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Full Length Article

Responses of Tomato to *Rhizoctonia solani* Infection under the Salinity Stress

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Abstract

Ten isolates of *Rhizoctonia solani* Kuhen were obtained from the roots and crown of different cultivars of tomato (*Solanum lycopersicum* L.). The phenotypic and microscopic characteristics of these isolates were examined. The pathogenicity of each isolate to tomato seedlings was evaluated *via* a pot experiment. Results showed that the isolates had varying pathogenicity. Ten isolates were identified, four of which exhibited severe pathogenicity. The effects of three salinity levels (2, 6 and 12 dS m^{-1} NaCl) on fungal growth, and the effects of the interaction between pathogenicity and these salinity levels on the germination indicators, phenotypic growth and biochemical characteristics of three varieties of tomato, namely, Salimah, Bushra and Yassamen both in the field and the laboratory were assessed. The combined stress of the pathogenic fungus *R. solani* with the increase in NaCl concentration had a stronger pathogenicity to the tomato plant than individual stress alone. As a result, the germination indicators and all phenotypic traits of the plants substantially decreased. As salt concentration increased, the contents of chlorophyll a, b and total chlorophyll decreased. By contrast, the contents of carotenoids and anthocyanins increased and those of carbohydrates and proline in the leaves considerably increased. Analysis of the interactions between the *R. solani* treatments and the salinity levels revealed a strong correlation between the salinity levels and H_2O_2 accumulation. Our findings proved that the pathological effect of *R. solani* was observed to be more significant on tomato varieties under salinity treatments.

Keywords: Biochemical responses; Fungi; Lipid peroxidation; Photosynthetic pigments; Salinity

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated vegetable crops worldwide. In terms of nutritional composition, tomato fruits contain 3% carbohydrates, 1.2% protein, 1% total fats, minerals (calcium, magnesium, phosphorous, potassium, sodium, zinc and manganese.), and different contents of vitamins (vitamins A and C, thiamine, riboflavin, niacin, pantothenic acid and pyridoxine) (Perveen *et al.* 2015; Melfi *et al.* 2018). Most cultivated tomato cultivars are exposed to infection with soil-borne pathogens, the most important of which is the fungus *Rhizoctonia solani*, one of the fungal pathogens transmitted through the soil and affects a wide range of plant families (Al-Hammouri *et al.* 2013). This pathogen also causes diseases in other members of family *Solanaceae*, especially in potato (Rafiq *et al.* 2020, 2021).

Effective control of this pathogen is difficult owing to the diversity of its host range, persistence of sclerotia formation in soil, lack of genetic resistance and limited efficacy of chemical fungicides (Zachow *et al.* 2011). Sumalatha *et al.* (2018) explained that some infected plants show symptoms of sunken watery spots that later turn into irregular brown spots on stems with the appearance of local necrosis on the bark. Mayo-Prieto *et al.* (2020) observed that the mycelium of this pathogen penetrates wound areas, leading to the rupture of the outer layer of the host's epidermis.

Tomato production faces enormous problems worldwide including lack of water resources, soil salinity and other abiotic stresses (Fahad *et al.* 2017; Zhou *et al.* 2019). These stresses have a detrimental effect on plant growth and development as they interfere plant morphological, physiological, biochemical and molecular responses (Rai *et al.* 2013; Abass 2016). Salt stress affects all major processes of agricultural crops such as germination, growth, photosynthesis, respiration, water content, nutrient imbalance, oxidative stress and yield (Yasin *et al.* 2018). Arif *et al.* (2020) reported that salinity enhances the content of reactive oxygen species in plant cells as a consequence of ion toxicity and ionic imbalance, which results in osmotic and ionic stress that disrupts the balance of nutrient absorption and damages the membranes

and various internal structures. Fungal activity is affected by salt stress (Rilling 2004). Asghari *et al.* (2008) confirmed that salinity restricts the growth of mycelium through the harmful effect of salts. Similarly, Peat and Fitter (1993) indicated that salinity decreased the number of spores and availability of carbohydrates necessary for fungal growth. Likewise, Juniper and Abbott (2006) found that inhibits spore germination and mycelium growth. Salih and Al-Maarich (2016) reported that the pathogenic *R. solani* isolates RS1 and RS2 can grow under saline conditions with concentrations ranging from 6–16 dS m^{-1}. The present study was conducted to investigate the biochemical response patterns of three tomato plant cultivars, namely, Salimah, Yassamen and Bushra to the interaction between the fungal pathogen *R. solani* and three NaCl levels under laboratory and greenhouse conditions.

Materials and Methods

Plant materials

The seeds used in this study were tomato seeds of three varieties (Salima, Yassamen and Bushra) obtained from local markets and predominantly cultivated in Zubair fields.

