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EVALUATION OF THE EFFECTIVENESS OF THE ALCOHOLIC EXTRACT OF SOME DESERT PLANTS IN CONTROLLING THE *RHIZOCTONIA SOLANI* FUNGI, CAUSER TO THE SEED ROT DISEASE AND THE DEATH OF OKRA SEEDLINGS *IN VITRO*

Asmaa A. Al-Araji and Fadhal A. Al-Fadhal
Agriculture College, University of Kufa, Iraq.

Abstract

This study was carried out to evaluate the efficiency of the alcoholic extract for six types of desert plants (Citrulus, Tamarix, Camel Thorn, Taraoui, Cactus and Malva) inhibiting the growth of *R. solani* causer to the seed rot disease and the death of okra seedlings in vitro. The concentration toxicity (5, 10, 20, 25) % of plant extract was tested to inhibit the growth of pathogenic fungi. Results showed superiority of Malva extract, the percentage of inhibition of the fungus growth was 79.5 and 62.4% at the first and second concentrations, and the percentage of inhibition of the fungus reached 75.4, 77.3, 78.1 and 83.5% respectively, with respect to the Malva extract. Desert plant extracts as well as the superiority of the inhibition of the fungi growth when preserved in commercial corn oil, where the preserved Citrulus extract in oil gave the highest percentage of inhibiting the fungi, which amounted to 84.6 and 81.6% for the first and second concentrations, and the percentage of inhibition of fungi reached 77.8, 83.0, 84.9 and 85.7, respectively as for the Malva extract preserved in corn oil. Which refers to the effectiveness of both alcoholic extract and an alcoholic extract preserved in oil of the Citrulus and Malva plants in the controlling of the R. *Solani* fungi, causer to the seed rot disease and the death of okra seedlings and the possibility of using it in the controlling of the pathogen.

***Keywords*:** Seed rot, Okra, *Rhizoctonia solani*, alcoholic extract.

Introduction

The okra crop (*Abelmoschus esculentus* L.) is an important summer vegetable crop in Iraq (ICSO, 2016), It belongs to the Malvacea family and believes that its native origin is Sudan, Egypt, Abyssinia and Eritrea (Matloob *et al.,* 1989). Okra is grown in various parts of the country with the aim of benefiting from it because of its great economic importance and it is important in the diets of the Iraqi family as its fruits contain a high nutritional value as it has a percentage of protein estimated at 16.17%, 2.07% fat, 60.90% carbohydrates, energy 326.93% in addition to important nutrients such as zinc 51, iron 371, as well as calcium 107 ppm (Hussain *et al.,* 2010). Okra production in Iraq suffers from loss of yield as a result of facing many biotic and abiotic factors (Stephan and Abu-Gharbieh, 2010). Pathogenic fungi attack the seeds and seedlings of okra, causing their rot and seedlings death in the early stages of their growth. The most important of these fungi are *Rhizoctonia solani* and *Fusarium solani* (Sultana et al., 2005; Esam; and Al-Obaidy, 2009; Santos *et al*., 2012). Many of the means used to control the causes of fungal pathogens Among the most important of these means are chemical pesticides, but the accumulation of these pesticides in the soil it leads to the death of large numbers of neighborhoods in the soil, and it can also leak into water sources and affect aquatic organisms and transmitted through the food chain of other living organisms (Al-Saadi, 2002). Therefore, alternatives to chemical pesticides were used, and among the best alternatives are plant extracts, which are safe for humans and animals and are environmentally friendly as they inhibit pathogens and stimulate plant growth, so they were used as an alternative to the intensive use of chemical pesticides (Yassin *et al.,* 2013). Because of the limited studies that relate to the use of desert plant extracts in the growth of some pathogenic fungi that cause seed rot and seedling diseases, the study aimed to evaluate the efficiency of the alcohol extract to control the fungi that cause the death of the seedlings of okra and the rot of its seeds And its impact on plant growth standards and controlling the pathogens of seedlings fall diseases using alternative methods of chemical pesticides and limit their use because they cause environmental pollution which may produce resistant fungi frequently used.

