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ANTI-FUNGAL ACTIVITY OF *ALTHAEA OFFICINALIS* L. TISSUE CULTURE EXTRACTS

Baan M. Twaij and Adel H. Alwan

Department of Biology, College of Science, University of Mustansiriyah, Baghdad, Iraq.
E-mail : msc.baan@uomustansirihyah.edu.iq, phdadel@uomustansirihyah.edu.iq

Abstract

This study was conducted to investigate the effect of the alcoholic and water extracts of the tissue plant to inhibiting the growth of the pathogenic fungi *Rhizoctonia solani* and *Fusarium oxysporam*. The results showed that the alcoholic extract was significantly higher inhibition compared to the control plant, 100% inhibition of 60 mg / 100 ml, 80 mg / 100 ml on both fungi. The effect of hot water extract was 84.5% and 90% for *Rhizoctonia solani* at 60 mg / 100 ml, 80 mg / 100 ml respectively. 86.1% and 89.7% for *Fusarium oxysporam* at 60-80 mg/100 ml, respectively.

Key words : *Rhizoctonia solani*, *Fusarium oxysporam*, tissue culture.

Introduction

The fungal diseases are important for human life because of their direct impact on their economic and food resources, the most important crops are vegetables. The common fungus of these crops is *Rhizoctonia solani*, which is highly proliferative and is widespread and is a soil fungus that causes damping off and mild rot rot, in its primitive stages (Berbee, 2001). Another fungus is *Fusarium oxysporam*, which affects vegetable crops that cause widespread root rot. In addition to being a disease causing plants, fungus is useful in the recycling of important elements in nature by living on the remains of organic matter (Smith and Read, 1997).

Because chemicals are the basis for the control of pathogenic fungi of plants and their effect on the pollution of the environment and the impact on human and animal health, there has recently been a need to use safe alternatives, the most important of which is the use of secondary metabolites of medicinal plants that have a direct effect on those organisms (Shameel, 1990). Natural plants are still an essential element in human life and we use many of them in our daily lives, including *Althaea officinalis* L., which belongs to the family (marshmallows). It is one of the medicinal plants used since the ancient times. The leaves of the plant *Althaea officinalis* L., Medical treatments is a perennial herb 60 to 120 cm, the stem is erect and has short engraved leaves. *Althaea officinalis* L. is a medicinal plant used to treat certain conditions such as blood vessels, rhinitis, oral cavity, gastric ulcers, platelet aggregation, cystitis, and annoying coughing (Ogunniyi, 2006; Shah *et al*., 2011), the contents or components of the seal plant.

The marshmallow plant contains 30% of the salty substances, which are the substances with palliative properties. It also contains sucrose, lecithin, citrate, asparagine, 37% and 11% gels, flavonides and phenolic. The amount of concentrations of the material in the roots and leaves and peak with the beginning of flowering and flowers and flowers contain a large proportion of salts and contains starch and sugars pentagram and hexa and pectin and tannins and traces of essential oil pilot and also contains Malfacin, Asparagine, Hepecin, MalvaYen, Malvidol, Malvan (Blumenthal *et al*., 2000).

It is preferable to apply different techniques such as plant tissue culture technology and it is possible to produce plants with the required specifications. This technique allows an increase in biomass or production of metabolic compounds using several techniques in callus or morphogenetic culture (Sutovska *et al*., 2009) these include Bioreactor, Micropropagation and genetic engineering (Hage-Sleiman *et al*., 2011). Plant tissue culture can produce economically viable whole plants free of disease without dependence on a particular season.

Materials and Methods

Isolation of fungi

The fungal isolates were obtained using the Brown (1999), method, the soil drop method, which is to dissolve one gram of soil contaminated with fungus, in 250 ml of distilled water and well prepared for homogenization for two minutes. Addition of 1 ml vaccine to the water solution, 25^0C for two days after which a piece of it is taken and transferred to the center of the solid PDA medium in dishes and incubated at 25° for a week to obtain pure culture.

Collection of plant parts

The plants were collected from the Mustan Siriyah University Gardens in addition to collecting parts of them from the Rashidiya orchards in Baghdad.

Preparation of the plant tissue culture medium

The solid media medium (MS) was prepared by dissolving all the substances in the composition in a quantity of distilled water, agar adding by 8g / L and 30g /L sucrose and then placed on a hot plate magnetic stirrer for moving media and boiling, PH 5.6-5.8 recorded. Fill the medium in vials with dimensions of 8 × 2.5cm by 10ml / vials and media sterility with Autoclave for 20 min.

