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Antifungal activity of alcoholic extract of *Juglans regia* against phytopathogenic *Rhizoctonia solani*

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Abstract A current study was conducted at University of Diyala, College of Agriculture, during 9\2016-3\2017 to evaluate in-vitro different concentrations (5, 10, 15 and 20%) of the stem bark of *Juglans regia* L. against three isolates of phytopathogenic fungi *Rhizoctonia solani* were isolated from (cauliflower ,tomato and eggplant) through poisoned food technique. The findings showed that fungal growth of three *R. solani* isolates were decreased with increasing of *J. regia* concentration with significantly differences between them, a minimum of mycelia growth of three isolates of *R. solani* was 3.5 cm at 5% concentration and decreased gradually to 0.4 cm at 20% concentration, *R. solani* isolates were showed significant differences in radial growth, *R. solani* (Eggplant) was susceptible to all concentrations from other isolates in radial growth (2.1 cm) followed by *R. solani* (Tomato) 3.5 cm and *R. solani* (cauliflower) 3.9 cm whereas data further exhibited a significant interaction between concentrations of *J. regia* with isolates of *R. solani*, all of the tested concentrations were inhibited the growth of pathogen strains, % inhibition of *R. solani* growth were 94.8% at concentration 20% followed by 90.7, 75.9 and 60.7% at concentrations (15, 10 and 5%) respectively, inhibition percentage of different *R. solani* isolates were with variable degrees of sensitivity to concentrations of *Juglans regia* (76.6, 60.4, 56.2%.) at (*R. solani*, Eggplant), (*R. solani*, Tomato) and (*R. solani*, cauliflower) respectively, a significant interaction between concentrations (10, 15 and 20 %) of *J. regia* with isolates of *R. solani* were most effective to suppressed mycelia growth of all the *R. solani* strains that recorded 100%.The concentrations of 10, 15 and 20 % were most effective to restrict the growth of all the pathogen isolates that recorded 100% in inhibition percentage of radial growth of *R. solani* (Tomato) and *R. solani* (Eggplant).

Keywords *Juglans regia*, *Rhizoctonia solani*, poisoned food technique

Introduction

Rhizoctonia solani is soil-borne fungus that causes losses in many crops [1]. *R. solani* causing several of seedlings diseases with high losses of up to 80–100% [2,3,4]. *R. solani* cause necrosis of root, cortex tissue, root rot, damping-off, subsequent death [5]. Chemical pesticides application have hazardous impacts of including problems of environmental pollution, public health, causing resistance in disease agents and toxic effect on no target organisms, In spite of synthetic pesticides are the most effective method of the disease control [6,7]. There are environmentally safe methods are needed to replace or reduce chemical pesticides. Biocides are easily biodegradable and environmentally safe [8]. Some of plants was containing natural antimicrobial compounds[9,7]. Several works have demonstrated in laboratory trials that different plant tissues, such as leaves, roots, flowers and seeds possess

inhibitory properties against fungi, bacteria, and insects [10]. *Juglans regia* was used for therapy as antifungal, antiseptic and antibacterial [11,12].

Objective of this study was to assess antifungal activity of *Juglans regia* stem bark *in vitro* against three isolates of *R. Solani*.

Materials and Methods

Isolation of *R. solani* Isolates

The stem bark of *Juglans regia* was collected from local markets in Baqubah. Three isolates of phytopathogenic fungi were isolated from (cauliflower, tomato and eggplant).

Isolation process was done from small pieces of root for each plant by cut from areas of healthy and diseased roots and surface sterilized with Sodium hypochlorite NaOCl (20%) for 3 minutes then using sterilized distilled water. Roots were put on sterilized paper to remove excess water then transferred to medium of potato-dextrose agar after sterilized at 121.6 ºC at 15 lbs pressure/inch^2 for 20 minutes in an autoclave. The petri dishes were kept at 25 ± 1^{o}C for 7 days, fungi colonies were identified depending on characteristics of morphology and reproductive using taxonomic keys [13,14] by Dr. Sattar Aziz in Agriculture College, University of Baghdad.

Preparation of Plant Extracts

The selected samples of *J. regia* Stem bark were prepared after drying and crushed in a grinder to a fine powder, 250 gm powder of tested plant part was macerated with 500 ml of ethanol 96% and placed for 24 hours in shaker then after filtration by cloth then transferred to centrifuge at (3000 rpm) for 10 min .The supernatant was taken and dried in the oven at 45 ℃, preparation of extract was described by [15] with some modifications.

