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RESEARCH PAPER

Evaluation of Rosemary extract and some fungicides activities against selected tomato fungal pathogens in Erbil and Sulaimani Governorates Kurdistan Region/ Iraq

Kamalaldeen Mohammad Fatah Hawrami[1], Zirak Fake Ahmed[2]
[1]Department of Plant Protection, College of Agricultural Engineering Sciences. Salahaddin University-Erbil, Kurdistan Region, Iraq .
[2] Department of Biology, College of Education,Salahaddin University-Erbi ,Kurdistan Region, Iraq

A B S T R A C T:
Tomato plants are subject to attacks by different types of fungi most of which are destructive and cause plant death. Extensive researches were carried out attempting to find safe and easily degradable bio-fungicides with minimum adverse effects. Therefore, naturally originated indigenous plant products are generally considered excellent alternative phytopathogen controlling agents. In recent study we evaluated the antifungal action of ethanol, methanol, acetone, and water Rosemary extracts against *Alternaria alternata*, *Fusarium solani*, *Rhizoctonia solani*, and *Phoma destructiva* fungi isolated from diseased tomato plants and identified by DNA based molecular identification technique. With the exception of the low inhibitory action of the extracts against *Fusarium solani* (5.5188 - 8.7762%), all extracts showed an excellent antifungal action against *Alternaria alternata* (40.1-44.9%), *Rhizoctonia solani* (13.8-19.2%), and *Phoma destructiva* (25.4-33.5%). The ethanol extract of Rosemary showed the best inhibition action among all tested solvent extracts.

KEY WORDS: Tomato diseases, Pathogenicity, plant extract, in vitro and molecular identification..
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1. INTRODUCTION:

Tomato (*Lycopersicon esculentum* Mill.) plant belongs to the *Solanaceae* family is considered as one of the most economically important crops coming in the second level in production and consumption over the entire world after cereals.
Tomato fields are subject to attacks by different types of fungi most of which are destructive and cause plant death like, *Alternaria solani* the causal agent of early blight, root rot and wilts caused by *Fusarium solani* and *Fusarium oxysporum f.sp. Lycopersici* respectively**,** *Rhizoctonia solani* seed and seedling damping off, damping-off and root rot caused by several *Pythium* species (Saba et al., 2010; Gulzar et al., 2018).

* **Corresponding Author:**
Kamalaldeen Mohammad Fatah Hawrami
E-mail: kamalaldeen.fatah@su.edu.krd
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Cultural practices, resistant varieties and pesticides, despite their limited success, have been used to minimize tomato crop diseases. Chemical efficiently reduce the damage caused by plant diseases, but in the last decades there was global awareness about excessive and improper use of chemical which makes it undesirable because of the harmful effects they have on the humans and animals' health, environment, and non-target organisms (Maji and Chakrabartty, 2014a).
Extensive research was carried out in an attempt to find safe and easily biodegradable bio-fungicides with minimum adverse. These compounds are found in nature and are related to the plant's defense mechanisms which enable them to metabolize active compounds that protect plants against insect and phytopathogens attacks. These metabolites are harmless. Therefore, naturally originated indigenous plant

products, like plant extracts, essential oils, gums, resins, etc. were tested in-vitro and in-vivo and generally are considered excellent alternative phytopathogen controlling agents (Joseph et al., 2002; Mazid et al., 2011; Rashid, 2016; Jimenez-Reyes et al., 2019; and Akinlolu et al., 2021)

Rosemary (*Rosmarinus officinalis* L.), originally comes from the Mediterranean Region where it grows on dry sunny slopes, has 1-3 closely related species growing in the region. It is a medicinal plant belongs to the Lamiaceae family, commonly known as rosemary, 60-120 cm tall, evergreen, aromatic shrub with needle-like leaves. It is a worldwide cultivated and used spice (Ilyina et al., 2017; Kompelly et al., 2019). It has been used in folk medicine as an oral preparation to relieve renal colic, dysmenorrhea, and muscle spasms. In addition, it has been widely accepted as a spice with a high antioxidant, antifungal, antiviral, antibacterial, anti-inflammatory, antitumor, antithrombotic, antidepressant, and antiulcerogenic (Genena et al., 2008; Malvezzi et al., 2020).