Salinity treatments

Three levels of sodium chloride salt were used during field and laboratory experiments (2, 6 and 12 dS m^{-1}). Distilled water was used to prepare the irrigation water with the desired NaCl salinity based on an initial survey.

Isolation and Identification of *R. solani*

The plant parts were collected from the affected areas of some tomato fields and nurseries planted in Zubair and Safwan districts, which showed symptoms of seed rot and seedling death, represented by rotting of seeds and stems of seedlings with a brown discoloration of the roots from light to dark, as well as wilting and yellowing of the leaves, especially the lower ones. The isolation on PDA medium containing Chloramphenicol (250 mg L^{-1}) was done (Mohammed-Ameen *et al.* 2021); all inoculated plates were incubated at 25 ± 2ºC. The identification was confirmed depending on the characteristics of the fungal colony, the nature of branching of the new mycelium, the structures that it forms, the ability to form sclerotia, the formation of barrel cells and the presence of double-hole septa, using the taxonomic key of Parmeter and Whitney (1970).

Fungal nuclear staining

All fungal isolates were stained according to Runion and Kelley (1993) using aniline blue and lactophenol.

Pathogenicity test

The fungal inoculum was prepared using millet seeds according Smiley *et al*. (2005); the pathogenicity trails were done on the seeds of Salimah tomato variety in petri dishes and pots experiment according to Bolkan and Butler (1974). For petri dishes trail, after seven days of incubation at 25 ± 2ºC the seed germination percentage was calculated; and for pots trails both seed germination and seedling damping off were measured; as well as; plant height, fresh and dry weight of shoot and root system after 45 days of inoculation.

The effect of salinity levels on fungal growth

The PDA medium was prepared using sterile distilled water containing saline levels of 0, 2, 6 and 12 dS m^{-1}, with the antibiotic Chloramphenicol at a concentration of 250 mg L^{-1} and then sterilized with an Autoclave, after the sterilization period, poured into sterile Petri dishes and inoculate with a 0.5 cm diameter disc of PDA medium of each *R. solani* isolate and incubated at a temperature of 25 ± 2ºC for three days. The radial growth was measured every 24 h by taking the average of two perpendicular diameters passing through the center of the disc and until the growth in the control treatment reached the edge of the plate. The percentage of radial toxicity was calculated according to the following equation (Abass 2017):

$$Radial\ toxicity\ (\%) = \frac{C - T}{C} x100$$

Which C: fungal growth in control; T: fungal growth in treatment. Additionally, the fungal dry growth inhibition undergoes the effect of salinity was done according to Muhsin (1990) using PD broth.

Tomato varieties responses to *R. solani* under salinity stress

The response of three varieties of tomato was tested, namely Salima, Yassamen and Bushra. In this experiment, sandy soil from one of the tomato farms in Zubair was used and washed well to remove excess salinity and dried with peat moss at a ratio of 1:3 then the soil was sterilized with an Autoclave for One hour twice on two consecutive days. The inoculum of the pathogenic fungus RS3 loaded on local millet seeds was added at a rate of 1% (w/w) to the sterilized soil, and it was planted in plastic pots (25 cm diameter), one pot contained 2 kg of sterile soil, and three days after adding the fungal inoculum to the soil mixture. Three tomato cultivars were sown at a rate of 20 seeds pot^{-1} sterilized with 10% sodium hypochlorite solution for 2–3 min. As for the control treatment, it was cultivated with the same steps without any addition; and irrigated the pots with the saline levels used in the study 2, 6 and 12 dS m^{-1} and

after two weeks of planting, the percentages of germination and tomato seedlings damping off were calculated and the infection rate was calculated. The interaction effect of pathogenic fungi and salinity levels was studied on some indicators of plant growth, such as plant height and fresh and dry weight for each of the shoot and root systems after 45 days of planting. The experiment lasted for 60 days and at the end of the experiment, some biochemical indicators were measured, including.

Photosynthetic pigments

The pigments chlorophyll a, chlorophyll b and total chlorophyll were estimated and extracted based on the method of Arnon (1949) and the content of carotenoids and anthocyanins by Asare-Boamah *et al.* (1986) and expressed in the unit (mg g^{-1}).

Proline content

Proline content in leaf tissues was measured by reaction with ninhydrin chromatically at 520 nm (Bates *et al*. 1973).

Hydrogen peroxide content

H_2O_2 levels were measured in control and stressed laves tissues according to the procedure of Sergiev *et al.* (1997). The hydrogen peroxide content was calculated using the standard hydrogen peroxide curve.

Malondialdehyde content

MDA was used as a marker for membrane lipid oxidation. MDA was extracted at 5% (w/v) with trichloroacetic acid (TCA), absorbance was measured at 532 and 600 nm, and the MDA concentration was calculated using the Extinction Coefficient (Heath and Packer 1968).

