An in Vivo Essay of Effect the Seeds Extracts of Castor Plant in Growth of the Two Pathogenic Fungi Fusarium Oxysporum and F. Solani

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Abstract

The research was conducted to evaluate the capability of the ethanolic, water and oily extracts of castor seeds (Ricinus communis L.) in inhibition the radial growth of the two pathogenic fungi Fusarium oxysporum and F.solani.

Results demonstrated high inhibition efficacy of the ethanolic and water extracts on the growth of the studied fungi. But this efficacy differed per the concentrations. Whereas, to the ethanolic extract, at the concentrations (2, 4, and 6 mlg/ ml), the percentages of inhibition ratio to the fungus F. oxysporum were (55.6, 62.0 and 70.1%), respectively, after seven days post incubation, and these percentages attained to 45.9, 57.2 and 67.4 %, respectively, to the fungus F.solani.

While at the same concentrations to the water extract, the percentages of inhibition ratios were (70.8, 74.3 and 85.0%), respectively, to growth of the fungus F. oxysporum, and (56.0, 71.0 and 86.3 %) to the fungus F.solani, respectively.

On other hand, results of the oily extract showed high inhibition ability, where the inhibition rate was 100% when the extract was used by the two concentrations 50 and 75 µl for both two studied fungi.

Key words: *Ricinus communis L., Fusarium oxysporum, F.solani, ethanolic extract, oily extract and water extract.*

I INTRODUCTION

Castor plant (*Ricinus communis* L.) is one of the major aromatic plants which have an important medicinal value to many diseases [1]. This plant belong to Ephorbiaceae, its origin is at Ethiopia in Africa.

Castor plant grows on wide range in all equatorial and semi equatorial regions, in addition to warm moderate regions around the world [2].

This plant is perennial shrubbery, has a fast growth, in some cases, castor grows as a 6 meter long tree. Leaves are big, palmate, lobed to about 5-12 part, green or red in color, its diameter ranges between 30 – 60 cm [3].

Castor is monoecious unisexual, its flowers are small, greenish to yellowish in color, locate in inflorescences [4].

Male flowers locate in the middle part of the branch, yellow in color, where female flowers locate at apex of the higher part, red in color.

Fruit is big, its dimensions ranged between 8 -18 mm as length, and 4- 12 mm as width. Three seeds were found inside each fruit [3].

Seeds are ovule, small, with large cavity. When the seeds ripen, the cavity loses its water, then protein reserves accumulate inside it, and divide to several cavities, each of them develops to castor –oil aloron.

Castor – oil consist of stearic acids, dihxdroxy stearic, linoleic oleic in addition to small amount of ricinolraic [5].

On other hand, the fungus *Fusarium* considers one of the most importance fungi which infect wide range of plants and economically crops, and cause many diseases to these plants as the vascular wilt and root rot in both open fields and protected cultures [6] [7].

Fusarium is facultative pathogenic fungus, persist as saprophytic or pathogenic on plant tissues, have a wide range of hostes [8] [9]. The two species *F. oxysporum* and *F.solani* are the main responsible of the most of *Fusarium* diseases [10], [11], [12].

Because of the medical importance of castor plant and its of many active compounds that inhibit the pathogenic fungi, in additional to the huge damage that caused by each of *F. oxysporum* and *F.solani* in our coastal region, the present study was performed to evaluate the in vitro inhibition capability of extracts of seeds of castor plant against *F. oxysporum* and *F.solani*. Also to detect the best solution and its role in increasing or decreasing the inhibition capability, so it was studied effect each of ethanolic, water and oily extracts in growth of *F. oxysporum* and *F.solani*.

II MATERIALS and METHODS

A. The fungal isolates

This search was performed at laboratories of Science Faculty in Tishreen University, Syria, during 2016- 2017.

The two fungi *F.oxysporum* and *F.solani* were isolated from roots and stems of pepper plants which showed the symptoms of infection of vascular wilt and root rot as yellowing and general wilt.

Depending to several keys as [13] [14] [15] [16] [17], *F. oxysporum* and *F.solani* were identifying. Fungal isolates cultured on PDA medium (potato dextrose agar), and incubated at 26±2°C for 7 days, finally saved on 4°C until use.

B. Preparation the castor extracts:

(1 Ethanolic Extract

Seeds of castor plant were collected and brought to the laboratory. 20g of the seeds were gritted to become as a powder. The powder put into Soxhlet extractor with 200 ml of ethanol at 40°C for 24 hours [18]. After 24 hours, it was filtered by filtering paper Whatman No. 1. This procedure was repeated several times to get enough amount of extract. At last, the extract was dried by rotary evaporator at 45°C to get the extract in dry form. It saved at 4 °C in darkness closer containers until use.

.(2 Water Extract

About 100g of castor seeds were added in electric mixer with 1L of sterile distiller water for 15 minutes. Then the mixer removed to Hot Plate Magnetic Stirrer at 45- 50 °C for 48 hours, then filtered by filtering papers.

The extract was moved to the centrifuge and centrifuged fast 3000 cycle / minute, this procedure repeated three times to remove settlings. The final extract put in water bath 60°C. Finally it saved on 4°C until use.

3. Volatile Oil

To obtain the oil from castor seeds, method of Water distillation as in re. [1] was followed.

100 g of dry seeds were gritted and put into the Clevenger flask with L distiller water. Water distillation was done by heating the flask for 3 -4 hours to get the biggest amount of oil.

To separate the oil, separator funnel was used, where extraction solution was added in the funnel, and let it until the oil layer raise to the surface. The oil then saved at 4 ° C until use.

C. Effect of ethanolic and water extracts on radial growth of the fungi F. oxysporum and F. solani:

Several concentrations of each extract (2, 4 and 6 mlg/ ml) were prepared in glass flasks. PDA medium prepared and infused in Petri dishes after adding the concentrations by 4 replicates of each concentration/ extract. The control treatment was prepared without extract.