Essential oils make up to 2.5% of rosemary leaves and apical shoots (Ilyina A. et al. 2017). These oils showed antimicrobial activity against some food spoilage organisms (Genena et al. 2008). These oils also showed antifungal activity against plant pathogenic fungi like *Penecillium italicum, Fusarium culmorum,* and *Fosarium oxysporum* by inhibiting spore germination. This inhibition action may reach 100% against some plant pathogenic fungi depending on the concentration (Sofiene et al., 2019).

This study aimed to test and evaluate the antifungal effect of ethanol, methanol, acetone, and aqueous extracts of rosemary plant against different pathogenic fungi isolated from diseased tomato plants in vitro.

2 Materials and methods

2.1 Collection of tomato plants

Fifty tomato plants showing disease symptoms were collected from a number of tomato fields in Erbil and Sulaimani Governorates/ Kurdistan Region/ Iraq during the period from 1st. April 2020 to 30th. September 2020. The samples were placed inside nylon bags and kept in a cold box and transported to the postgraduate laboratory/ Department of Plant Protection/ Collage of Agricultural Engineering Science/ Salahaddin University where they were kept in the refrigerator at 4ºC till processing time.

2.2 Isolation and purification of the fungi

The plant samples were thoroughly washed with tap water to remove all foreign material. After visual examination, small pieces (4-5 mm^2) were taken from the area that showed moderate symptoms, and all parts of each plant (roots, stem, leave and fruits) were included in this process. All collected pieces were then separately surface sterilized by soaking for 2 minutes in 1% sodium hypochlorite, and then they were soaked in sterilized distilled water to remove any Sodium hypochlorite residuals.

After blotting on sterile filter paper, the specimens were placed onto potato dextrose ager (PDA) medium in disposable Pitry plates. In order to prevent bacterial growth, 1% (w/v) of each of streptomycin and tetracycline was added to the PDA medium before pouring it into the Petri plates. The plates then were incubated at 25±2ºC. After 3-7 days the plates were examined visually and under dissecting microscope (HumaScop/ Human/ Germany) to inspect colony features and differentiated growing colonies. Then 1cm plug of each differentiated colony was cut using a heat sterilized cork borer. The plugs were moved separately using heat sterilized needle and placed onto a new PDA plate and incubated at 25º±2C until the fungus grow and nearly fill the Pitry plate. This process was repeated until pure colonies were obtained.

2.3 Pathogenicity test

Spore suspension ($3X10^5$CFU/ml) was prepared from each *Alternaria alternata, Fusarium solani* and *Phoma destructiva* fungal isolate while for *R. solani* the inoculum was prepared cultivation of the fungus hypha in potato dextrose broth according to the method described by (Silva et al., 2020). Prepared inoculums then were inoculated on 45 days old local seedlings

grown from locally cultured tomato seed. The seeds have been surface disinfected using 1:1(v/v) mixture of 70% Ethanol/ 5% Sodium hypochlorite for 2 minutes and then rinsed two times in sterilized distilled water (Sauer and Burroughs 1986, with slight modification), before sawing them in 144-hole plastic plug tray filled with (1:1) clay-peat soil mixture. The trays then were placed in a plastic house under ordinary daylight and watered every day or when required. After 4 weeks 3 of the apparently healthy growing seedlings were transferred into a plastic pot containing about 1 kilogram of mixed clay-peat soil (1:1v/v). The inoculation was made when the seedlings were 45 days old by making 2 inch deep hole in the soil at the rhizosphere area using a plastic rood, then 1.5 ml of the spore suspension poured into the hole. For the control treatment sterile distilled water poured in the holes, a second set was left with no inoculation. Three replicates were used for each treatment. The experiment was carried out in a completely randomized design (CRD).