Assay of Antifungal Activity

the activity of plant extract as antifungal was conducted by technique of poison food [16]. Plant extract concentrations 5, 10, 15, 20 % were prepared by adding 2 g from plant extract in solid state to 20 ml of sterile distilled water to make stock, 2 ml from stock was added to 100 ml of PDA medium to make concentration 5%, the same procedure was followed up by adding (4, 6 and 8 ml) from stock to 100 ml of PDA medium to make concentrations (10, 15 and 20%) respectively and poured in Petridishes, 5 mm disc from culture of *R. solani* were put at the center of the Petridishes then after incubated at 24±1 °C for seven days, cross diameters of mycelial growth were measured and recorded ,each treatment included three replicates were maintained. Treatment of control was without the extracts, a formula of inhibition Percentage of mycelia growth was used the following equation [17].

$$IP = \frac{DC - DT}{DC} x\ 100$$

where,

IP = inhibition percentage

DC = Diameter of colony in control

DT = Diameter of colony in treatment

data was designed in Factorial Experiments **[18].**

Results and Discussion

The results showed that fungal growth of three *R. solani* isolates were decreased with increasing of *J. regia* concentration with significantly differences between them; a minimum of radial growth was 3.5 cm at 5% concentration of plant extract and decreased gradually to 0.4 cm at 20% concentration. Isolates of *R. solani* were showed significant differences in radial growth, *R. solani* (Eggplant) was susceptible to all concentrations from other isolates in radial growth (2.1 cm) followed by *R. solani* (Tomato) 3.5 cm and *R. solani* (cauliflower) 3.9 cm whereas data further exhibited a significant interaction between concentrations of *J. regia* with isolates of *R. solani* (Table 1).

Table 1: Effect of various concentrations of alcoholic extract of *J. regia* L. stem bark *in vitro* on radial growth of *R. solani* isolates

***R. solani* isolates**	**Concentrations of *J. regia***					**Means of *R. solani* isolates (cm)**
	5%	**10%**	**15%**	**20%**	**control**	
R. solani (Tomato)	5.3	3.5	0.0	0.0	9.0	3.5
R. solani (Eggplant)	1.5	0.0	0.0	0.0	9.0	2.1
R. solani (Cauliflower)	3.8	3.0	2.5	1.4	9.0	3.9
Means of Concentrations (cm)	3.5	2.1	0.8	0.4	9.0	

CD (0.05) Concentrations 0.2
CD (0.05) *R. solani* strains 0.1
CD (0.05) Concentrations×*R. solani* strains 0.4

It was observed that all of the tested concentrations were inhibited the growth of pathogen strains (Figure1, 2 and 3).

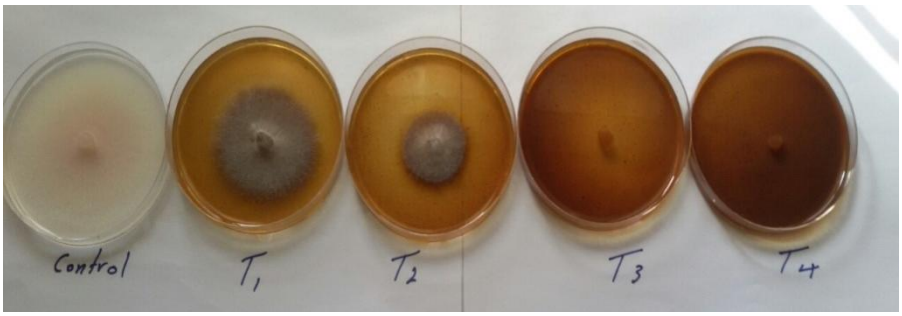

Figure 1: Effect of different concentrations of alcoholic extract of J. regia L. stem bark on R. solani (Tomato)