A center of each dish was inoculated by 1 cm piece of 7 days old colony of *F. oxysporum* or *F. solani*. Dishes were incubated at 26±2°C for 7 days.

Daily records were taken, and percentage of inhibition was calculated using the following counteraction:

Inhibition Rate %= [(mean of control colony diameter– mean of treatment colony diameter)/ mean of control colony diameter].

D. Effect of oily extract on radial growth of the fungi F. oxysporum and F. solani:

The oil was prepared by several concentrations (25, 50 and 75 µl), and added to PDA medium, then the center of each plate inoculated by the fungus *F. oxysporum* or *F. solani*, and incubated at 26 °C ± 2.

Four replicates were prepared of each treatment. Percentages of inhibition ratio were calculated as above.

E. Statistic Analysis

The significant differences between treatments were compared by One–way repeated analysis of variance (ANOVA). LSD were performed post comparisons. The level of probability was set at P>0.05.

III RESULTS AND DISCUSSION

A.) Effect of ethanolic extract on radial growth of the fungi F. oxysporum and F. solani

Results showed significant effect of ethanolic extract by all used concentrations (2, 4 and 6 mlg/ ml) on growth of the studied fungi. The effect differed per concentration, the highest inhibition ratio associated with 4 and 6 mlg/ ml. Whereas, It was noticeable an existence of direct relation between inhibition rates and increasing the concentration (Table 1). Significant differences between treatments were increased by time. In the seventh day, the fungal growth in control treatment was 8.7cm. The inhibition ratio attained 70.1 % and 67.4 % at the concentrations 6 mlg / ml, to the fungi *F. oxysporum and F. solani*, respectively. Whereas, at 4 mlg / ml, the ratio become 62.0 and 57.2% to the fungi *F. oxysporum and F. solani*, respectively.

The inhibition ratio of fungal growth decreased a little at 2 mlg / ml (55.6 and 45.9%) (table 1).

Table (1): Effect of ethanolic extract of castor seeds on the two pathogenic fungi *Fusarium oxysporum* and *F.solani*

Treatment	Inhibition ratio (%) ± SD		LSD 5%
	F.oxysporum	*F.solani*	
2 mlg / ml	55.6 ± 4.10b* A#	45.9 ± 2.23cB	4.23
4 mlg /ml	62.0 ± 9.06abA	57.2 ± 3.54bA	6.0
6 mlg /ml	70.1 ± 2.11aA	67.4 ± 1.28aA	3.4

Control	0c	0d	-
LSD 5%	9.11	8.15	-

*Means followed by different letters in a column (concentration) indicate significant differences, # means followed by different uppercase letters in a line (fungus) indicate significant differences

B.) Effect of water extract on radial growth of the fungi F. oxysporum and F. solani

Results showed effect of the water extract by used concentrations on inhibition the growth of the two fungi, it was notable that the concentration 6 mlg / ml was the highest effective (85 and 86.3 %) for the *fungi F. oxysporum and F. solani*, respectively (Table 2).

Results demonstrated that water extract caused more higher inhibition ratio than the ethanolic extract, and its effect was more stronger on the fungus *F. oxysporum* than on the fungus *F. solani*, without significant differences in all treatments (table 2).

Table (2): Effect of water extract of castor seeds on the two pathogenic fungi *Fusarium oxysporum* and *F.solani*

Treatment	Inhibition ratio (%) ± SD		LSD 5%
	F.oxysporum	*F.solani*	
2 mlg / ml	70.8 ± 5.21 b* A#	56.0 ± 1.02 c B	7.11
4 mlg /ml	74.1 ± 11.01 b A	71.0 ± 3.20 b A	10.02
6 mlg /ml	85.0 ± 5.14 a A	86.3 ± 4.35 a A	5.46
Control	0c	0d	-
LSD 5%	10.02	11.6	-

*Means followed by different letters in a column (concentration) indicate significant differences, # means followed by different uppercase letters in a line (fungus) indicate significant differences

C. Effect of oily extract on radial growth of the fungi F. oxysporum and F. solani:

Results showed total inhibition of growth th**e** two fungi when volatile oil was used at 50 and 75 µl, where inhibition ratio of fungal growth attained 100 % to the studied fungi (table 3).

As a result, oily extract was the most efficacy in inhibition growth of *Fusarium* species compared to water and ethanol extracts.

Table (3): Effect of volatile oil of castor seeds on the two pathogenic fungi *Fusarium oxysporum* and *F.solani*

Treatment	Inhibition ratio (%) ± SD	
	F.oxysporum	*F.solani*
25 µl	60.3 ± 7.29 b*	59.6 ± 1.15 b
50 µl	100 ± 0.0 a	100 ± 0.10 a
75 µl	100 ± 0.01 a	100 ± 0.0 a
control	0c	0c
LSD 5%	23	21.2

The results showed high effect of all studied extracts of castor seeds in inhibition the growth of pathogenic fungi *F.oxysporum* and *F.solani*. The effect relashion direct with time and increasing the used concentration.

Re. [1], [2] and [5] refer to the important role that castor seeds extracts play in inhibition phytopathogenic fungi growth and give us a safety solution to reduction spread of these danger fungi.

The conclusion of the study was:

High effect of castor seed extracts (ethanolic, water and oily) in inhibition growth of the pathogenic fungi *F.oxysporum* and *F.solani*.

Superiority volatile oil on other studied extracts in inhibition ratio.

The effect correlate strongly with increasing the concentration and time.

Due to the encouraging results of this study, inhibition capability of extracts of castor seeds must be studied against several phytopathogenic fungi which exist in our local region.

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