Materials and Methods

In the study, isolates of the pathogenic fungi of *R. solani* was used obtained from the laboratories of the protection department - University of Kufa, originally isolated from the okra seedling by method of live plant traps.

Alcoholic Extract Preparation

The method of Shtayeh and Abu ghadeib (1999) was followed:

20 g of dry matter powder were taken for the leaves of the plant (Citrulus, Tamarix, Camel Thorn, Taraoui, Cactus and Malva) separately, and put in a glass beaker 500 ml and add to it 200 ml of ethyl alcohol with a concentration of 70%, it was then placed in a shaker incubator at 35 °C for 24 hours and it was filtered using medical gauze, then discarded by the centrifuge at 3000 rpm for 10 minutes, then it was dried in the oven (Oven at 40 °C.), then kept in dark and tight tubes in the freezer at a temperature of -18 °C until use.

Preparation of plant extract concentrations

The concentrations necessary for the tests were prepared by dissolving 2 g of powder and the preparation according to the method in the previous paragraph for each study plant in 10 ml of phosphate buffer saline solution (PBS) to obtain a solution (Stock solution) and using the general dilution law C1V1 = C2V2 concentrations (10, 15, 20, 25) were prepared for each plant and separately, with a comparison focus (zero) and sterilized using the Millipore filter with holes of 0.22 micrometer.

Alcoholic extract effect test: The technology of the nutritional medium was used to study the toxic effect of alcoholic plant extracts where concentrations (0, 10, 15, 20, 25)% were used with the sterile PDA medium for each concentration used and for each plant extract separately and repeated each treatment 3 times and pollinated each dish with a 0.5 cm disk From the fungus R. solani, the comparison treatment was without extract and with three replicates as well and then incubated at a temperature of 25 ± 2 ° C for a period of 5 days. The effect of the extracts on the studied fungi was tested by calculating the percentage of inhibition according to the Abbot equation for the inhibition, the Abbot equation by (Shaaban and Al-Mallah, 1993):

$$\% \text{ of inhibition} = \frac{\text{Rate of fungal growth diameters in comparison} - \text{Rate of fungal growth diameters in treatment}}{\text{Rate of fungal growth diameters in comparison}} \times 100$$

Test the effect of alcoholic extract preserved in oil on the fungi used in the study in Petri dishes

Nutritional medium technology was used to study the toxic effect of vegetable alcoholic extracts preserved in oil in concentrations (0, 10, 15, 20, 25)% with sterile PDA medium for each concentration used and for each plant extract separately and repeated each treatment 3 times and pollinated each dish with a diameter disk 0.5 cm from the fungi of *R. solani*. As for the comparison treatment, it was without extract and with three replicates as well, and then incubated at a temperature of 25 ± 2 °C for a period of 5 days. The effect of extracts kept in oil on the studied fungi was tested by calculating the percentage of inhibition according to the inhibition equation.

Statistical Analysis

All the experiments of the study were carried out according to the complete randomized design (C.R.D) and the mean of the coefficients were compared according to the method of the least significant difference of L.S.D and at the probability level of 0.01 for the implemented laboratory experiments (Al-Rawi and Khalaf Allah, 2000), and the results were analyzed using the statistical analysis program (Genstat12th Edition).

Results and Discussion

Pathogenicity test The results of the pathogenic ability of *R. solani* isolates showed their ability to reduce germination rate of okra seedlings, reaching 40% compared to the control treatment of 100%, which indicates that this fungus has high pathogenicity and a role in the ability to cause infection and disease development. Where the fungi of *R. solani* attack the seeds of the host that leads to the killing of embryos, as well as the secretion of many enzymes that analyze cellulose and chitin, so the fungi of *R. solani* is one of the most important causes of seed rot and seedling death in the world (Agrios, 2007).