After 45 days the plants were examined to evaluate their pathogenicity. A disease severity scale (Table 1), was used to score the disease severity 7-15 days after the inoculation of the seedlings (Latha et al., 2009; Sibounnavong et al., 2010). The disease severity % was calculated using the following formula (Maharjan et al. 2015).

Disease severity % = (Scoring of different rating/ (Number of samples × highest score)) ×100

Then small sections (1×1 cm) were taken from each diseased plant (leaves, stems or roots) and cultured on PDA medium and incubated in the same way mentioned previously. Macroscopic and microscopic morphological characteristics were investigated after 3-7 days to confirm that the isolated fungi are the same as fungi that were used in the inoculation process to satisfy Koch's postulates criteria (Rashid, 2016; Teixeira et al., 2017).

2.4 DNA extraction and molecular identification of fungal isolates

Fungal DNAs were extracted using FavorPrep Tissue Genomic DNA Extraction Mini Kit (FAVORGEN BIOTECH CORP.)

Nuclear ribosomal internal transcribed spacer (ITS) has been selected as the DNA barcode for fungi (Mahmoud and Zaher 2015). The ribosomal RNA (5.8S rRNA) of the extraction product was amplified according to the protocol described by White et al. (1990) using the following primers:

ITS1forward primer (5´TCC GTA GGT GAA CCT TGC GG 3´)

ITS4 reverse primer (5´TCC TCC GCT TAT TGA TAT GC 3´)

Using PTC-200 Gradient thermocycler (Bio Research), PCR amplification was carried out in 50 µl of reaction mixture containing the following components (White et al., 1990): 25 µl of 2x Taq DNA Polymerase Master Mix (AMPLIQON A/S Stenhuggervej 22), ITS1 (2 µl), ITS4 (2 µl), DNase free water (17 µl), DNA template (4 µl).

The process was carried out according to the master mix manufacturer (AMPLIQON A/S Stenhuggervej 22). The PCR programming steps were as following:

One cycle of initial heating: 95 °C for 3 minutes

35 cycles of (95 °C for 30 seconds, 60 °C for 30 seconds,72 °C for 30 seconds) one cycle of 72°C for 5 minutes.

The amplified extraction products were visualized using 1% Agarose gel electrophoresis technique as described by (Gold Biotechnology, 2018).

2.4.3 DNA Sequencing

The PCR products of each isolate were sequenced using ABI Prism Terminator Sequencing Kit (Applied Bio system) at Macrogene Company/ South Korea. 5.8S rRNA chromatograms were edited and base calls checked using Finch TV program software developed by Geospiza, Inc. The 5.8SrRNA gene sequence with 500bp size was applied to Basic Local Alignment Search Tool (BLAST) available at the National Center for Biotechnology Information (NCBI) website (https://blast.ncbi.nlm.nih.gov/Blast.cgi), in

order to compare isolates sequences and alignment of them with other biological sequences in order to find out the similarity between query isolates and reference species. Partial of 5.8S rRNA gene sequence from our samples were submitted in National Center for Biotechnology Information (NCBI).

2.5 Collection of plant materials

Rosemary plants were collected from a number of gardens and nurseries in Erbil province/ Kurdistan Region/ Iraq and kept in cold box transported to the laboratory. The plant materials initially were washed thoroughly under tap water in order to remove any foreign materials, and after being cut into small pieces they were shade dried at 35 - 40°C. When completely dried, the samples were grinded using an electric grinder and sieved using a flour sieve. The obtained powder was kept in tightly closed container, labeled with required information and saved in a dark, dry, cold place until next processing step. A sample of the Rosemary plant was subjected to DNA extraction and molecular identification at Macrogene Company of South Korea, the same center where fungal isolates were identified.

