mechanism that causes chlorophyll degradation. (Gonzales et al., 1997). Scientists are looking for natural and harmless alternatives to synthetic herbicides and pesticides.

Natural or synthetic antibiotics inhibit the growth of bacteria by affecting the membrane and cell wall structure, folic acid metabolism or the functioning of molecular building blocks. The high amount of phenolic, flavonoid or alkaloid contents of the plants used in the study significantly affected the growth of both bacteria and fungi development. In addition, in current study, water extracts of four different plants given with irrigation water were applied to both weed and cultivated plants. Moreover, plant extract with different solvents were tested for antimicrobial activity.

2. Material and Methods

2.1. Selection of plants and preparation of extracts

By using the experience of plant ecologist and field observations, four plants with different characteristics (invasive, aromatic) were selected for application. For target seedlings, 2 cultivated and 2 weed species were decided. The plants for application and target seedlings and are given in Table 1.

Table 1. Donor and target plants.

Invasive	Aromatic
Lepidium draba L	*Thymus kotchyanus*
Acroptilon repens	*Inula peacockiana*
Target plants	
Culture Form	Weed
Pisum sativum L. (Pea)	*Amaranthus retroflexus* L. (Pig weed)
Hordeum vulgare L (Barley)	*Portulaca oleraceae* L (Common Pursalene)

Plant were collected from different localities. They were washed with tap water and then with distilled water to remove dust and contamination and air-dried in the shadow for 10 days. At the end of the period, plants were cut into small pieces and 1 % of aqueous extracts were prepared (Türker et al., 2008).

2.2. Chlorophyll and phytohormone content analysis

0.1 g of leaf samples were thoroughly crushed in 80 % acetone. The crushed samples were filtered through filter paper made up to 100 ml. The absorbance values of the obtained pigment extracts at 645 and 663 nm were measured in a spectrophotometer. Absorbance values were substituted in the formulas. Chlorophyll and carotenoids contents were calculated (Lichtenthaller, 1987).

Chlorophyll a = 12.7 × A 663 – 2.69 × A 645
Chlorophyll b= 22.9 × A 645 – 4.68 × A 663
Total chlorophyll = 20.2 × A 645 + 8.02 × A 663
Total Carotenoid = 4.07 × A(450) – (0.0435 × chlo.a amount + 0.367 × chlo.b amount)

80 % of methanol (+ 4°C) was added to 300 mg powdered samples. Samples were homogenized in an ultra-tissue shredder for 10 minutes, and kept in the dark for 24 hours. The samples were filtered through Whatman No:1 filter paper and passed through 0.45 µml PTFE filters. It was dried at 35 °C by evaporator and dissolved in 0.1 M KH_2PO_4 (pH 8) buffer. The samples were centrifuged at 5,000 rpm for an hour at 4 °C to separate the fatty acids. The supernatant was taken from the tubes with an automatic pipette and left in a beaker. In order to separate the phenolic compounds and colorants, 1 gram of insoluble Polyvinyl Polypyrilidone (PVPP, Sigma) for each sample was prepared and mixed thoroughly and filtered (Hernandez-Miana, 1991). Filtered samples were passed through Sep-Pack C18 reverse phase filters and injected into HPLC.

2.3. Application of plant extract on development of target seedlings

The climate room was used for the study, the room and the pots to be used were sterilized. The humidity of the room was provided by water vapor and the light by fluorescent lamps. Environment conditions; Humidity was 60%, average light was 10000 lux, day temperature was 27 ºC, night temperature was 18 ºC. 14-hour photoperiod was provided. The temperature, humidity and light value of the climate room were checked daily with the RAM DT-8820 Environment Meter device. Sterilized pots were filled with a homogeneous mixture of 2; 2; 1

ratio from the fertilizer mixture. Ten seeds of pea and barley seeds and 50 seeds of weeds each were placed in pots.

Target seedlings were irrigated by 100 ml of aqueous extracts of four plants after emerging of seedlings. Extracts were prepared daily, pH and osmotic potential values of the extracts were measured. The control group was irrigated with tap water at the same rate. The application was conducted for 15 days. Seedlings were harvested and ground with liquid nitrogen in a mortar. Tissue samples stored in a ultra freezer at –80 ºC until analysis.

2.4. Antimicrobial assay

Reference *Bacillus cereus* (CCM 99), *Staphylococ aureus* (ATCC 6538), *Pseudomonas syringae* (ATTC 123) and *Escherichia coli* (ATTC 11230) bacterial strains were used for antibacterial effect. Disk diffusion method was used for antibacterial effect. The water, methanol and hexane solvents were prepared. 10 % extracts were kept in a shaker overnight in the dark. The extracts filtered through filter papers and then passed through 0.45 µm filters for sterilization. The water extracts obtained for all plants were directly used, however methanol and hexane extracts were completely dried at room temperature (Rigano et al., 2006). As a control application, streptomycin antibiotic was placed in the middle of the petri dishes of Muller Hinton agar medium. Antibacterial zone diameters in petri dishes were measured and photographed. Zones were compared with the streptomycin and antibacterial effects were determined (Burt, 2004).