Table 1 : R pathogenicity test of fungi of *R. solani*

Treatment	% Germination
Rhizoctonia solani	40
Control	100
LSD=0.05	16.03

Effect of some desert plant extracts on the percentage of growth of *Rizoctonia solani* in Petri dishes:

The effect of alcoholic extract on the percentage of growth of *R. solani*

The results indicate, as shown in Table (2), that there were no significant differences at the probability level of 0.05 among all the concentrations studied for the alcoholic extract (10, 15, 20, 25)% and there was an inhibitory effect of the rate of concentrations according to the study results on the growth of pathogenic fungus *R. solani* as it became clear also the rate of extract of all studied plants had an inhibitory effect on the growth of the pathogenic fungi of *R. solani* where the rate of Citrulus plant extract and the rate of Malva plant extract exceeded the rest of the studied plants where the percentage of inhibition of the fungus reached 63.07% and 78.57%, respectively, when a study of the effect of interaction shows that the concentration of 10% for Citrulus and 25% for the Malva plant extract has given the highest rate of inhibition of the growth of fungi of *R. solani*, where the percentage of inhibition of 95.5% and 83.5% respectively. The effect of the alcoholic extract of plants on the growth of *R. solani* has been reported in many studies. Kareem (2000) found that the raw alcoholic extract of the flower buds of the carnation plant, with a concentration of 1000 ppm, was inhibiting the growth of the fungi of *R. solani*. It also indicated (Mazzola *et al*., 2001) that the use of powder and alcohol extract of the cauliflower and *Brassica oleracea capitata* leaves inhibited a number of fungi that caused the root rot of the broad bean plant, including the fungus *R. solani*.

The effect of alcoholic preserved in oil on the percentage of growth of fungi of *R. solani* in Petri dishes

The effect of preserved alcoholic extract in oil on the percentage of growth of fungi of *R. solani*

The results of Table (3) indicate that there were no significant differences at the probability level of 0.05 between all concentrations studied for the alcoholic extract preserved in oil (10, 15, 20, 25)%, there was an inhibitory effect on the rate of concentrations according to the results of the study on the growth of pathogenic fungi of *R. solani*. It was also found that the extract rate of all studied plants had an inhibitory effect on the growth of the pathogenic fungi of *R. solani*, the rate of citrulus extract and the rate of citrulus extract were superior to the other studied plants, as the percentage of inhibition of the fungus reached 81.3% and 82.8%, respectively. When studying the effect of interaction, it was found that the concentration was 10% for citrulus extract and the concentration of 25% for the Malva plant extract gave the highest rate of inhibition of the growth of the fungi of *R. solani*, the percentage of inhibition amounted to 84.6% and 85.7%, respectively. (Kosalec *et al*., 2005) indicated that there were significant differences between the effect of anise extracts and feverfew oil against the fungal skin and yeasts as anise oil appeared inhibitory efficacy against these fungi compared to aqueous extracts. Inhibition of fungi has been indicated by storing the extract in oil in several sources, (Batta, 2016) and (Mcclatchie *et al*., 1994).

Table 2 : The effect of alcoholic extract of desert plants on the percentage of growth of *R. solani* in petri dishes

% Concentration	Inhibition% Plants						Rate
	Citrulus	Camel Thorn	Tamarix	Taraoui	Cactus	Malva	
10	79.5	9 .12	8.1	23.7	48.5	75.4	41.35
15	62.4	12.6	8.2	15.2	45.1	77.3	36.8
20	59.7	18.5	9.4	17.9	49.9	78.1	38.58
25	50.7	30.7	8.7	9.9	53.4	83.5	39.81
Rate	63.07	18.67	8.6	16.67	49.22	78.57	
LSD=0.05		Concentration= 7.22		Extracts= 8.84		Interaction= 17.69	

Table 3 : Effect of alcoholic extract preserved in oil in the percentage of growth of fungi of *R. solani* in Petri dishes

% Concentration	Inhibition% Plants						Rate
	Citrulus	Camel Thorn	Tamarix	Taraoui	Cactus	Malva	
10	84.6	31.1	33.8	25.5	38.1	77.8	50.3
15	81. 6	31.6	31.2	24.6	49.0	83.0	52.0
20	79.7	41.9	37.0	22.7	55.7	84.9	49.1
25	79.2	44.6	40.6	3.5	61.9	85.7	53.5
Rate	81.3	37.3	35.6	19.1	51.2	82.8	
LSD=0.05		Concentration= 8.99		Extracts= 1.01		Interaction= 22.01	

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