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# EFFECT OF SALT AND WATER STRESS ON ROOT INFECTION BY *Macrophomina phaseolina* AND ION COMPOSITION IN SHOOT IN SORGHUM*

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## Abstract

The interaction of salt and water stress to infect the roots by *Macrophomina phaseolina*, and affect the ion composition and growth of sorghum (*Sorghum bicolor)* was studied in a greenhouse experiment (19-35˚C). Treatments consisted of 4 levels of salinity (0, 1400, 2100 and 2800 mg of NaCl $kg^{-1}$ soil) and three water stress levels (3, 7 and 10 irrigation intervals). Infested soil containing 100 viable microsclerotia $g^{-1}$ of a melon isolate of *M. phaseolina* and non-infested soil were used for all treatments. The experiment was arranged in a completely randomized design with four replications. Six-week-old sorghum seedlings after their transferring to infested and non-infested soil were exposed to salt stress, after which, water stress was started. Shoot dry weights were reduced by increasing salinity levels. This reduction was more pronounced in infested soil than in non-infested. Increasing irrigation intervals reduced salt injuries. Shoot and root colonization by *M. phaseolina* significantly increased by increasing salinity levels up to 1400 mg of NaCl $kg^{-1}$ soil. Moreover, salinity and *M. phaseolina* interaction increased the concentrations of $Na^+$ and $Cl^-$ compared to salt stress *per se*, but negatively correlated with increasing irrigation intervals. Concentration of $K^+$ was in contrast with $Na^+$ and $Cl^-$. Also, disease symptoms appeared only in the highest irrigation intervals (7 and 10 days). Consequently, more infected crown and root were observed by increasing irrigation intervals and NaCl levels up to 1400 mg $kg^{-1}$ soil.



**Keywords:** Matric potential, Osmotic potential, Charcoal rot, *Sorghum bicolor*, Drought.

See Persian text in (Page۲۳۷).

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## Introduction

*Macrophomina phaseolina* (Tassi) Goid is a soil-borne, microsclerotia producing fungus that causes root and stem rot on a large number of host plants including sorghum, sunflower, corn, melon and beans in tropical as well as subtropical regions (Mihail 1992). This fungus usually infects plants that are subjected to severe stresses induced by drought and high temperatures (Nischwits *et al*. 2002; Olaya *et al*. 1996). Environmental stresses can predispose plants to disease from relatively weak parasites, due to their negative effects on host physiology. Drought and salinity are regarded as the major predisposing factors in plant diseases (Ma *et al*. 2001, Triky-Dotan *et al*. 2005).

Drought stress is a limitation to crop productivity. Under this condition, removal of water from the membrane disrupts the normal bilayer structure and results in the membrane becoming exceptionally porous when desiccated. Stress within the lipid bilayer may also result in displacement of membrane proteins and this contributes to loss of membrane integrity, selectivity, disruption of cellular compartmentalization and a loss of activity of enzymes, which are primarily membrane based. In addition to membrane damage, cytosolic and organelle protein may exhibit reduced activity or may even undergo complete denaturation when dehydrated. The high concentration of cellular electrolytes due to the dehydration of protoplasm may also cause disruption of cellular metabolism (Mahajan and Tuteja 2005).

Soil salinity problems are widespread often in arid and semi-arid regions of the world. In the field, salt stress is usually accompanied by water stress. Irrigation of crop plants with poor quality water often leads to salt build up and water stress (Richardson and McCree 1985). The components of drought and salt stress cross talk with each other as both these stresses ultimately result in dehydration of the cell and osmotic imbalance. Virtually every aspect of plants physiology as well cellular metabolism is affected by salt and drought stress (Mahajan and Tuteja, 2005). In saline soils, although water is present it is unavailable to plants because it is retained by the ions in the soil, such as $Na^+$ and $Cl^-$ (Perez-Lepza *et al*. 2009). High salt concentrations decrease the osmotic potential of soil solution creating a water stress in plants. In addition to osmotic stress, salinity imposes on plants other stresses such as ion toxicity, as a result of ion entry in excess of appropriate compartmentation, and nutrient imbalances, as commonly seen in the displacement of potassium by sodium. The main damage to plants, however, could result from osmotic stress imposed externally due to high ion concentrations in the soil or internally when excess salt uptake resulted in high salt accumulation in the intercellular spaces (Zhang *et al*., 2006).