Total soluble carbohydrates

The method described in Watanabe *et al.* (2000) was followed to estimate the carbohydrate content in leaf tissues by interacting with the anthron reagent and measuring the absorbance at a wavelength of 620 nm and the carbohydrates were estimated using the standard glucose curve.

Statistical analysis

With three salt levels (2, 6 and 12 dS m^{-1} NaCl) and three tomato varieties (Salimah, Yassamen and Bushra), the experiments utilized a completely randomized factorial design. All of the tests were triplicates, and the data was analyzed using SPSS-22 software for two-way analysis of variance (SPSS Inc., Chicago, IL., USA). To examine significant variations between means, the least significant difference (LSD) was employed. A P value of less than 0.05 was used to determine statistical significance.

Results

Isolation and identification of *R. solani*

Ten different isolates of *R. solani* (herein designated as RS1-RS10) were isolated from different fields in Safwan and Zubair, Iraq.

Morphological and microscopic characteristics of *R. solani* isolates

The 10 *R. solani* incubated for 2 weeks on PDA culture medium in the dark at 25 ± 2°C. Examination of their phenotypic characteristics revealed that they differ in appearance, consistent with the findings of Yadav and Tiwari (2005), Lal and Kandhari (2009) and Misawa and Kuninaga (2010) These aforementioned studies reported that *R. solani* colonies differ in terms of growth, morphology and colours, as well as in terms of their density and spread on the surface of the culture medium. According to their microscopic features, the 10 *R. solani* isolates were found to have a different ability to form swollen barrel-shaped cells called monilioid cells or different manner of the hyphae branching (Fig. 1).

Nuclear staining of *R. solani* isolates

Microscopic examination showed that the number of nuclei in the newly emerged hyphae cells of eight isolates, namely, RS1, RS2, RS3, RS4, RS6, RS8, RS9 and RS10 was more than two nuclei per fungal cell; the number of nuclei was between 3 and 9 nuclei/fungal cell (Table 1 and Fig. 2). By comparison the average number of nuclei in RS5 and RS7 was 2 nuclei/fungal cell (Fig. 2). Moreover, these two isolates were not pathogenic to the tomato plants.

Pathogenicity trails

Results of pathogenicity trails on Petri dishes (Table 2) showed that most of the *R. solani* isolates examined herein remarkably reduced the percentage of germination of tomato seeds on WA medium by 16.6–66.6% compared with the control treatment, which reached 100% germination rate (Table 2). The exception was the RS5 isolate (90.0% germination rate), which was not significant differences ($P < 0.05$) from that of the control treatment.

Pot experiments obtained similar results. The difference in germination rates between the isolates and the control treatment was not statistically significant. The control treatment achieved 86.6% germination rate. By comparison the RS4, RS2 and RS3 isolates had the lowest germination rate (40.0, 33.3 and 20.0%, respectively). The

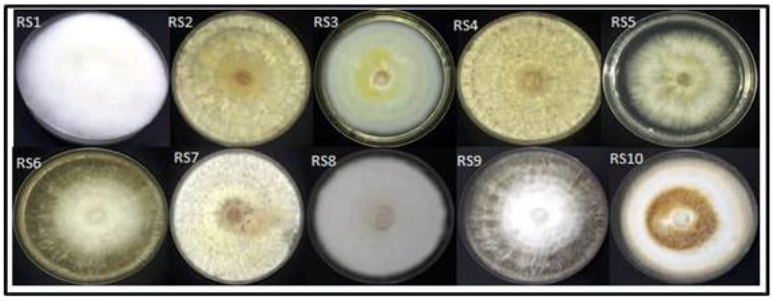

Fig. 1: Colour and shape of colonies *Rhizoctonia solani* isolates from soil and roots of tomato plants on PDA culture media in the incubator at 25°C for one week
*The letters RS stand for *Rhizoctonia solani* and the number beside them represents the isolate number

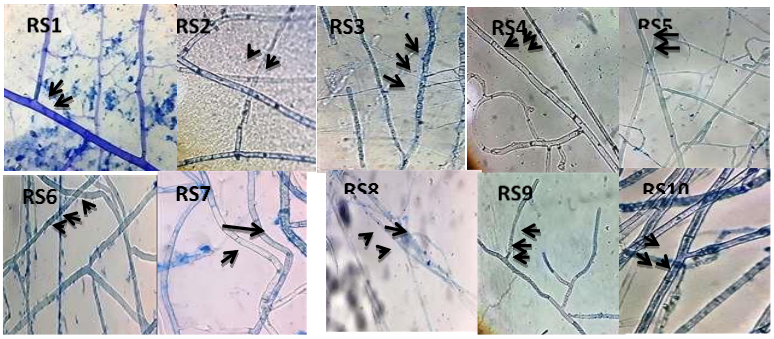

Fig. 2: Nuclei numbers in hyphae of *Rhizoctonia solani* isolates (X40)
* The letters RS stand for *Rhizoctonia solani* and the number beside them represents the isolate number

