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Study on the inhibitory effect of some wild plant extracts on the growth of pathogenic fungi in Sawa Lake

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Abstract

The Desert and Sawa Lake Research Center, a laboratory affiliated with Muthanna University in Iraq In 2022, conducted a laboratory experiment to evaluate the effectiveness of different plant extracts. : Artemisia herba-alba, Heliotropium bacciferum, Citrullus colocynthis, Achillea santolina, and Ricinus communis at different concentrations (1000 , 2000 AND 5000) ppm on the growth of the pathogenic fungi Fusarium solani, Aspergillus niger, and Trichothecium roseum. The findings indicate that the inhibitory effects of plant extracts on pathogenic fungus vary greatly.

The maximum inhibition level of the aforementioned fungus was seen in the alcohol extract of Artemisia herba-alba at a concentration of 5000 ppm, yielding inhibition rates of 1.4, 1.7, and 1.1 cm for the examined fungi. The position refers to the alcoholic extract of the Heliotropium bacciferum plant, exhibiting fungal diameters of 2.5, 3.1, and 2.7 cm. Other extracts demonstrate inferior efficacy compared to these two, while the aqueous extract of the same plant is less effective in inhibiting the growth of pathogenic fungi than the alcoholic extract. The alcoholic extract of Artemisia herba-alba conferred comprehensive protection to radish seeds against pathogenic fungus. In the presence of harmful fungi, the seed

germination rate for all three fungi attained 100%, and in the extract of Heliotropium bacciferum, the aforementioned respective concentrations are 72%, 84%, and 75%.

key words: plant extracts, pathogenic, inhibitory effect.

Introduction

Fungi are regarded as significant pests that adversely influence crops at various growth stages, diminishing both crop quantity and quality. The application of chemical pesticides is the most expedient and preferred method employed by farmers when their crops are afflicted, often overlooking the detrimental effects of pesticide usage. These effects include the adverse impact on natural biological predators of pests, numerous issues arising from their toxicity to humans and animals if these chemicals infiltrate the environment, elevated costs associated with their application, and the potential development of resistance in certain pests against pesticide efficacy, along with their considerable contribution to environmental pollution [1].

Active compounds in wild flora provide significant advantages, prompting researchers to uncover these unidentified molecules, as wild plants harbour crucial antibacterial agents whose application has expanded across numerous domains in recent years. It possesses abilities that combat numerous harmful bacteria, particularly fungus. It facilitates seed germination and mitigates fungal infections [2]. Wild plant extracts serve as an alternative to chemical pesticides by inhibiting fungal growth and reducing their proliferation, as these extracts contain metabolic compounds, some of which are poisonous to pests, particularly pathogenic fungus. Preferred characteristics include low bacterial toxicity and good specificity[3]. Recent data indicates that wild plants may possess chemicals with significant inhibitory effects on the growth of many harmful bacteria. The objective of this study was to acquire a comprehensive opportunity to examine the characteristics of some pathogenic bacteria. Wild flora and their clear identification. It exerts inhibitory effects on several harmful fungus Phenolic compound molecules secreted by wild plants act as inhibitors of the growth of pathogenic fungi by affecting the growth of fungi and reducing their pathogenicity.

Objective of the study:

1. Separating a few untamed plants from the Muthanna desert and determining the active ingredients they contain

2. Assessing the impact of wild plant active ingredients on the development and pathogenicity of certain plant-pathogenic fungus

Material and methods

1- Isolation of pathogenic fungi

The experiment was performed at the laboratory of the Desert and Sawa Lake Research Centre at Muthanna University, Iraq, utilising fungal species (Fusarium solani, Aspergillus niger, and Trichothecium roseum). The colonies, previously isolated from the waters and beach of Sawa Lake, were identified based

on their morphology, diameter, height, and microscopic features including form, size, vector, and spore type. Keys obtained from sources were utilised to study and classify fungus [4 and 5].

2- Assessing the extent of fungal pathogenicity

The levels of fungal pathogenicity were assessed using the following method [6]radish seeds were surface-sterilized with 75% alcohol for 2 minutes, subsequently rinsed thoroughly with sterile distilled water, and then placed in a 9 cm diameter petri dish containing sterile nutrient medium (Potato Dextrose Agar) at a density of 10 seeds per plate. A disc with a diameter of [7] is inoculated at the centre of the petri dish and assumes a circular form after two days. The toxins (Fusarium solani, Aspergillus niger, and Trichothecium roseum) are extracted from the peripheries of newly formed colonies cultivated on PDA media. Ratios of three replicates of treatments, considering comparisons with treatments where seeds were cultivated on nutritional media in an identical manner and without exposure to pathogenic fungus. The germination rate of the seeds was determined following a 6-day culture period at a temperature of 25±2°C.