Sorghum is often grown in areas with relatively low rainfall, high temperatures and saline soils (Netondo *et al.* 2004). Tolerance to salinity is variable among crops (Sanogo 2004). Plant responses to salinity stress depend upon various factors, such as the duration and degree of the stress and growth stage (Triky-Dotan *et al*. 2005). Most agronomical crops do not function well at a salinity level of 5 dS $m^{-1}$ or higher (Mass 1986). Sorghum's ability to be productive in comparison with other cereals in saline and drought-prone environments has been attributed to several different morphological and physiological traits (Smirnoff, 1998). Osmotic adjustment defined as a net increase in tissue solute concentration in response to salt and water stress has been proposed as a beneficial salinity and drought tolerance mechanism for several crop species including sorghum (Girma and Krieg 1992). Accumulation of organic solutes mainly proline, free amino acids, and carbohydrates are among the nonspecific mechanisms, which increase under a range of stressful conditions (Khan 2007). This mechanism has

a positive effect on the daily carbon balance of a stressed plant, since it allows the plant to photosynthesize down to lower leaf water potentials than would otherwise have been possible (McCree 1986).

Charcoal rot is a major disease problem under drought and saline conditions (Diourt *et al*. 1995). The effects of salt on plant disease may result from its effect on one or more of biotic components involved in the disease namely the pathogen, the host, microbial activity in soils, or abiotic components of soil (Triky-Dotan *et al*. 2005). Water potentials from -1.2 to -1.5 MPa have been shown to increase predisposition to root rot pathogens. Edmunds (1964) found that charcoal rot was severe in sorghums plants when inoculated near maturity at temperatures of 35-40˚C and 25% available soil moisture. Water stress can predispose the plant to *M. phaseolina* whenever the defense mechanism of the plant is impaired (Waller 1986).

The objective of the present study was to determine the effects of several NaCl levels and irrigation intervals on the growth and mineral composition of sorghum and its susceptibility to charcoal rot pathogen.

## Materials and methods

### Soil

A dry virgin, sandy clay soil (pH 7.9, organic matter 2.2%, field capacity 16.52% (w/w), permanent wilting point 3.21% (w/w) and electrical conductivity ($EC_e$) 0.83 dS $m^{-1}$ was collected from the Agricultural Experiment Station, Shiraz University in Badjgah, 16 km north of Shiraz, Iran. To determinate the maximum accessible moisture for each pot, the soil moisture curve was obtained at a preliminary experiment using cell pressure apparatus (Fig. 1) (Cramer *et al*. 1985). Field capacity (FC) and permanent wilting point (PWP) were found to be 0.0305% and 17.2%, respectively.

The maximum accessible moisture for each 5000 g pot was 709ml according to equation 1:

water volume (ml) = water density / soil weight (g) × (FC-PWP)
(0.1723 - 0.0305) × 5000g = 709ml

### Inoculum production

A melon isolate of *M. phaseolina* (MP) was collected in summer from the Borazjan area, in Boushehr province in southern Iran. Microsclerotia of *M. phaseolina* were obtained free of culture medium by aseptically placing a small agar block from an actively growing culture in a flask containing sterile potato dextrose broth (PDB) (Short and Wyllie, 1978). The flask was incubated at room temperature for 3 months until a thick mat composed predominantly of microsclerotia forms on the surface of the broth. The mat was removed, washed three times using sterile distilled water, and dried at 35°C. The dried microsclerotial mat then were ground with a mortar and pestle giving a black powder of microsclerotia. The germination of microsclerotia was calculated using water agar medium (WA) prior to the experiment. The microsclerotia were mixed with 1000 g sterile air-borne sand and stored at 4°C. Before inoculation, the mixture was added to 104 kg of soil required for infesting soil with the pathogen (to reach 100 viable microsclerotia $g^{-1}$ soil).