Water and methanol extracts were used for antifungal assay by disc dilution method. PDA (potato dextrose agar) medium was used for the growth of fungal cultures (*Fusarium oxysporum*, *Rhizoctania solani* and *Aspergillus niger*) and fungi were incubated for ten days. Samples from fungi were added to PDA media with plant extracts and control groups. Thus, it was determined how plant extracts affect fungal growth. At the end of the incubation, the plant extract media and the control growth zones were measured and compared. (Khalil et al.,. 2005; Parvez et al., 2004).

3. Results and Discussion

3.1. Morphologic effects

Aqueous extracts of the plants were used instead of irrigation water caused decrease in the growth rate of the target seedlings. Additionally, it was observed that chlorosis and lesions were occurred on the leaves**.** (Fig. 1).

Figure 1. Lesion on pea leaf caused by *Inula peacockiana* plant extracts.

The effects of aqueous extracts were variable. Although aqueous extracts did not affect barley and pea growth, Inula and Thymus extracts negatively affected weed development, especially *Amaranthus* seedlings (Fig. 2).

Figure 2. General morphologic effects of plants extracts on target seedling.

3.2. Pigment effects of extracts

When the chlorophyll values in barley seedlings were examined, it was observed that a decrease in chlorophyll values occurred in *T. kotschyanus* and *I.peacockiana* applications. There was an increase in total carotenoid levels in *L. draba* and *P. armeniaca* applications in pea seedlings. In addition, *L. draba* extract application caused an increase in chlorophyll values in *Amaranthus* seedlings (Table 2).

Table 2. Pigment contents of target seedlings exposed to plant extracts.

Plant extracts	Chlol a	Chlo b	Total Chlo	Carotenoids
		***Amaranthus retroflexus* L. (Pig weed)**		
Lepidium draba	16,66*	5,71	22,37	2,98*
Acroptilon repens	13,02	5,60	18,62	1,77
Thymus kotschyanus	12,64	5,06	17,71	1,79
Inula peacockiana	12,85	5,55	18,41	2,20
Control	12,84	4,59	17,44	1,90
		***Portulaca oleraceae* L (Common Pursalene)**		
Lepidium draba	4,64	1,64	6,28	0,82
Acroptilon repens	4,72	1,65	6,37	0,85
Thymus kotschyanus	5,82	1,68	7,51	1,09
Inula peacockiana	4,93	1,44*	6,37*	0,95
Control	5,84	2,44	8,29	0,78
		***Pisum sativum* L. (Pea)**		
Lepidium draba	17,40	7,856	25,24	3,39
Acroptilon repens	14,93	6,98	21,91	2,82
Thymus kotschyanus	13,92	6,30	20,22	2,65
Inula peacockiana	14,19	6,35	20,55	2,79
Control	17,93	9,69	27,62	2,95
		***Hordeum vulgare* L. (Barley)**		
Lepidium draba	13,27	4,90	18,17	2,39
Acroptilon repens	15,06	5,62	20,67	2,65
Thymus kotschyanus	10,453	3,66	14,11	1,96
Inula peacockiana	10,118	3,67	13,78	1,83
Control	13,28	5,052	18,32	2,38

P<0.05

Recent studies reported that especially phenolic compounds target the Mg-cletase enzyme, which causes the reduction of chlorophyll, and thus affect photosynthesis (Yang et al., 2004). In the current study, it is seen that especially *Inula peacockiana* applications cause a decrease in chlorophyll levels. This may be due to the effect of phenolic compounds on the biosynthesis pathway**.** The changes in the amounts of carotenoids indicated that the seedlings are under stress and especially in *L. draba* applications made cause stress in pig weed seedlings.

3.3. Phytohormone effects of extracts

In general, plant extract applications caused a decrease in gibberellic acid levels and an increase in abscisic acid levels. It was determined that all applications except *L. draba* and *I. peacockiana* extract applications in pea plant caused lower GA amount compared to the control. Similarly, high ABA levels were observed in the same extract applications. In addition, an increase in the amount of zeatin was determined in the

application of *A. repens.* In IAA amounts, lower than control amounts were measured in all applications. It was observed that GA levels increased in *L. draba* and *T. kotschyanus* applications *to H. vulgare* and *Amaranthus* seedling compared to the control group (Table 3).

Table 3. Hormone contents of target seedlings exposed to plant extracts.

