Effect of plant extracts *Ammi* and *Rheum* on plant pathogenic fungus *Helminthosporium* sp

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Abstract

Penicillium, Aspergillus, Rhizopus, Altrnaria, and Helminthosporium, were genera of fungi that isolated and identified from local black Barley *Hordeum vulgare* were obtained from local market of Erbil city. The percentage of infection were 14 ,16 ,8 ,9 ,and 20 % respectively ,the fungus *Helminthosporium* sp showed highest infection so it was chosen to control it by plant extracts. Flower part of Pick tooth plant *Ammi visinaga* and root part of plant Rhubarb *Rheum ribes* two plants which have been used for this study. The chemical compounds that isolated from both plants were phenolic compounds, alkaloids, and tannins. Anti-fungal activity of plant extracts was tested at concentrations 0.01 and 0.04 g/100ml of medium against pathogenic fungus. The results showed that phenolic compounds and alkaloids at 0.01 and 0.04 g/dl of the medium for both plants, exhibited maximum inhibition of mycelium growth, and significantly decreased the dry and fresh weight of culture and spores germination compare with control treatment, while the tannins appeared less activity against mycelium (dry and fresh weight) and also spores germination when compared with other substances.

Introduction

Fossil records revealed that the human use plants as traditional medicine dates back to middle Paleolithic age, approximately 60000 years ago[1]. Medicinal plants have provided a good source of a wide variety of compounds such as phenolic compounds, nitrogen compounds, vitamins, terpenoids, and some other secondary metabolites which are rich in valuable bioactivities such as antioxidants, inflammatory, antitumor, antimutagenic, antibacterial, and antiviral activity [2].

Plant extracts have been used successfuly to control diseases in plants and tuber crops [3, 4]. A number of antifungal compounds of diverse skeletal patterns have been found in plants, these compounds belong mainly to six broad chemical groups such as phenol and phenolic acids, coumarins and pyrones, flavonoids, isoflavonoids, steroids, alkaloids, and miscellaneous compounds [5]. Barley (*Hordeum vulgar*) infected by different types of fungi and caused to it different diseases such as root and shoot rot, powdery mildew, leaf stripe, net blotch, scald, covered smut, and loose smut [6].

The purpose of the present study was to investigate the antifungal activity of alkaloids, phenols and tannins that extracted from *Rheum* and *Ammi* plants against pathogenic plant fungus *Helminthosporium* sp under laboratory conditions.

Materials and methods:

Isolation of fungi:

Three kg of black barley were collected from local market, one kg for each sample. The samples were mixed together, and 100 seeds were randomly transferred to ten Petri dishes containing potato dextrose agar medium (PDA). For this study ,surface sterilize of seeds were done by sodium hypochlorite (6% NaOCl)for two minutes., washed three times with sterile distilled water then dried with sterilized filter paper, ten seeds were transferred to each Petri dishes containing sterilized potato dextrose agar medium (PDA),and in order to suppress bacterial growth 30mg/L streptomycin was added to isolating medium. All plates were incubated at 25C^{o} . The colonies which developed on seeds were carefully counted; all fungi were purified and identified according to keys of genes [8 and 9].

Preparation of plant extracts:

Ammi sp belongs to family Umbellifaceae and *Rheum* sp belong to family polyonaceae, were chosen to be tested against phytopathogenic fungus that caused diseases to plants. Alkaloids, phenolic compounds and tannins were extracted according to the following procedures.

Alkaloid extraction:

Sample of 20gm of dried powered plants materials (flower part of Pick tooth, and root part of Rhubarb, each plant separately) were suspended in 100 ml surfactant solution in glass conical flask, and sonicated for 2.5 h in an ultrasonic bath at a constant temperature of 25^{o}C. The extract was separated by simple filtration and the residue washed with 20ml of distilled water. The filtrate was acidified with sulfuric acid solution (2%) to pH (3-4) and the alkaloids were precipitated with 15 ml of Mayer's reagent. The precipitate was dissolved in an alkaline solution of sodium carbonate (5%) and then extracted with chloroform $CHCl_3$ (three times). The organic (chloroform layer was washed with water to neutral pH, dried with Na_2SO_4 and concentrated to dryness under reduced pressure to obtain crude alkaloids [9].

Extraction of condensed tannins:

Sample of (20 gm dry weight) was weighed into a glass conical flask, the tannins were extracted with 70% aqueous acetone, stirred for 15 minutes, sonicated for 2h.at 25C, separated, finally tannins were precipitated from filtrate with 50% aqueous methanol, and then centrifuged at (3000gx for 10minutes), resultant extract was washed with 50% aqueous methanol (two times), then the extract was evaporated to dryness [10].