Sterilization of plant parts

Sterilization of leaves and stems

Mature *Althaea officinalis* L, washed with running water for 15 min, then submerged with ethanol 70% concentration for 30 sec and then washed with sterilized distilled water several times and then submerged with sodium hypochlorite solution with 1.5% added Twee- 20 as a diffuser for 10 min, the plant parts were washed several times with distilled water to remove the effect of the sterile material and then cut off the plant parts distributed in pre-prepared seedling.

Development of callus

The plant parts separated the leaves and stems from the sterile plant and were planted in the pre-prepared solid MS medium, which were supported by plant hormones. The first combination to develop the callus from the stems and leaves of the plant is 2.4-D [0, 1, 2, 3 mg / l] and BA [0, 0.5, 1, 1.5mg / l] the second combination was IAA [0, 1, 2, 3 mg / l] and BA [0, 0.5, 1, 1.5 mg/l].

The leaves and stems were separated from the adult plants by 10 replicates of each type. The induced callus was maintained at 3-4 weeks. All seedlings were incubated at 25 ± 1° C in light and dark conditions.

Study of the effect of plant extracts on fungi

To study the effect of plant extracts prepared on fungus and then follow method (Mondall *et al*., 2009), which is the measurement of the diameter of the fungal colony growing on the seed medium added to the extract after the work of the concentration of the extract after sterilization through the millipor filter on a diameter of 0.22 Mn and compare the resulting measurements with the control dish Which contains mushrooms on the center of the plant without extract and then incubate dishes at 25°C for a week and measured the growth of mushrooms daily and the application of the measurement of the rate of inhibition percentage.

Planting medium

The PDA was used to isolating and purification of fungi and to examine the effect of the plant extracts on the fungus. It is prepared by melting of 200 gm of potatoes plus 20 gm of sugar and 20 gm of sugar dextrose per liter of distilled water. Then autoclaved under pressure / 15 / 1b / 20 minutes and is poured into dishes and kept for use.

Detection of phenols

The phenolic material is detected in the plants with 30 gm of the plant powder in 1 ml of water and added up to 3 drops of 2.5% of the hydrolysis of the ferric chloride. The color change and sediment formation are observed and most phenols produce red, blue or purple (Smetanska, 2008).

Detection of Anthraquinone

It is an aromatic organic compounds detected by adding 5 gm of powder in a test tube and 2ml of dichloromethane is added and re-extracted several times until it becomes colorless. Absorption was measured along a 420 nm wavelength using the spectrophotometer. (Shihata, 1951).

Detection of Flavonoids

Flavonoids are tested and found in the extract with 1gm extract in 4ml of ethanol and acetone at 3: 7 v / v for 1 h at 37^0C (Harbone, 1984). The sediment is reclaimed under the same conditions. 1 mol of NaOH is added to the mixture Absorption is calculated at 506 nm with the optical spectrometer.

Detection of Glycosides

According to the (Ramachandra and Ravishankar, 2002) method used in the examination of the classics, and then mix two parts of the flask with the plant extract as well as boiling in a water bath for 15 minutes until the appearance of red color indicates the positive examination.

Detection of tannins

In this test prepare the ferric chloride solution with

1% concentration of 1gm of $FeCl_2$ in 100 ml of distilled distilled water. In order to determine the effect of tannins and positive examination, the appearance of greenish color is observed when mixing the solution with an equal size of the plant extract (Ramachandra and Ravishankar, 2002).

Preparation of plant extracts

Preparation of the hot water extract

The hot water extract of the plant is prepared according to Smetanska (2008) method with some modifications. 100 gm of the tissue is added and 500 ml of sterilized sterile distilled water is added to the incubator for 30 minutes at 30^0C. Then leave the solution for 72 hours at room temperature then filtered using filter papers. The filtered solution shall be placed in centrifugal tubes 3000 cycles for 5 minutes, then put in rotary evaporator to concentrate the extract and save for use.

Preparation of alcohol extract

The same method is used to prepare the hot water extract but replace the distilled water with the concentrated ethanol. 80% (Alaa, 2016)

Statistical analysis

The results were analyzed using a very significant difference (L.S.D.) with a probability score of 0.05 to test the significance of the differences between the results.

Results and Discussion

Introduction callus

Planting of plant parts leaves and stems separated from the adult plant had an effect on the percentage of callus development. As the callus was not induced from the stems and leaves of the plant of the seal and for all interference in light and dark conditions at good rates, the response of the leaves to the callus development was relatively superior in giving the response to the callous development at the concentration 1mg / l 2,4-D and 0.5mg / l BA, where the response was very weak compared to the second combination, despite the attempts to maintain the calcination of the leaves and was maintained every 3 weeks and by 10 replicates, so they were removed from the results and work and adoption of the second combination of the development of calcareous N stems and leaves.