2.6 Preparation of plant extracts

For aqueous extraction boiled water was used, while acetone, ethanol and methanol used for organic extraction. The extraction was made by adding the solvent to the plant powder in the ratio of (10:1) in a tightly screw cupped glass container, to speed up the extraction process; the bottles were shaken on a locally made shaker for 24 hours at 100rpm. Thereafter the extracts were filtrated by passing them through an absorbent wound dressing sheet (ViTri Medical/ Sweden) and Whatman No.1 filter paper (Merck/Germany) using Buchner`s funnel set connected to a vacuum pump system. Each filtrate then was poured into a pre-weighted clean container labeled with required information. All containers then were placed in an electric oven (Memmert, Germany) at 50±2ºC till the extracts were completely dry.

2.7 Preparation of the stock and test extract solutions

An initial stock solution with a 100mg/ml concentration was prepared from each dried extract by dissolving 1gm of the dried extract in 10 ml of (1:1v/v) mixture Dimethylsulphoxide (DMSO)/ Methanol (Rashid, 2016). After centrifugation at 5000rpm for 5 minutes, the stock solutions were sterilized by passing them through a 0.45µm sterile syringe filter (Sartouris/ Germany) and preserved in sterile screw-capped tubes.

2.8 Preparation of the fungicides (control positive)

The fungicides Avalon, Zoxis, and Topaz50wp (Table 2) were bought from the local market and used as positive controls. Fungicides solutions used in this study were prepared as described by the manufacturer instruction.

2.9.1 Antifungal activity

The agar well diffusion method described by (Olakunle et al., 2019), was followed with minor modification to determine the antifungal activity of each prepared stock solution. Four wells were made in the PDA medium 1 cm away from the Petri plate margin using a 6mm diameter sterile cork borer, then 100µl of ethanol, methanol, acetone, and aqueous stock extract solutions. 100 µl of Avalon, Zoxis, and Topaz50wp fungicides solution (positive controls), distill water and DMSO/methanol mixture (negative control), were poured into the wells of separate plates. The petri plates then were covered and kept at room temperature for 24hrs to allow the extracts and controls to diffuse through the agar. Then a 5 mm fungal plug was cut from the leading edges of a seven-day-old culture of each isolated fungus using sterile cork borer, then using a sterile needle the plug was removed and placed in the center of the assigned Petri plate. Then the petri plates were covered and incubated at 25 ±2ºC and observed daily until the negative control growth completely covers the entire surface of the agar. Then the inhibition zone around the well for each treatment was measured using a digital vernier caliper (PREISSEER/ Germany).

In this assay all treatments were performed in three replicates for each fungal isolate.

The percent of inhibition was calculated using the following formula:

$I\% = (N-T/N) \times 100$

Where:

I= Inhibition

N: Diameter of growth in the negative control.

T: Diameter of growth in the test.

2.9.2 The minimum inhibitory concentration (MIC):

Well diffusion method (Olakunle et al. 2019) was used to determine the minimum inhibitory concentration by pouring 100, 50, 25 and 12.5 µl from each extract in 4 wells made in the PDA medium using a sterile cork borer. Positive (fungicide) and negative controls (DW and DMSO/Methanol 1:1 mixture (DMSM)), were included. The plates were left at room temperature for 24 hours in order to allow the tested solutions to diffuse throughout the medium. Then, each plate was inoculated with the specified fungal isolate, sealed with parafilm, and incubated at 25±2°C for 72 hrs., or until the growth in the negative control completely covered the plate. For each treatment the experiment was performed in three replicates.

The inhibition zone for each treatment was then measured using a digital vernier caliper (PREISSEER/ Germany).The percent of inhibition was calculated using the same formula used in the previous assays.

3. Results:

3.1 Isolation and purification of the fungi

As a result of culturing and repeated purification, 73 pure fungal isolates diverse colony morphology were isolated from different parts of the 32 diseased tomato plant samples. Microscopic examination showed that some of these isolates were common contaminants and not related to tomato diseases like *Aspergillus, Rhizopus and Pencillium* spp., which only in suitable conditions may cause postharvest problems. These isolates were neglected and destroyed. Other isolates that morphologically resembles pathogenic fungi were kept pure and included in the subsequent processes.