Extraction of simple phenolic compounds

Plants(each plant separately) were grinded to powder and 25g was defatted by petroleum ether, the residue was extracted with 80% methanol using sonication for 2h.,the crude extract was separated by simple filtration, the filtrate was evaporated, and the residue was partitioned between chloroform ($CHCl_3$) and H_2O(using separation funnel). The dark brown residue left after evaporation of

chloroform ($CHCl_3$) was used as crude phenolic compounds (modified method of Harborn) [11].

Activity of plant extracts against mycelium growth of fungus:

0.01and 0.04g/ml of plant extracts and 100ml of Czapk dox agar medium were chosen to be tested against mycelium growth [12]. Plant extracts were added to the medium, mixed well, poured into sterilized plate, and sterilized water was added as the control, and then allowed to solidify. The center of each plate was inoculated with (5mm) diameter mycelia disc of the pathogen, taken from pure culture (7 days old) was inverted and placed on the fresh medium. All inoculating plates were incubated at 25°C for 6 days [13].

$$\text{Liner growth reduction \%} = \frac{\text{Growth in control} - \text{growth in treatment}}{\text{Growth in control}} \times 100 .$$

Wet and dry weight:

To determine the fungal rate growth (fresh and dry) Czapkes dox medium was prepared, distributed in conical flasks volume 250 ml, 100 ml of medium /flask. 0.01 and 0.04g/ml of plant extracts were added to sterilized cooled medium, and mixed well. Three replications were used for each treatment. All flasks incubated in shaker incubator at 25°C for 15 days with shaking at (100rpm), growth culture was filtrated, wet or fresh weight recorded and dry weight was calculated after oven drying at 80°C for 48h., sterile distilled water was added to control treatment instead of plant extracts [1**4].**

Effect of plant extracts on fungal spore germination:

Antifungal activity of *Rheum* and *Ammi* extracts on spores germination of *Heliminthosporium* sp. was tested by the slide technique, the plant extracts of both plants 0.01 and 0.04 mg/ml were placed in cavity of the dried clean slide as a film, a drop of the conidial suspension ($4x10^6$spores/ml) of *Heliminthosporium* sp, prepared in sterilized distilled water, was added to dried clean slide as film and thoroughly mixed. The cavity of slide was incubated in Petri dishes glass bridges chamber. Three slides were used as replication for each concentration, incubated for 24h at 25°C. The germination of spores was observed by three different microscopic fields and recorded after 48h to calculate the percentage of germination according to the following formula [15]:

$$\text{\% spore germination} = \frac{\text{Spore germination(no)}}{\text{Total spore(no)}} \text{ X } 100$$

Result And Discussion:

Pencillium sp, *Aspergillus* sp, *Rhizopus* sp, *Alternatia* sp. and *Helminthosporium*, were isolated from barley seeds, the infection percentage was 14, 16, 8, 9, and 20 respectively as show in (Table1). Investigators[16] indicated that at least 59 species represent 35 fungal genera were recorded from wheat seed, including *Alternaria, Epiccum, Aspergillus, Fusarium,* and *Drechslera*.[17] Reported that twelve fungal genera were isolated from five cultivations of wheat and two cultivations of barley seeds, the genera were *Aspergillus* spp *Rhizopus* spp, *Fusarium* spp, *Alternaria* spp, *Penicillium* spp, *Hileminthosporium, Mucor, a*nd *Epiccoum,* the same result recorded by [18].

Table (2) shows that all treatments were positive effect in reducing the linear mycelia growth of pathogenic fungus. The percentage value or reduction of mycelium growth shows that phenolic compounds and alkaloids have the highest effect on reduction, comparing with the control. [18] indicated that the inhibitory effect of the test extracts might be due to natural bioactive materials present in these extracts.[19]reported that the crude water extract of chickpea(*Cicer arietinum*) was tested against *Drechslera hawaiiensis* and *D.tetramera* showed the most significant antifungal activity.

Tables (3 and 4) show the effect of plant extracts on the fresh and dry weight of fungus, the result indicated that phenolic compounds of both *Rhem* and *Ammi* completely inhibiting the mycelium growth in liquid culture compared with control treatment for wet and dry weight 7.15 and 1.2 respectively. The percentage inhibition of spores germination of *Heliminthosporiu*m by Phenolic compound were more significant for *Rhemand* and A*mmi* 0,04gm/ml and 0.01gm/ml, but the tannins show the least effect on spore germination.