Table 1: The number of nuclei per cell in the *Rhizoctonia solani* isolates

Isolate Number	The nucleus number of each isolate		
	Minimum Number	Maximum number	Average number
RS1	3	5	4
RS2	4	7	5
RS3	3	9	6
RS4	6	9	7
RS5	2	2	2
RS6	3	6	5
RS7	2	2	2
RS8	3	7	5
RS9	4	7	5
RS10	6	9	7

*The letters RS stand for *Rhizoctonia solani* and the number beside them represents the isolate number

Table 2: The effect of *Rhizoctonia solani* isolates on tomato seed germination in Petri dishes

Isolate number	Seed germination %
Control	100.00
RS1	16.66
RS2	26.66
RS3	20.00
RS4	26.66
RS5	90.00
RS6	36.66
RS7	66.66
RS8	30.00
RS9	36.66
RS10	30.00
LSD (0.05)	15.31

*The letters RS stand for *Rhizoctonia solani* and the number beside them represents the isolate number

RS3 isolate showed the highest reduction in seed germination (80.0%) and seedling damping off (14.90%). The RS2 isolate had 66.66% seed germination and 9.95% seedling damping off. The RS4 isolate; the values were 60.0 and 7.19% for seed germination and seedling damping off, respectively. In the control treatment, the values were 14.0 and 0.00%, respectively. Therefore, the isolates whose rates of seed germination and seedling damping off were not substantially different from those of the control treatment were not (Table 3). Thus, RS3 isolate was superior over the other isolates. Moreover, some of the growth parameters including plant height and the fresh and dry weight of shoot and root systems (Table 3) were considerably reduced. Clearly RS3 was the most pathogenic among the 10 isolates, whereas the RS5 isolate was not pathogenic.

Effects of salinity levels on the growth of different *R. solani* isolates *in vitro*

The growth rates of the isolates were slightly affected by the increases in salinity levels (Fig. 3). The highest growth rate of 8.95 cm was recorded when salinity level was 12 dS m^{-1}. This rate was not significantly different from that of the control treatment (8.98 cm). When the salinity levels

Table 3: Pathological testing of isolates of the fungus *R. solani* in tomato seed germination (%) Seed decay (%), seedling damping off (%), plant height (cm) and fresh and dry weight of the shoot and root system (mg) in plastic pots

Isolate No.	Seed germination (%)	Seed decay (%)	Seedling damping off (%)	Plant height (cm)	Fresh weight (mg)		Dry weight (mg)	
					Shoot	Root	Shoot	Root
Control	86.66	14.00	0	15.16	548	49.66	38.33	6.50
RS1	46.66	53.66	3.40	13.30	459	38.66	29.33	2.66
RS2	33.33	66.66	9.95	10.41	400	36.33	28.66	4.50
RS3	20.00	80.00	14.90	8.90	301	12.00	14.33	2.33
RS4	40.00	60.00	7.19	11.33	483	38.00	32.33	4.70
RS5	86.66	13.66	0	14.50	540	45.00	35.00	5.40
RS6	80.00	20.00	0	11.16	433	30.50	15.20	3.20
RS7	60.00	40.00	0	13.16	523	20.00	36.33	4.20
RS8	73.33	27.00	0	10.50	477	33.80	29.86	3.10
RS9	73.33	27.00	0	11.40	343	35.60	24.00	5.00
RS10	53.33	46.66	0	13.70	413	34.20	26.43	4.30
LSD($P < 0.05$)	42.51	42.06	0.83	1.99	50.60	13.41	4.02	1.62

*The letters RS stand for *Rhizoctonia solani* and the number beside them represents the isolate number

Table 4: Effect of salinity levels (dS m^{-1}) on the radial growth rate (cm) of *Rhizoctonia solani* isolates

Salinity level (dS m^{-1})	*Rhizoctonia solani* isolates					Average of salinity level
	RS1	RS2	RS3	RS4	RS5	
Control	9.00	9.00	8.90	9.00	9.00	8.98a
2 dS m^{-1}	8.80	8.90	6.80	8.90	5.50	7.78c
6 dS m^{-1}	8.80	8.95	8.30	8.95	8.10	8.62b
12 dS m^{-1}	8.95	8.96	8.95	9.00	8.90	8.95a
Average of isolates	8.88a	8.95a	8.23b	8.96a	7.87c	