3.2 Pathogenicity Test

Results of testing pathogenicity showed that only 4 of the fungal isolates were pathogenic causing disease to inoculated tomato plants. Scoring disease severity (Table 3) showed that it lay between 3 and 5 scores, and the calculated disease severity percentage (DS %) lay between 60 - 80 %. Various types of disease symptoms commonly seen on diseased plants in tomato fields, including dumping off, wilting, yellowing, spotting, crown rot, blight, and plant death, were observed on inoculated plants (Figure 1). Re-isolation, macroscopic and microscopic examinations confirmed that the isolated fungi were the same used in the inoculation (Figure 2).

3.3 Molecular identification of Fungal isolates

Table (4) shows molecular identification results and the accession number given for each of the four isolated fungi genes after been compared with the nucleotides saved NCBI/GenBank. The results of sequence BLAST showed a 100% match of the Query cover and identic number with sequences saved in GenBank (Table 5 & Figure 3). The identified isolates were *Alternaria alternata, Fusarium solani, Phoma destructiva,* and *Rhizoctonia solani.* A phylogenetic tree of the identified fungi was constructed (figure 9).

3.4 Molecular identification of Rosemary plant

According to the results of molecular identification (Table 5, and Figure 4), the collected Rosemary plant was identified and submitted to the NCBI/GenBank as (*Salvia rosmarinus*), and given the accession number (OP270717). The phylogenetic tree for the gene sequence of Rosemary sample from Kurdistan Region/ Iraq was constructed using the Maximum Likelihood method based on the Tamura-Nei model in MEGA11 software and bootstrap analysis with 100 re-samplings. Partial DNA sequences of concatenated partial tRNA- Leu gene were used as input data.

3.5 In-vitro assay of antifungal activity

The antifungal activity of ethanol (ROE), methanol (ROM), acetone (ROA), and water (ROW) Rosemary stock solutions (100mg/ml) against four fungi isolates were tested (Table 6).

Statistical analysis of the results showed that the extracts had significantly effect on the growth of the fungal isolates. However, there was a clear discrepancy in the inhibitory activity of each solvent extracts against each of the four fungi. This discrepancy was related to the type of solvent used in the extraction and the treated fungus, with the exclusion of any effect of any external factor

because all treatments were performed in the same place and under the same conditions.
The results against *Alternaria* sp (Figure 3) showed that all extracts have inhibition action which resulted in a significant decrease in the fungal growth. The inhibition was very close to that caused by the fungicides, or even was better.
Ethanol, methanol, acetone and water, were effective and induced high inhibition of *Alternaria alternata* growth (44.86%), (47.15%), (42.66%) and (40.09%) respectively. While the percentage of inhibition of these extracts on the growth of *Fosarium solani,* was (5.5188%), (8.705%), (8.3368%), and (8.7762%) respectively, indicating a moderate to low antifungal activity.
The extracts showed a moderate to low antifungal actions against *Rhizoctonia solani*. The percentages of inhibition were ethanol (19.213%), methanol (13.818%), acetone (16.211%), and water (13.863%).
The extracts against Phoma destructiva, except for the ethanol, (33.533%), methanol, (25.389%), acetone (25.715%), and water (25.715%), extracts showed high inhibition activity.

3.6 The minimum inhibitory concentration (MIC)

Statistical analysis of this assay results (Table 7) revealed that, in general, the difference in the concentrations of each Rosemary extract causes no significant change in the inhibitory action of the extract (Table 7).