3- Preparation of plant extracts

Wild plants (Artemisia herba-alba, Heliotropium bacciferum, Citrullus colocynthis, Achillea santolina, and Ricinus communis) were procured from the Samawa Desert following the specified methodology. Clean the plant components, then disinfect them with a sodium hypochlorite solution (0.01) for two minutes to ensure surface sterilisation, and thereafter cut them into small fragments. Weigh 1g of each of the aforementioned plants, heat 10ml of distilled water, combine it in a 250ml flask, allow it to stand for 24 hours, filter using filter paper, and centrifuge to eliminate suspended contaminants [8]. Utilise the aforementioned process for the alcoholic extracts, substituting water with 70% ethanol. Subsequently, conduct a chemical analysis of the extracts to identify active compounds (Table 1). Finally, store both the aqueous and alcoholic extracts in opaque sterile glass bottles until required [9].

Table 1: Chemical disclosure of plant extracts used in the study

Extract	detection type	result
Artemisia herba-alba	Tannins	+
	Carbohydrate	+
	Glycosides	+
	Phenols	+
	Flavonoids	-
	Alkaloid	+
	Terpenes	-
Heliotropium bacciferum	Tannins	+
	Carbohydrate	+
	Glycosides	+
	Phenols	+
	Flavonoids	-
	Alkaloid	+
	Terpenes	-
Citrullus colocynthis	Tannins	+
	Carbohydrate	+
	Glycosides	-
	Phenols	-
	Flavonoids	-
	Alkaloid	+
	Terpenes	-
Achillea santolina	Tannins	+
	Carbohydrate	+
	Glycosides	-
	Phenols	-
	Flavonoids	-
	Alkaloid	+
	Terpenes	-
Ricinus communis	Tannins	+
	Carbohydrate	+
	Glycosides	-
	Phenols	-
	Flavonoids	-
	Alkaloid	+
	Terpenes	-

4- Antagonism between plant extracts and fungi

The toxic medium method was employed to assess the efficacy of plant extracts against pathogenic fungi. Three concentrations (1000, 2000, and 5000 ppm) of each extract were prepared, and 10 ml of each concentration was added to the previously prepared PDA culture medium individually. The flask was thoroughly agitated to ensure uniformity of the mixture, after which the medium was transferred into a 9 cm Petri dish. A 1 cm diameter disc was excised from the periphery of the fungal colony, and F. solani, A. niger, and T. roseum were inoculated separately, Incubate the petri dish in an incubator at 25±2°C for seven days. Establish three replicates for each treatment and conduct comparative analyses among the treatments. After 7 days (on days 4 and 10), measure the diameter of the expanding fungus.Determine the percentage of fungal inhibition using the subsequent equation:

$$\text{Inhibition } \% = \frac{\text{Control growth rate} - \text{treatment growth rate}}{\text{Control growth rate}} \times 100$$

5- Capability of plant extracts to safeguard radish seedlings from fungal pathogens

This experiment was conducted to evaluate the efficacy of the aforementioned plant extracts in safeguarding Raphanus sativus seeds from the specified fungus. A quantity of radish seeds was washed with distilled water, subsequently sterilised with a 0.01 sodium hypochlorite solution for two minutes for surface sterilisation, then rinsed with distilled water and placed on filter paper to remove excess moisture. Subsequently, the seeds were allocated to flasks containing plant extracts at a concentration of 5000 ppm, with a certain quantity of seeds inserted in each flask, each carrying a distinct extract, The seeds were immersed for six hours and subsequently placed in Petri dishes with PDA media at a density of ten seeds per dish. Each plate was inoculated with a 1 cm diameter segment from the periphery of the fungal colony for each fungus utilised in the study, with three replicates for each treatment. A comparative treatment involved placing seeds without prior soaking in the plant extract in dishes containing the fungi, each separately [10]. The plates were incubated at a temperature of 25 ± 2 °C for one week, after which the germination percentage of radish seeds in all plates was assessed.