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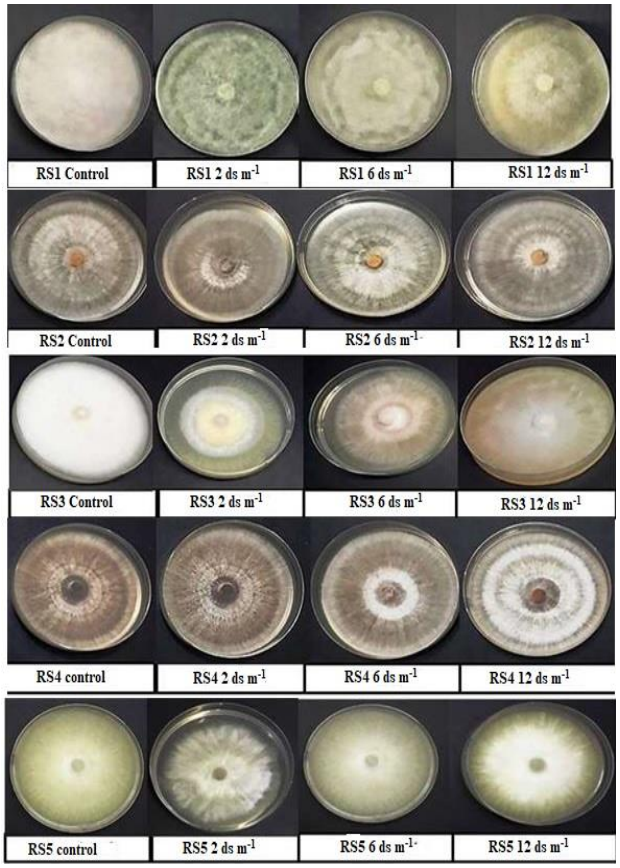

Fig. 3: Effect of salinity levels (dS m^{-1}) on the radial growth of *Rhizoctonia solani* isolates

* The letters RS stand for *Rhizoctonia solani* and the number beside them represents the isolate number

were 6 and 2 dS m^{-1} the radial growth rates of the isolates was inhibited (8.62 and 7.78 cm), respectively, (Table 4).

Responses of different tomato varieties to *R. solani* isolates and salinity stress

The isolates examined herein and the salinity levels of irrigation water had a significant effect ($P < 0.05$) on the percentages of seed germination and seedling death of the tomato varieties Salimah, Yassamen and Bushra (Table 4). The Bushra cultivar was more tolerant to salinity and fungal pathogen than the two other varieties. Its average germination rate was 70.43%, which was significantly different from that of Yassamen (61.50%) and Salima (60.75%). Notably, the germination rate decreased from 94.08% in the control treatment (*i.e.*, without salinity and pathogen infection) to 83.16% when the tomato plants were treated with the isolates and subjected to the salinity level of 2 dS m^{-1}. Moreover, the germination rate further decreased to 52.16 and 27.50% when the tomato plants treated with the isolates and subjected to salinity levels.

The interaction between the fungal isolates and the salinity levels was significantly decreased the seed germination percentage of the tomato varieties. Salimah was the most sensitive to the fungal isolate when the salinity level was 12 dS m^{-1}. The germination decreased as the salinity levels of the irrigation water increased, especially in the soil contaminated with the fungus *R. solani* (Table 5).

The pathogenic effect of the *R. solani* isolates increased as the salinity levels increased. The disease incidence in the control treatment was 5.36%. When the

Table 5: Effect of the pathogenic fungus *Rhizoctonia solani* and different salinity levels on germination indicators and phenotypic characteristics of tomato varieties