4. Discussion

The antifungal action of ethanol, methanol, acetone, and water extracts against four fungal isolates were tested. All solvent extracts efficiently inhibited the growth of *Alternaria alternata, Rhizoctonia solani*, and *Phoma destructive* with exception of the low action against *Fusarium solani.*
The antimicrobial action of *Rosmarinus officinalis* L. essential oils against phytopathogenic fungi including *Alternaria alternata, Colletotrichum* sp., *F. oxysporum*, and *Penecillium italicum* was evaluated (Saba et al., 2010; Barakat and Ghazal, 2016; and Bakhtiarizade and Souri, 2019). They stated that there was variability in the antifungal action of Rosemary oils and the rate of disease inhibition they induce in in-vitro and in-vivo experiments. They also stated that there was a relationship between the extract concentration and the inhibitory action of the extracts against each of the tested microorganisms. In addition they reported that doubling any extract concentration turned it from an ineffective extract that does not affect the growth of microorganisms to a strongly effective and fatal one.
Seint et al. (2011) studied the antifungal action of Water and 70% ethanol Rosemary extracts and stated that ethanolic extract of Rosemary inhibited 91.8% of the growth of *Rhizoctonia solani*, 72.6% of *R. oryzae*, and 43.7% of *R. oryzae-sativae* and these results are in agreement with our results. While water extract of rosemary inhibited the growth of *R. oryzae-sativae* by 10%, but was ineffective on *R. solani* and *R. oryzae*, these results are contrary to our results which showed a significant inhibitory action of the water extract of Rosemary against *Rhizoctonia solani.* Saba et al. (2010) studied the antifungal action of Rosemary extracts on the growth of *A. solani* and they confirmed the significant antifungal action of these extracts by causing a total inhibition of the mycelial growth and spore germination. They also stated that the percentage values of reduction in mycelial growth showed to be increased with the increasing extract concentration from 1 to 25% for *R. officinalis*. In the recent study we found that increasing the concentration of the extract from 12.5 to 100 mg/ml caused no statistically change in the antifungal activity of the Rosemary extracts.
Barakat and Ghazal, 2016 in their study evaluated the antifungal action of Rosemary essential oils against eight *Fusarium* species. Results of their study revealed that Rosemary essential oils were found to possess strong antifungal action against the treated fungi. Muthomi et al. (2017) in their study, among many other plants, evaluated the antifungal action of Rosemary against *F. oxysporum* and *R. solani* and they stated that the extract of Rosemary showed restricted activity against these two fungi. These findings come consistence with the results of our study.
Yulia, (2005) in his M Sc. Study evaluated the activity of Rosemary extracts and oils against several plants pathogenic fungi among them *Fusarium* sp. and *phoma* sp. and he stated that Rosemary extracts and oils showed low activity against these two fungi. These findings are consistence with our results related to *Fusarium*

sp., but not with results related to *Phom* sp. which showed a good antifungal action.
These antifungal effects may be due to the presence of specific phytochemicals in these extracts. At the same time the antifungal activity of the extracts was not significantly affected by the concentration. All the results strongly support the idea of utilizing Rosemary extracts specifically the ethanol extract as a natural fungicide to control tomato fungal pathogens.

5. Conclusion

From our study it clearly appeared that Rosemary ethanol, methanol, Acetone, and water extracts have very good antifungal action against each of *Alternaria alternata*, *Rhizoctonia solani*, and *Phoma destructive*, and even they showed a weak action against *Fusarium solani*.

6. Figures

Figure 1 Symptoms of wilting, spotting, and rooting appeared on inoculated tomato plants.

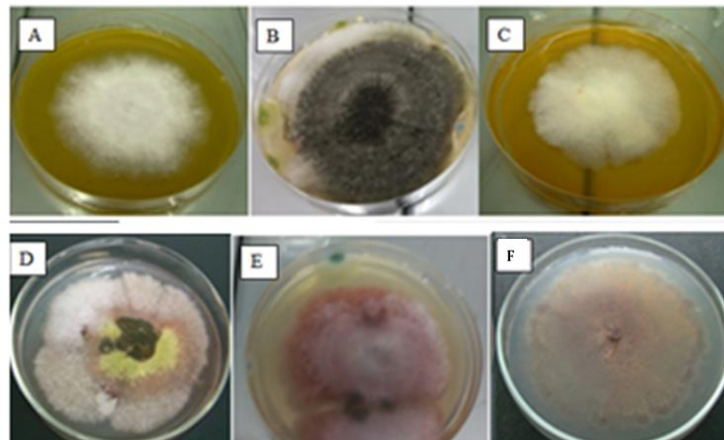

Figure 2 Re-isolated fungi: *Rhizoctonia* sp. (A&C), *Alternaria* sp. (B), *Fusarium* sp. (D&E), and *Phoma sp.* (F), (G).