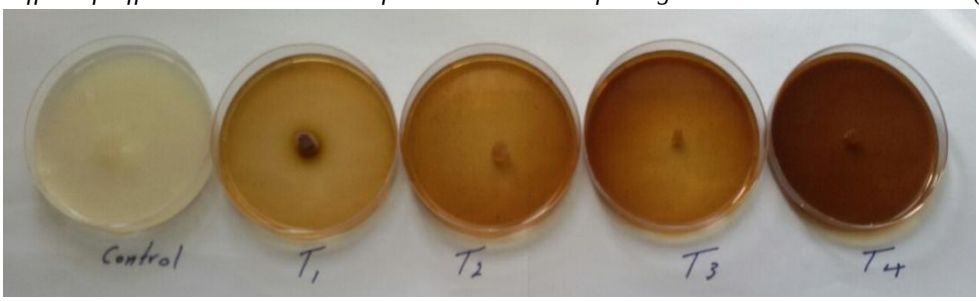

Figure.2: Effect of different concentrations of alcoholic extract of J. regia L. stem bark on R. solani (Eggplant)

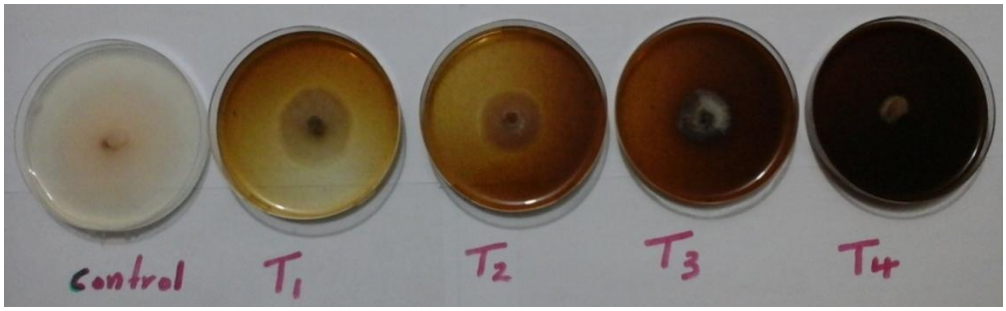

Figure.3: Effect of different concentrations of alcoholic extract of J. regia L. stem bark on R. solani (Cauliflower)

A concentration of 20% was recorded high significant in inhibition of *R. solani* strains 94.8% followed by 15, 10 and 5% concentrations which was 90.7, 75.9 and 60.7% respectively. *R. solani* (Eggplant) was susceptible to all concentrations from other isolates in percentage of inhibition of radial growth that recorded (76.6%) followed by *R. solani* (Tomato) 60.4% and *R. solani* (cauliflower) 56.2%. The concentrations of 10, 15 and 20 % were most

effective to restrict the growth of all the pathogen isolates that recorded 100% in inhibition percentage of radial growth of *R. solani* (Tomato) and *R. solani* (Eggplant) (Table 2).

Table 2: Effect of various concentrations of alcoholic extract of *J. regia* L. stem bark *in vitro* on % inhibition of radial growth of *R. solani* isolates

***R. solani* isolates**	***Juglans regia* concentrations**					**Means of *R solani* isolates %**
	5%	**10%**	**15%**	**20%**	**control**	
R. solani (Tomato)	41.1	61.1	100	100	0.0	60.4
R. solani (Eggplant)	83.3	100	100	100	0.0	76.6
R. solani (Cauliflower)	57.7	66.6	72.2	84.4	0.0	56.2
Means of concentrations %	60.7	75.9	90.7	94.8	0.0	

CD (0.05) Concentrations 0.4
CD (0.05) *R. solani* strains 0.3
CD (0.05) Concentrations×*R. solani* strains 0.8

The present findings are in agreement with the results of [19, 20] stated that *J. regia* L. stem bark contains constituents of chemical like juglone, sitosterol, ascorbic acid, quercetin-3-Larabinoside and is also known useful for the treatment of fungal infections [21].

The fruit extracts of *Juglans regia* have antifungal activity against *Candida albicans* and *Cryptococcus neoformans* [22,23]. Extracts of the bark was demonstrated the same antimicrobial activity [24]. Analyzes of this plant showed Phytochemical molecules which was rich in phenolic compounds which are represented mainly by flavonoids and naphthoquinones these molecules have power in inhibiting growth of fungi [25].

Conclusion

The findings from this study showed that alcoholic extract of *Juglans regia* L. stem bark by various concentrations was inhibited the growth of pathogenic isolates with various degrees of sensitivity. *Juglans regia* L. has antifungal activity and can be recommended for control of *R. solani.*

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