Variety	Treatment	Seed germination (%)	Seedling damping off (%)	Injury rate (%)	plant height (cm)	Fresh weight (mg)		Dry weight (mg)	
						Shoot	Root	Shoot	Root
Salimah	Control	88.50	8.44	5.72	9.44	244.75	42.25	89.00	5.12
	2 dS m^{-1} + RS	85.75	14.64	14.46	8.94	181.25	36.00	80.50	4.25
	6 dS m^{-1} + RS	48.75	35.83	30.83	8.21	125.00	28.00	34.00	1.75
	12 dS m^{-1} + RS	20.00	67.92	82.71	5.78	69.00	20.25	18.75	1.17
Yassamen	Control	95.25	5.23	5.20	7.75	183.25	27.50	30.50	3.37
	2 dS m^{-1} + RS	70.25	14.26	7.13	6.51	154.75	27.25	15.25	2.62
	6 dS m^{-1} + RS	52.25	52.25	26.70	6.36	81.25	15.00	11.37	1.50
	12 dS m^{-1} + RS	28.25	66.86	53.16	4.65	30.00	8.50	4.12	1.00
Bushra	Control	98.50	5.07	5.18	9.75	193.75	31.50	48.25	3.75
	2 dS m^{-1} + RS	93.50	10.71	5.41	7.63	158.25	21.50	40.50	3.25
	6 dS m^{-1} + RS	55.50	33.62	18.05	7.28	143.00	17.25	20.25	2.25
	12 dS m^{-1} + RS	34.25	47.23	38.20	6.90	111.25	13.00	9.93	1.75
LSD (*P < 0.05*)		4.31	3.57	2.95	0.58	27.48	5.23	5.86	0.68
Average of varieties	Salimah	60.75b	31.70b	33.42a	8.09a	155.0a	31.62a	55.56a	3.07a
	Yassamen	61.50b	34.65a	23.04b	6.31b	112.3b	19.56b	15.31c	2.12b
	Bushra	70.43a	24.15c	16.70c	7.88a	151.5a	20.81b	29.73b	2.75a
LSD (*P < 0.05*)		3.04	2.52	2.08	0.41	19.43	3.70	2.14	0.48
Average of treatments	Control	94.08a	6.24d	5.36d	8.97a	207.25a	33.70a	55.91a	4.08a
	2 dS m^{-1} + RS	83.16b	13.20c	8.99c	7.69b	164.75b	28.25b	45.41b	3.37b
	6 dS m^{-1} + RS	52.16c	40.56b	25.19b	7.28b	116.41c	20.08c	21.87c	1.83c
	12 dS m^{-1} + RS	27.50d	60.66a	58.02a	5.77c	70.08d	13.91d	10.93d	1.30c
LSD (*P < 0.05*)		3.52	2.92	2.41	0.47	22.44	4.27	4.78	0.55

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Table 6: Effect of the pathogenic fungus *Rhizoctonia solani* and different salinity levels of on the biochemical characteristics of tomato varieties

Varieties	Treatments	Chl *a* (mg g^{-1})	Chl *b* (mg g^{-1})	Total Chl (mg g^{-1})	Carotenoids (mg g^{-1})	Anthocyanin (mg g^{-1})	Carbohydrates (mg g^{-1})	Proline (μg g^{-1} FW)	H_2O_2 ($\mu M.$ g^{-1} FW)	MDA (nmole g^{-1})
Salimah	Control	*2.89	0.84	3.74	1.40	0.060	2.00	1.23	1.195	0.077
	2 dS m^{-1} + RS	2.70	0.79	3.49	1.43	0.065	0.52	1.22	1.263	0.146
	6 dS m^{-1} + RS	2.33	0.79	3.12	1.57	0.074	0.52	1.31	1.570	0.195
	12 dS m^{-1} + RS	2.10	0.78	2.88	1.58	0.083	1.03	1.46	1.578	0.547
Yassamen	Control	3.12	1.02	4.14	1.72	0.054	1.52	1.14	0.016	0.042
	2 dS m^{-1} + RS	3.17	0.99	4.16	1.77	0.055	2.02	1.16	0.614	0.167
	6 dS m^{-1} + RS	2.83	0.93	3.76	1.80	0.062	1.53	1.25	1.179	0.235
	12 dS m^{-1} + RS	2.66	0.93	3.59	1.83	0.078	1.53	1.35	1.310	0.417
Bushra	Control	3.55	1.04	4.60	2.00	0.091	2.03	1.23	0.136	0.044
	2 dS m^{-1} + RS	3.46	1.02	4.49	2.08	0.095	2.02	1.25	0.173	0.057
	6 dS m^{-1} + RS	3.25	1.02	4.27	2.09	0.109	1.52	1.31	0.263	0.072
	12 dS m^{-1} + RS	3.08	0.92	4.00	2.11	0.162	2.03	1.43	0.628	0.126
LSD (*P < 0.05*)		NS	NS	NS	0.07	0.004	NS	NS	0.006	0.005
Average of variety	Salimah	2.50c	0.80b	3.31a	1.49c	0.071b	1.02c	1.30a	1.402a	0.241a
	Yassamen	2.94b	0.97a	3.91b	1.78b	0.063c	1.65b	1.23b	0.780b	0.216b
	Bushra	3.33a	1.00a	4.34c	2.07a	0.115a	1.90a	1.30a	0.300c	0.075c
LSD (*P < 0.05*)		0.08	0.03	0.09	0.05	0.003	0.24	0.01	0.004	0.004
Average of treatments	Control	3.19a	0.97a	4.16a	1.75	0.070c	1.85	1.20c	0.449d	0.055d
	2 dS m^{-1} + RS	3.11a	0.93b	4.05b	1.76	0.070c	1.52	1.21c	0.684c	0.123c
	6 dS m^{-1} + RS	2.80b	0.91bc	3.72c	1.81	0.082b	1.19	1.29b	1.007b	0.168b
	12 dS m^{-1} + RS	2.61c	0.88c	3.49d	1.80	0.108a	1.53	1.41a	1.170a	0.364a
LSD (*P < 0.05*)		0.09	0.03	0.10	NS	0.003	NS	0.01	0.005	0.004

* The letters RS stand for *Rhizoctonia solani* and the number beside them represents the isolate number

tomato plants were treated with the *R. solani* isolates and when the salinity level was 2 dS m^{-1} the disease incidence was 8.99. When the salinity levels were 6 and 12 dS m^{-1}, the disease incidence was 25.19 and 58.02%, respectively.