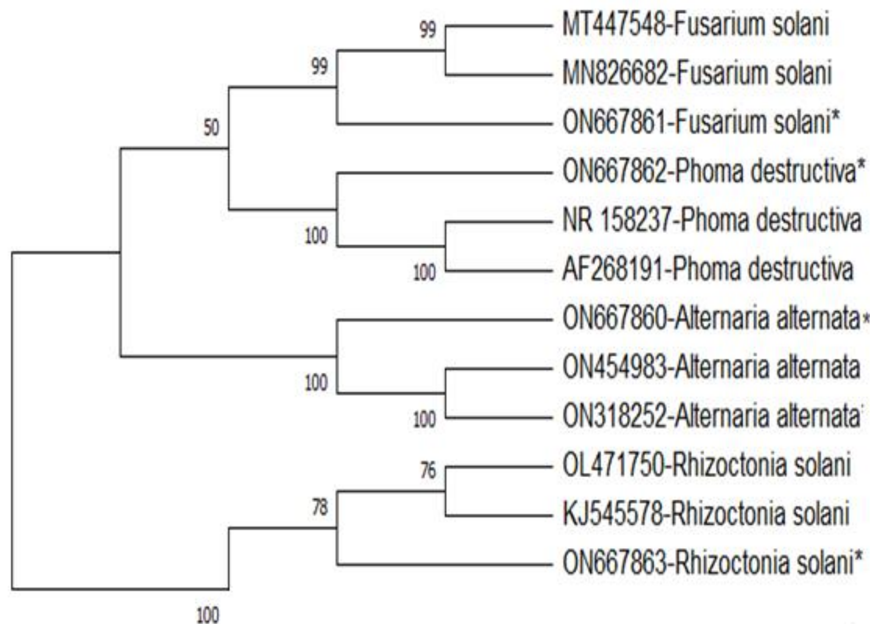

Figure 3 Phylogenetic positioning of fungal isolates (asterisks), according to the sequences of 5.8S rRNA employing maximum likelihood available in GenBank sequences

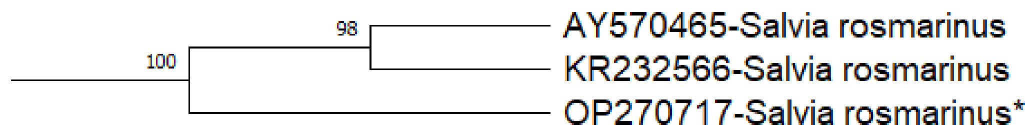

Figure 4 phylogenic tree constructed for the sequence of Rosemary plant sample/ Kurdistan Region/ Iraq (asterisks)

Table 1: Latha et al. (2009) and Sibounnavong et al. (2010) disease severity ratting scale

Score	Grade	Disease severity range
0	Healthy	0 % of the plant infected
1	Initial	1-20 % of the plant infected
2	Low	20-40% of the plant infected
3	Medium	40-60% of the plant infected
4	High	60-80% of the plant infected
5	Very high	80-100% of the plant infected

Table 2 Fungicides used as positive controls

Fungicide		Active ingredient	Manufacturer
F1	Avalon 400	Almephenom+ Smetrobine	Greenriver Industry Co. ‹Ltd./ China
F2	Zoxis SC	Azoxystrobin	Arysta LifeScience Benelux/ Belgium
F3	Topaz50wp	Tolclofos methyl+ Thiram	Ascot/ UK

Table 3 Calculated disease severity % observed on inoculated plants

Code	Suspected pathogen	DS %*
F1	Alternaria sp.	66.66
F2	Fusarium sp.	73.33
F3	Phoma sp.	80
F4	Rhizoctonia sp.	73.33