Results in table (5) shows that all treatments significantly decrease the percentage spore germination of *Heliminthosporium* compared with the control. Plant extracts of *Amim* at 0.04gm/ml for their phenolic compounds, alkaloids and tannins were more effective in reducing spore germination which where 11.00, 26.00 and 34.00 respectively, while the plat extract of *Rhume* at 0.01gm/ml was less effective in reducing the spore germination for tannins, alkaloids and phenolic compounds which were 66.66, 46.33 and 38.00 respectively compared with control which was 87%.[20] Indicated that the crude plant extract of *Ammi* and *Rheum* has been used against pathogenic fungi isolated from pine seedling, the investigate demonstrated that the crude extract of *Rhem* was reduce significantly the mycelium growth of pathogen fungi *Rhizoctonia* ,*Pythium* and *Fusaruim.*

The inhibition of mycelium growth may be attributed to the loss of emerge or in other words due to respiration inhibition [21].The extracts could be used as protecting biopesticide against plant pathogenic fungi that caused different disease at different plants.

Table (1): isolation of fungi from barley *Hordeum sp.* Seeds

Fungi	Infection%
Penicillium sp.	14
Aspergillu ssp.	16
Rhizopus sp.	8
Alternaria sp.	9
Helminthosporium sp.	20

Table (2) effect of plant extracts (*Rheum* and *Ammi*) of mycelium growth of *Helminthosporium sp.* Incubated at 25 °C for 6 days

Plants	Concentration gm/dl	Alkaloids	Phenol	Tannins
Rheum	0.01	*0.80	0.00	2.50
	0.04	0.00	0.00	2.10
Ammi	0.01	0.00	0.00	2.30
	0.04	0.00	0.00	1.70
Control	0.00	6.50	6.50	6.50
LSD	0.01	0.42		
LSD	0.05	0.31		

* Each number represents 4 replications

Table (3) effect of plant extracts (*Rheum* and *Ammi*) on the fresh weight of *Helminthosporium sp.* Incubated at 25 °C for 6 days

Plants	Concentration gm/dl	Alkaloids	Phenol	Tannins
Rheum	0.01	*2.20	0.00	2.40
	0.04	1.18	0.00	1.00
Ammi	0.01	1.78	0.43	2.10
	0.04	0.80	0.00	1.10
Control	0.00	7.15	7.15	7.15
LSD	0.01	0.60		
LSD	0.05	0.44		

*Each number represents 4 replications

Table (4) effect of plant extracts (*Rheum* and *Ammi*) on the dry weight of the fungus *Helminthosporium sp.* Incubated at 25 °C for 6 days

Plants	Concentration gm/dl	Alkaloids	Phenol	Tannins
um	0.01	*0.80	0.00	0.80
	0.04	0.25	0.00	0.30
Ammi	0.01	0.45	0.10	0.80
	0.04	0.10	0.00	0.30
control	0.00	1.20	1.20	1.20
LSD	0.01	0.29		
LSD	0.05	0.21		

*Each number represents 4 replications

Table (5) effect of plant extracts (*Rheum* and *Ammi*) on spore germination of the fungus *Helminthosporium sp.* Incubated at 25 °C for 6 days

Plants	Concentration mg/ml	Alkaloids	Phenol	Tannins
Rheum	0.01	46.33	38.00	66.66
	0.04	35.00	22.33	59.66
Ammi	0.01	41.66	22.33	40.00
	0.04	26.00	11.00	34.66
Control	0.00	87.00	87.00	87.00
LSD	0.01		9.59	
LSD	0.05		7.07	

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تأثير مستخلص النباتات (*Rheum ribes ,Ammi Visinaga*) على الفطر الممرض للنباتات

He lminthosporium sp

(تاريخ الاستلام: ٢٢ / ٣ /٢٠٠٩ ، تاريخ القبول: ٨ / ٦ / ٢٠٠٩)

الملخص

عزلت وشخصت الفطريات التالية على مستوى الجنس من بذور شعير الاسود *Hordeum Vulgar* التي جمعت من أسواق مدينة اربيل والاجناس كانت*Alternaria,Rhizopus, Aspergillus, Penicillium ,Helminthosporium*, و بلغت نسبة الإصابة للفطريات المعزولة ١٤ ،١٦ ،٨ ،٩ ، و ٢٠% على التوالي. إن الفطر *Helminthosporium* سجل أعلى نسبة الإصابة ولهذا السبب أختير كأحد مسببات المرضية للعديد من النباتات لغرض السيطرة عليه من خلال مستخلص النبات لمجموعة النوورة الزهرية الخلة (*Ammi Visinaga*)Pick tooth ومستخلص الجذري للنبات*Rumer Crispus*. ان المركبات االكيمياوية التي عزلت منها كانت القلويدات والفينولات والتانينات والتي االستخدمت بتركيزين ٠,٠١ و ٠,٠٤غم/ ١٠٠مل من الوسط البطاطا دكستروز اكار، اظهرت النتائج بان مركبات القلويدات والفينولات وبكلا التركيزين ادت الى تثبيط الكلي لنمو القطري لمستعمرات الفطر او في الوزن الطري والجاف وكذلك في تخفيض الانبات للسبورات بشكل معنوي للمعاملات المذكورة.