Furthermore, the *R. solani* isolates, increasing salinity levels and their interactions remarkably decreased the plant height and the fresh and dry weights of shoot and root systems (Table 5).

Biochemical analyses of the tomato varieties, responses to pathogen infection, increasing salinity levels and their interactions revealed that the contents of chlorophyll a, chlorophyll b and total chlorophyll significantly decreased. As the salinity level further increased from 2 dS m^{-1} to as 12 dS m^{-1}, the contents of these pigments further decreased (Table 6). However, the opposite trend was observed in the contents of carotenoids

and anthocyanins (Table 6).

As a response to pathogen attack, salinity treatment and their interactions, the contents of carbohydrates and proline accumulated at high levels. Proline accumulation is one of the most important mechanisms that plants resort to under the influence of salt stress. The concentration of proline substantially increased with the increase in salinity levels.

Similarly, the contents of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) in the tomato varieties significantly increased in response to pathogen attack, salinity treatment and their interactions. The content of H_2O_2 in tomato leaves significantly as salinity levels increased. Infection with the isolates at the salinity level of 12 dS m^{-1} resulted in the highest H_2O_2 content of 1.170 μmol g^{-1}. When the salinity levels were 6 and 2 dS m^{-1}, H_2O_2 content was 1.007 and 0.684 μmol g^{-1}, respectively.

In the tomato leaves, the content of MDA, which is the product of the peroxidation of polyunsaturated fatty acids in cell membrane, increased as salinity levels increased because of high level of oxidative stress. The average MDA content substantially increased from 0.123 μmol g^{-1} to 0.364 μmol g^{-1} as the salt level increased from 2 to 12 dS m^{-1} in the presence of the *R. solani* isolates. The interaction between salinity level and the *R. solani* isolates had a significant effect on the MDA content.

Discussion

R. solani isolates were isolated from different parts of tomato plants collected from various areas in Basrah Province, Iraq. On average, the RS5 and RS7 isolates, had 2 nuclei per ungal cell. Thus, these isolates were not pathogenic to the tomato plants. By comparison, other isolates had multiple nuclei. Hence, they were pathogenic to the tomato plants. These results were consistent with those found by Mirmajlessi *et al.* (2012) and Mustafa *et al.* (2021) who reported that multinucleate *R. solani* isolates are pathogenic to plants. Several studies indicated that binucleate *R. Solani* isolates are non-pathogenic and thus could be used in the biocontrol of pathogenic isolates (Elsharkawy *et al.* 2014). Pathogenicity trails proved that most of the *R. solani* isolates examined herein had high pathogenicity effects. The present work and previous studies confirmed that fungal isolates have the ability to reduce the germination rate of the plant seeds (Li *et al.* 2019).

The difference in the pathogenicity among the isolates examined in this study could be attributed to variation in the amount of toxic substances they secrete. Although these toxic substances, are chemically similar they differ quantitatively. Wyllie (1962) suggested that the difference in the pathogenicity of *R. solani* isolates may be due to their different capabilities to parasitize on the seeds directly. Highly pathogenic isolates cover seeds with mycelium, thereby preventing them from germinating. The protease enzyme plays a major role in determining the pathogenicity of *R. solani* (Ramezani 2008).

The superiority of the RS3 isolate over the other isolates may be due to difference in the ability to secrete degrading enzymes such as cellulase and pectinase. Moreover, it could be attributed to the secretion of amylases enzymes that leads to cell killing and turn the colour of seeds into dark brown (Ravjit *et al.* 1999; Mahmoud *et al.* 2007). Furthermore, it could be attributed to differences in their ability to secrete some phytotoxin compounds that can kill seed embryo, such as phenyl acetic acid and its hydroxylated derivatives, beta-hydroxy acetic acid and para-hyderoxyacetic acid (Mandava *et al.* 1980).

The RS3 isolate was the most pathogenic whereas the RS5 was not pathogenic. This seeming discrepancy was also noted by other researchers. The effects of different isolates of the *R. solani* on the growth of tomato plants vary, some have negative effect, whereas others promote plant growth (Macnish *et al.* 1995; Inoue *et al.* 2002).

The results of the evaluation of the effects of salinity levels on fungal growth obtained in this study, were consistent with the findings of Regragui and Lahlou (2005); Mustafa *et al.* (2021). The interaction between the fungal isolates isolated herein and the salinity levels was significant decreased the seed germination rate of the tomato varieties examined herein; This result may be due to the fact that salinity reduces and delays germination, a condition that increases the chance of fungus attacking the seeds (Li *et al.* 2019).