Results and Discussion

Pathogenicity of fungal isolates against radish seeds

The results indicated (Table 2) that the three fungal isolates, Fusarium solani, Aspergillus niger, and Trichothecium roseum, were all pathogenic. Trichothecium roseum exhibited the highest percentage inhibition of radish seed germination at 100%, followed by Fusarium solani at 80%, and Aspergillus niger at 70%, This indicates that all fungal isolates exhibit significant pathogenicity relative to the control treatment, likely attributable to the production of various metabolites and mycotoxins, which may inhibit seed germination. The pathogenic fungus may secrete one or more compounds, including carbohydrates, peroxidase enzymes, fatty acids, amino acids, or toxic substances that contribute to seed decay [11].

Table 2: Effect of fungal isolates on germination rate of radish seeds

fungal isolation	% Germination
Trichothecium roseum	**0**
Fusarium solani	**20**
Aspergillus niger	**30**
Control	**100**

Antifungal efficacy of plant extracts against pathogenic fungi

The results presented in Table 3 indicate that the alcohol extracts from each plant utilised effectively inhibit the growth of pathogenic fungi (F. solani, A. niger, T. Roseum). Notably, at a concentration of 5000 ppm, there is a significant difference observed when compared to the control treatment. At the specified concentrations, with the highest values observed in A. herba-alba plants, the fungal diameters measured were 1.4 cm, 1.7 cm, and 1.1 cm, respectively. The alcoholic extract of H. bacciferum ranked second, with the average diameter of the pathogenic fungi measuring 2.5, 3.1, and 2.7 cm, respectively. In a similar manner, the diameters of the fungi for the remaining extracts of C. colocynthis, A. santolina, and R. communis were recorded as follows: (4.5, 4.3, and 5.1 cm), (5.0, 4.2, and 4.0 cm), and (5.5, 6.4, and 6.2 cm), respectively.

All water extracts demonstrated minimal effects in comparison to alcohol extracts. A. herba-alba extract demonstrated the most significant inhibitory effect on the growth of pathogenic fungi, with diameters measuring (3.1, 2.8, 3.4 cm), followed closely by H. bacciferum. The diameters of the fungi measured were (4.5, 5.4, 4.2 cm) respectively. In contrast, the extract of C. colocynthis demonstrated a reduced impact on pathogenic fungi, which exhibited diameters of (7.7, 8.4, and 7.9 cm respectively). The aqueous extracts of A. santolina and R. communis demonstrated no significant effect on inhibiting the growth of pathogenic fungi when compared to the control treatment.

The inhibitory activity of the plant extracts of A. herba-alba and H. bacciferum may be attributed to the glycosides and phenolic compounds listed in Table (1), which include active compounds such as Santhonin and Thugone. The results align with the findings of [12 and 13]indicates that glycosides and essential oils present in plants, along with other compounds, may assist in inhibiting various microorganisms, particularly fungi. This effect is directly proportional to the concentration of the extract and the type of solvent used. The results align with what was mentioned in [14].

Table 3: The ability of plant extracts to inhibit the growth of fungi (*F. solani*, *A. niger* and *T. roseum*) after 7 days of incubation at a temperature of 25 ± 2

Extract			Fungi diameter cm after 7 days		
	Type	Concentration Ppm	*F. solani*	*A. niger*	*T. roseum*
A. herba-alba	alcoholic	1000	3.5	3.8	3.0
		2000	2.8	2.9	1.9
		5000	1.4	1.7	1.1
	aqueous	1000	5.5	5.0	6.0
		2000	4.2	3.3	4.8
		5000	3.1	2.8	3.4
H. bacciferum	alcoholic	1000	4.4	5.2	5.0
		2000	3.1	4.5	3.4
		5000	2.5	3.1	2.7
	aqueous	1000	6.0	7.3	5.9
		2000	5.2	6.0	5.0
		5000	4.5	5.4	4.2
C. colocynthis	alcoholic	1000	5.5	5.8	7.2
		2000	5.1	4.9	6.0
		5000	4.5	4.3	5.1
	aqueous	1000	8.2	9.0	9.0
		2000	8.0	8.7	8.5
		5000	7.7	8.4	7.9
A. santolina	alcoholic	1000	7.7	6.8	6.2
		2000	6.3	5.5	5.1
		5000	5.0	4.2	4.0
	aqueous	1000	9.0	9.0	9.0
		2000	8.8	8.5	8.5
		5000	8.5	8.2	8.2
R. communis	alcoholic	1000	7.2	7.9	8.1
		2000	6.4	7.0	7.0
		5000	5.5	6.4	6.2
	aqueous	1000	9.0	9.0	9.0
		2000	9.0	9.0	9.0
		5000	8.6	8.5	8.6
Control			9.0	9.0	9.0
L.S.D			0.91		

The efficacy of plant extracts in safeguarding radish seedlings from harmful fungus.