Where the stem response was superior in giving the highest percentage for the development of callus and at the concentration of 0.5mg / l BA and 2mg / l IAA and adopted this combination in the maintenance of callus induced from the legs of the plant and the return of the seal and was maintained every 4 weeks and by 10 replicates, The callus to develop callus very few and certain interventions, and took a longer period of time to develop compared to the legs, and the quality of callus was needed to sustain continuous intervals of time and was Callas color brown and colored the center of the

Table 1 : Development of callus from the stems of the plant and the seal of the seal.

Concentration of BA Mg/l	Concentration of IAA Mg/l				Mean
	0	1	2	3	
0	0	0	10g	35fg	11.25d
0.5	8h	34 de	52c	88a	45.75a
1	13h	38ef	40d	63b	35.5b
1.5	9h	20h	42fg	30d	19.25c
Mean	6.25d	18c	33.75b	53.75a	

Table 2 : Chemical compounds test in plant extract.

No.	Chemical com.	Hot water extract	Ethanolic extract
1	Glycosides	+	+
2	Anthraquinon	+	+
3	Tannins	+	+
4	Flavonoid	+	+
5	Phenolic	-	+

Table 3 : Effect of plant extract (hot water, ethanolic) on fungi growth.

Extract	Percentage of inhibition mm				
ConcentrationMg/ml	*Rhizoctonia solani*		*Fusarium oxyporum*		LSD P≤0.05
	Hot water. extract	Ethanolic extract	Hot wat. extract	Ethanolic extract	
2.5	23.00	59.37	31.90	72.00	8.78
5	27.10	71.55	43.62	75.19	12.10
10	40.61	75.17*	47.18	84.41**	14.04
20	49.99	83.50**	58.59	88.52**	9.58
40	66.80	88.92***	72.10*	92.19***	11.11
Control	0.00	0.00	0.00	0.00	0.00
LSD P ≤ 0.05	6.14	9.77	11.13	15.79	

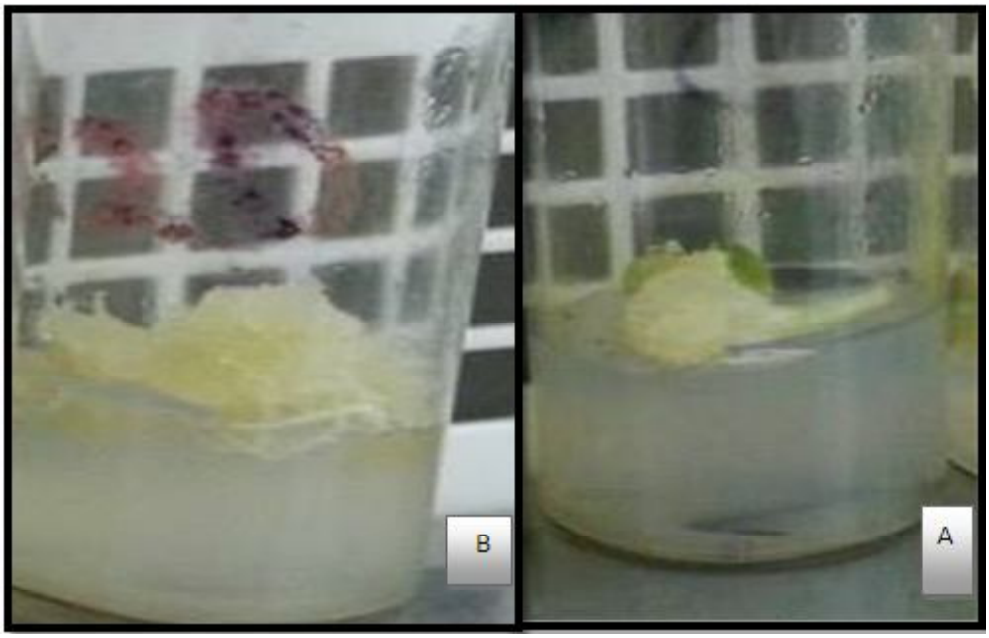

Fig. 1 : Stimulates the callus from the stalks of the plant and the seal: The figure of the callus induced from the stems of the plant and the seal of the seal before its maintenance (A(B) the image of the callus induced from the stalks of the plant and the seal after sealing.