Growth parameters, including plant height and the fresh and dry weights of shoot and root systems, significantly decreased. Kaya and Kirnak (2001) and Kutuk *et al.* (2005) indicated that increasing soil salinity levels decrease the fresh and dry weights of the shoot and root systems of tomato plants, because of the decrease in the ability of the plants to absorb water and nutrients as the ions involved in the composition of salts leach into plant tissues, thereby impeding water transfer (Yeo 1998).

The decrease in the contents of chlorophyll a, chlorophyll b and total chlorophyll could be the results of the generation chlorophyllase, which is responsible for chlorophyll degradation. Furthermore, the decrease in contents of these pigments could be that result of changes in the composition of the chloroplasts at high salinity levels, thereby, that degraded plastid proteins and reduced chlorophyll contents thereby inhibiting electron transport (Tuna *et al.* 2008).

The contents of carotenoids and anthocyanins increased in response to pathogen attack and salinity stress. Previous studies indicated that salinity increases carotenoids content in tomatoes, because salt stress enhances carotenoids accumulation. Krauss *et al.* (2006) showed that the reduced leaf area caused by growth inhibition under salinity stress leads to increased carotenoid accumulation. Anthocyanin pigments represent a subgroup of plant flavonoids and play important roles in

plants as photoprotective pigments in shoot tissues for ultraviolet and high light absorption, and they also serve as antioxidants. Anthocyanins accumulate in plant tissues in response to various types of abiotic stresses, including osmotic stress, salinity, and high temperatures (Pourcel *et al.* 2007; Mouradov and Spangenberg 2014).

Carbohydrates and proline accumulated at high levels in response to pathogen attack, salinity treatment and their interaction. Previous studies reported that high salinity levels and fungal attack increase carbohydrates production and accumulation (Sarwar and Ashraf 2003). The content carbohydrate increased in the tomato leaves as the salinity level increased. Microorganisms adapt to salt stress by accumulating organic compounds (proline, glycine and betaine) and inorganic compounds soluble in cells, including potassium cations (Sagot *et al.* 2010).

Proline accumulation is one of the most important mechanisms that plants resort to under the influence of salt stress. Its accumulation in plants under excessive salinity levels is a primary response to maintain osmotic pressure in cells due to the decrease in the activity of oxidative enzymes (Sudhakar 2001). Proline concentration considerably increased as salinity levels increased. Proline plays the role of an effective osmotic protector and is the key to protection against external stresses and is also known as a salt tolerance limiter (Dogan *et al.* 2010).

The increase in salinity levels resulted in higher MDA contents in the plant leaves. MDA is the product of the peroxidation of polyunsaturated fatty acids in cell membrane, and its content in plants increases under a high level of oxidative stress (Pan *et al.* 2006). The interaction between salinity level and the *R. solani* isolates had a remarkable effect on the MDA content. This result was consistent with that of Giannakoula and Ilias (2013) who observed that lipid peroxidation increased in tomato plants treated with 150 m*M* NaCl. As NaCl content increased, MDA production was higher by twofold than that of the control treatment. Moreover, H_2O_2 concentration linearly changed with the increase in NaCl content. Kaushik and Roychoudhury (2014), and Foyer (2018) indicated that the production of reactive oxygen species in affected plants affects physiological aspects and growth by increasing damage to membranes (lipid peroxidation), proteins, carbohydrates, nucleic acids and plant pigments while decreasing seed viability and root growth. In the manner, pathogens can destroy the defence system of host plants and successfully establish.

Conclusion

Ten *R. solani* isolates were isolated form symptomatic leaves and crown parts of tomato plants. Pathogenicity test proved the virulence effect of *R. solani* isolates on the sensitive tomato variety Salimah. There salinity levels as 2; 6 and 12 dS m^{-1} of water salinity were selected to examine their effect on tomato physiology and their responses to pathogen attack. Results revealed the pathogenic effect of *R. solani* alone or in interaction with salinity levels on tomato varieties on germination and plant height, fresh and dry weight of shoots and roots. Additionally, a decrease in the chlorophyll content with the increase of salt concentrations was observed, and an increase in the carotenoids, anthocyanins, carbohydrates and proline contents in the leaves. A significant correlation between the salinity levels and H_2O_2 accumulation was revealed at the interactions treatments between *R. solani* and salinity levels.

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Author Contributions

AAM conducted the experiments and collected the samples, KMA interpreted the results and statistically analyzed, MHA supervised research and provided guidelines for writing manuscript and write the manuscript.

Conflicts of Interest

All authors declare no conflicts of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable in this paper.

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