The results indicated (Table 4) that all alcoholic extracts at a concentration of 5000 ppm conferred protection to radish seeds Raphanus sativus against fungus (F. solani, A. niger, and T. roseum) during the germination phase, exhibiting significant differences from the control treatments included. The seeds were included into the fungal isolate without prior treatment with the extract, The highest germination rate of 100% was observed in the extract of A. herba-alba, while the alcoholic extract of H. bacciferum yielded germination rates of 72%, 84%, and 75%, respectively, at the

same concentration for all previously mentioned pathogenic fungi. The extracts of C. colocynthis, A. santolina, and R. communis exhibited limited efficacy, with germination percentages of 31%, 35%, and 23% respectively against F. solani. For A. niger, the germination percentages were 21%, 29%, and 20%, respectively. The lowest germination rates were observed with T. roseum, yielding percentages of 18%, 21%, and 14%, respectively. The water extracts of the identical plants had minimal to negligible effects on pathogenic fungus in comparison to the alcoholic extracts.

The observed differences in the inhibitory efficacy of plant extracts against pathogenic fungus may result from the diverse nature and concentrations of the active chemicals and components involved. A. herba-alba and H. bacciferum contain glycosides, tannins, and alkaloids, which have a chemical composition and inhibitory activity that influence the growth and survival of certain fungi. The physiological effects of the effective compounds that inhibit fungal growth may stem from a reduction in carbohydrate and total protein content, while simultaneously enhancing the efficacy of the enzymes dehydrogenase, succinate dehydrogenase, and fumarase, and diminishing the activity of the enzyme catalase in fungi. This leads to increased toxicity and a subsequent reduction in fungal growth rate, corroborating the findings reported by [15].

Table 4: Effect of plant extracts on germination of radish seeds with and without fungi (*F. solani*, *A. niger*, and *T. roseum*) after 7 days of incubation at a temperature of 25 ± 2.

Extract			% Seed germination after 7 days and in the presence of fungi %			% Seed germination without the presence of fungi
			F. solani	*A. niger*	*T. roseum*	
A. herba-alba	alcoholic	1000	30.50	41.10	36.60	100
		2000	69.50	72.40	68.50	
		5000	100	100	100	
	aqueous	1000	2.50	3.10	4.70	100
		2000	6.60	7.20	8.80	
		5000	11.00	11.00	13.00	
H. bacciferum	alcoholic	1000	25.10	30.20	27.20	100
		2000	48.20	50.3	50.60	
		5000	72.00	84.00	75.00	
	aqueous	1000	0.00	2.00	0.00	100
		2000	4.10	6.10	4.00	
		5000	9.00	11.00	9.00	
C. colocynthis	alcoholic	1000	10.20	9.00	3.00	100
		2000	17.40	14.40	11.20	
		5000	31.00	21.00	18.00	
	aqueous	1000	0.00	0.00	0.00	100
		2000	2.20	2.50	2.10	
		5000	7	8	6	
A. santolina	alcoholic	1000	11.20	10.60	9.00	100
		2000	18.50	15.50	13.30	
		5000	35.00	29.00	21.00	

	aqueous	1000	0.00	0.00	0.00	100
		2000	3.30	2.10	2.20	
		5000	8.00	6.00	6.00	
R. communis	alcoholic	1000	7.00	6.30	3.50	100
		2000	13.20	10.10	7.60	
		5000	23.00	20.00	14.00	
	aqueous	1000	0.00	0.00	0.00	100
		2000	1.00	2.20	0.90	
		5000	4.00	6.00	4.00	
Control			0	0	0	100
L.S.D			0.87			

Conclusion

The current study concludes that natural plants, particularly desert flora, exert a significant inhibitory effect on pathogenic fungi due to their chemical constituents that restrict fungal growth and induce mortality. Experimental results demonstrate that all alcoholic extracts exhibited a pronounced and substantial efficacy in inhibiting fungal proliferation compared to the aqueous extracts of the same plants.

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