Plant extracts	Gibbrellic A.	Zeatin	Indol Asetic A .	Abscisic Acid
		***Amaranthus retroflexus* L. (Pig weed)**		
Lepidium draba	3,07[a]	13,45[a]	12,35[a]	9,19[a]
Acroptilon repens	14,5[b]	5,78[b]	8,97[b]	9,15[a]
Thymus kotschyanus	5,789[a]	6,78[b]	16,87[ac]	9,76a
Inula peacockiana	21,05[bc]	11,32[ab]	11,2[ab]	6,87[ab]
Control	19,5[bc]	19,98[ac]	9,45[a]	5,7[b]
		***Portulaca oleraceae* L (Common Pursalene)**		
Lepidium draba	9,47[a]	12,34[a]	5,49[a]	4,8[a]
Acroptilon repens	13,6[a]	7,22[b]	8,94[ab]	6,9[a]
Thymus kotschyanus	8,96[ab]	14,76[a]	9,76[b]	5,678[a]
Inula peacockiana	3,625[b]	13,1[a]	14,04[bc]	11,08[ab]
Control	15,62[a]	11,25[a]	13,2[bc]	7,05[bc]
		***Pisum sativum* L. (Pea)**		
Lepidium draba	20,6[a]	4,825[a]	4,295[a]	3,505[a]
Acroptilon repens	6,17[b]	10,695[ab]	3,8825[a]	10,465[b]
Thymus kotschyanus	2,937[b]	3,697[a]	2,5725[a]	7,407[ab]
Inula peacockiana	15,95[a]	4,175[a]	3,55[a]	13,3[b]
Control	23,35[a]	2,6925[a]	15,67[b]	6,65[ab]
		***Hordeum vulgare* L. (Barley)**		
Lepidium draba	13,527[a]	6,45[a]	7,894[a]	5,225[a]
Acroptilon repens	10,45[b]	5,678[a]	6,908[a]	6,175[a]
Thymus kotschyanus	13,28[a]	6,58[a]	3,214[b]	6,87[a]
Inula peacockiana	7,6[b]	3,67[b]	5,678[ab]	9,875[b]
Control	7,325[b]	7,45[a]	9,678[ac]	7,88[ab]

P<0.05

The general effects of secondary metabolites are effects on the structures of precursors and enzymes involved in hormone biosynthesis. Changes in hormone values can affect the whole plant metabolism. In this study, especially *Inula peacockiana* extracts increased ABA and IAA levels in *Portulaca oleracea* seedlings and caused a decrease in GA levels. Ferulic acid increases the ABA level and causes changes in the synthesis pathway of ABA via signaling (Wang et al., 2005). The opposite effect is indicated in other weed species of *Amaranthus retroflexus*. A stable situation was observed in cultivated plants. GA levels was decreased in some

applications and there was a decrease in ABA levels. This difference in hormone and other analyzes revealed that weeds activate different mechanisms against the plant extract irrigation. Also mixtures of various substances, often complex, and these mixtures can have synergistic or antagonistic effects, which can be modified by the chemical, physical and biotic properties of the soil (Reigosa et al., 2013)

3.4. Antimicrobial effect of extracts

Different plant extracts inhibited the growth of bacteria such as *Bacillus cereus, Staphylococ aureus and Pseudomonas syringae*. The highest inhibition effect was especially on the plant pathogen *Pseudomonas syringae* bacteria (Fig.3).

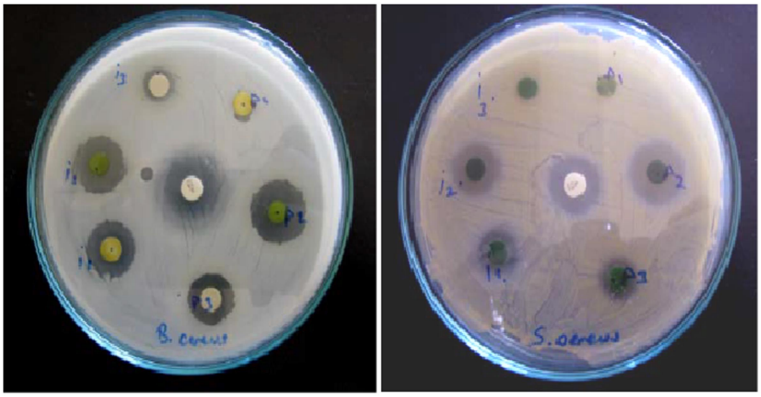

Figure 3. Antibacterial effects of *Inula peacockiana* extract.

Zones of some plant extracts were close to the streptomycin antibiotic, but the plant extracts could not inhibit the growth of *Escherichia coli* bacteria. The highest antibacterial effect was observed in *Inula peacockiana* plant extract, and the lowest effect was observed in *Lepidium draba* plant extract.