***Results are the mean of three replicates reading**

Table 4 Molecular identification and submitted fungal partial 5.8S rRNA gene of the four query sequences in NCBI/GB

code	Identification (ID)	Accession number	Query Cover %	Identic No %	Genbank Access No.
F1	*Alternaria alternata*	ON667860	100	100	ON667860
					ON318252
F2	*Fusarium solani*	ON667861	100	100	MT447548
					MN826682
F3	*Phoma destructiva*	ON667862	100	100	NR_158237
					AF268191
F4	*Rhizoctonia solani*	ON667863	100	100	OL471750
					KJ545578

Table 5 Identification of Rosemary plant used in the present study and accession number

Sample Code	Plant Identified	Accession Numbers	Query Cover %	Identic Number %	Genbank Accession No.
P3	*Salvia rosmarinus*	OP270717	100	100	AY570465
					KR232566

Table 6 Effect of the Rosemary extracts on the growth of isolated fungi

Fungi sp.	Extract	Inhibition	
		Zone (mm)	Percent %
Alternaria	**ROE**	**35.89**	**44.9**
	ROM	**37.73**	**47.2**
	ROA	**34.13**	**42.7**
	ROW	**32.07**	**40.1**
	FC1	**33.08**	**41.4**
	FC2	**42.25**	**52.8**
	FC3	**42.25**	**52.8**
	DW	**4.75**	**5.9**
Fusarium	**ROE**	**9.7**	**5.5**
	ROM	**12.3**	**8.7**
	ROA	**12**	**8.3**
	ROW	**12.3**	**8.8**
	FCI	**31.4**	**32.7**
	FC2	**27.8**	**28.2**
	FC3	**29.3**	**30**
	DW	**5.3**	**0**
Rhizoctonia	**ROE**	**35.393**	**19.2**
	ROM	**16.413**	**13.8**
	ROA	**16.449**	**13.9**
	FC1	**30.25**	**31.2**
	FC2	**33.417**	**35.2**
	FC3	**33.583**	**35.4**
	DW	**5.417**	**0**
	ROW	**18.318**	**16.2**
Phoma	**ROE**	**26.6**	**33.5**

	ROM	**20.1**	**25.4**
	ROA	**20.4**	**25.7**
	ROW	**21.2**	**26.8**
	FC1	**33.5**	**42.3**
	FC2	**32.3**	**40.8**
	FC3	**31.8**	**40.1**
	DW	**5.8**	**7.3**

Table 7 Effect of extract concentration on the inhibition activity (MIC)

Fungi	Solvent	Volume of stock solution (100mg/ml)			
		100µl	50µl	25µl	12.5µl
Alternaria alternata	Ethanol	37.7a	37.1a	33.4a	35.4a
	Methanol	35.5a	40.9a	38.7a	36a
	Acetone	34.2a	39.6a	32.1a	30.63a
	Water	34.9a	32.6a	29.5a	31.3a
Fusarium solani	Ethanol	11.3a	9.8a	10.1a	7.7a
	Methanol	10.73a	11.1a	13.2b	14b
	Acetone	12a	12.7a	11.7a	11.2a
	Water	11.2a	12.9a	11.8a	13.4a
Rhizoctonia solani	Ethanol	22.1a	20.1a	20.2a	20.5a
	Methanol	17.2a	15.5a	16.7a	16.4a
	Acetone	20a	19a	14.7b	12.2b
	Water	18.9ab	16.2a	20.1b	18.1ab
Phoma destructiva	Ethanol	21. 3a	24.6a	24a	20.8b
	Methanol	21a	15.6b	23.2a	15.7b
	Acetone	20.5ab	18.4a	20.3ab	22.4b
	Water	22.8a	22.5a	21a	19a

*Inhibition zones (mm) with the same letters have no significant difference between each other. The results represent the mean of three Inhibition zone replicates (IZ. LS$\bar{x}$)

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