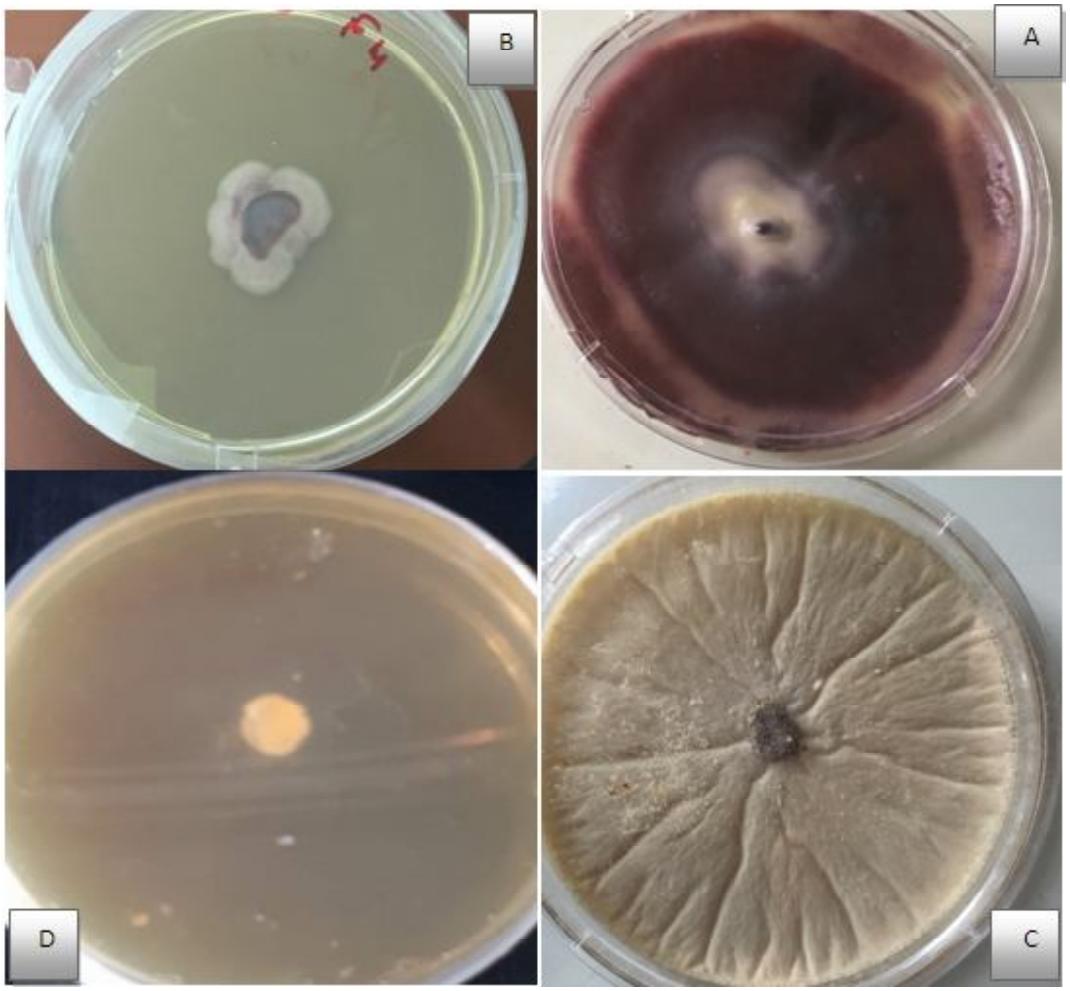

Fig. 2 : Effect of plant extracts on fungi: a) *Fusarium oxyporum* control. b) *Fusarium oxyporum* treated with ethanolic plant extract, c) *Rhizoctonia solani* control d) *Rhizoctonia solani* treated with ethanolic plant extract

plant brown as a result of the secretion of phenolic materials so it was not referred to the table for lack of access On the results of c Dah for the development of callus and were excluded from subsequent testing (Table 1, fig. 1).

Detection of active chemicals in the plant extract of the seal

Tests on plant extracts showed several secondary metabolites of significant efficacy as shown in Table 2.

Effect of plant extracts on plant pathogenic fungi

The results of the test of the effect of the crude extracts of the plant exceeded the alcoholic extracts by the inhibitory effect of the pathogenic fungi and the two types used in the experiments of this research as shown in table 3 and fig. 2.

The studies indicate that the plant of the seal of the seal of the medicinal plants important for this was selected for the experiments of this research and the work of plant extracts with two types of alcoholic and aqueous to know the effect of the type of solvent on the extracted extract gave the alcoholic extracts strong inhibition of the used users, the effect of the extract of the plant and the answer to the conclusion to the presence of compounds (Hartmann *et al*., 2002) showed that these compounds have an effect on the growth of fungal and their evolution through its effect on the cellular wall and plasma membrane. (Hussein *et al*., 2015). Noted that the volatile compounds found in the extract o conidia) and fungus mushrooms *Fusarium oxysporum*.

This explains that water extracts are less soluble than most active substances (phenols, terpenes, flavonoids, etc.), which have inhibitory effect on the growth of pathogenic fungi, while organic solvents have a great ability to dissolve many of the active substances of this plant. This corresponds to (Pornphan *et al*., 2016; Dwaish *et al*., 2018).

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