Table 4. Zone diameter of plant extract application on bacteria strain.

	B. ceresus	*S. aereus*	*P. syringae*	*E. coli*
A. repens (Methanol)	1.7	1.6	1.2	-
A. repens (Water)	1.4	1.1	-	-
*I. peockiana (*Hexane)	1.5	1.1	1.2	-
I. peacockiana (Methanol)	1.6	1.4	1.7	-
I. peacockiana (Water)	1.2	-	1.4	-
T. kotschyanus (Hexane)	0.9	0.9	-	-
T. kotschyanus (Methanol)	-	1.0	0.8	-
L. draba (Methanol)	-	-	1.2	-
Streptomisin (Kontrol Antibiyotik)	1.5	1.4	1.8	1.1

When the antifungal results were examined, *Inula peacockiana* plant extract had the highest antifungal effect for *Rhizoctonia solani*. The lowest effect was observed in the medium with *Lepidium draba* plant extract. In the development of *Fusarium oxysporum* fungus, the water extracts of the plants caused different growth zone

diameters, but all of them showed lower growth than the control group. In the antifungal assay *with Aspergillus niger*, the plant extracts could not inhibit the fungal zone growth, but spore formation was largely suppressed in methanol extract of plants (Fig. 4).

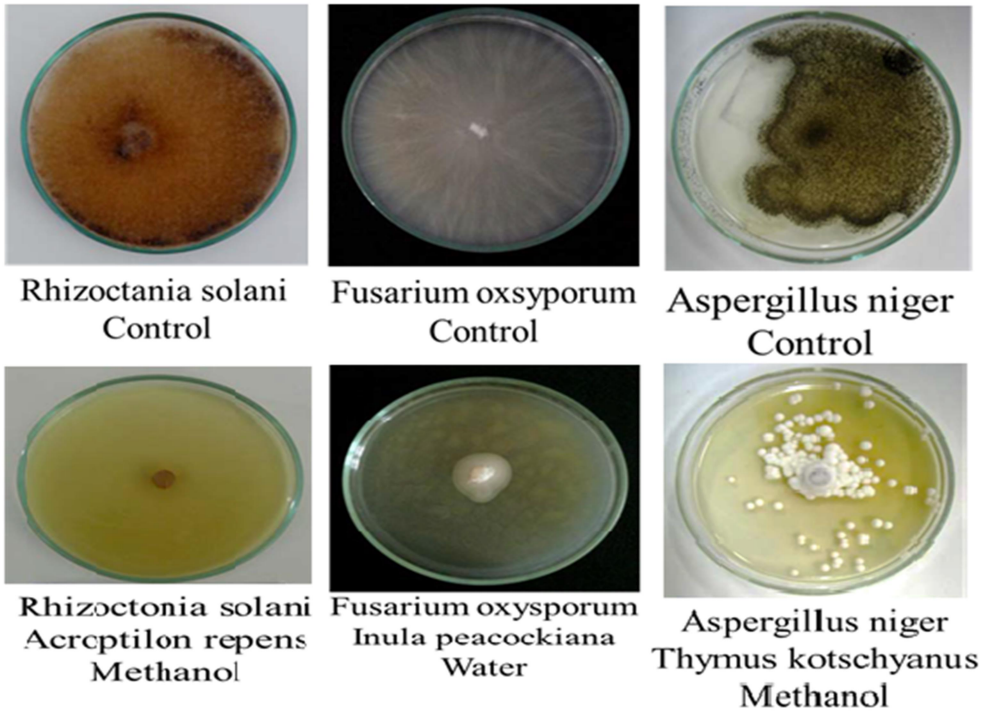

Figure 4. Antifungal effects of plant extracts.

F. oxysporum and *R. solani* fungi are plant pathogens and cause product losses. The plants used in the study have high antifungal effects Similar results reported that the substances in plant extracts inhibited fungal growth more than synthetic fungicides (Khalil et al., 2005; Guleria and Kumar, 2006; Tegegne et al., 2007). Previous preliminary phytochemical analysis revealed, plant extracts possess allelochemicals like alkaloids, terpenoids, tannins, and saponins (Gatto et al., 2021) and it is a promising source of antioxidants as well as antibiotics too (Goswami and Ray, 2017)

4. Conclusion

When the effects of all studied plants were examined in general, the allelopathic potentials and antimicrobial effects of *Inula peacockiana* and *Acroptilon repens* plants were higher than the other plant extracts. The results obtained from this study indicated that the effects caused by the plant extracts depend on the metabolite content and diversity, the solvent type and the genetic potential of the target plant or organism. The investigation of the phytochemical composition and isolation of new compounds having agricultural and medicinal potential from these plant extracts could be considered as further studies.

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Conflicts of Interests

Authors declare that there is no conflict of interests

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