### Inoculation and salt treatment

Ten germinated sorghum seeds were sown in each pot containing 1000 g dry soil (15 cm in diameter and 15 cm high). After 6 weeks, all 10 plants were transferred to larger pots (25 cm in diameter and 25cm high) containing 5000g infested (100 viable microsclerotia $g^{-1}$ soil) and non-infested soil. Salt treatments consisted of 0, 1400, 2100 and 2800 of mg NaCl $kg^{-1}$ soil. Sodium chloride was gradually added to the soil in aqueous solution for 7 d to reach the desired NaCl levels. For uniform water distribution, the pots were irrigated through a nylon tubing placed diagonally in the soil with a closed end and several, 1-2 mm diameter, holes on the

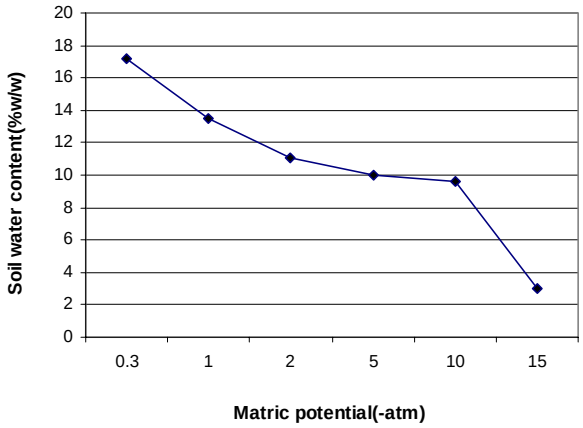


**Fig. 1. Soil moisture curve for the pot soils.**

tube (Banihashemi and deZeeuw 1975). The whole salt solutions were retained in the pots and maintained in field capacity by periodical weighing.

## Irrigation treatments

Three irrigation treatments using distilled water at 3, 7 and 10 d intervals were imposed after salt treatment and pots were irrigated with distilled water to a field capacity by periodical weighing throughout the experiment (Table 6). The number of total irrigation used during the experiment in 3, 7 and 10 d treatments were 16, 7 and 5 times, respectively. The experiment was conducted in a greenhouse with supplemental light provided by fluorescent and incandescent illuminations. The air temperature during the experiment fluctuated between 19˚C and 35˚C, however it often was above 30˚C during the day.

## Data Collection

At the end of the experiment (9 wk after inoculation), 15-day-old sorghum seedlings were cut at the soil line and roots were washed free of soil. The shoots were dried at 70˚C for 48 h, weighed and ground, dry-ashed and $Na^+$ and $K^+$ concentrations were determined by flame photometry. The $Cl^-$ concentration was determined following the method of Chapman and Pratt (1961). The interactions among salt, water stress, and infection by *M. phaseolina* were evaluated by measuring the percentage of crown and root segments colonized. Crowns and roots were thoroughly washed free of soil and surface sterilized for 2 min in 0.5% sodium hypochlorite. Twenty five pieces (0.5 cm) of crown or root were randomly selected from all plants separately in each pot and plated on potato dextrose agar (PDA). All plates were incubated for 10 d at 35˚C and the percentages of crown and root segments colonized by *M. phaseolina* were recorded.

## Statistical Analysis

The experimental layout was a 4 × 3 × 2 factorial arrangement in a completely randomized design with three replications. Analysis of variance for the effects of irrigation intervals, salt levels, disease infection and their interactions was done using MSTATC software.

# Results

The analysis of variance of the effects of irrigation intervals, salt, charcoal rot and their interactions on the growth and ion concentrations is shown in Table 1.

Results reported here indicate that increasing NaCl level led to a significant ($P \leq 0.001$) rise in $Na^+$ and $Cl^-$ concentrations of shoot (Tables 2 and 3). Whereas, there was a

**Table 1. Analysis of variance for growth responses and ion concentrations in shoot (s) of sorghum under four salt levels, three irrigation treatments and with or without fungus.**

| Source | df | Mean Square | | | |
|---|---|---|---|---|---|
| | | Shoot dry weight | Na (s) | K (s) | Cl (s) |
| **Salt (S)** | 3 | 26*** | 3.1*** | 0.2*** | 81.7*** |
| **Water (W)** | 2 | ns | 0.5*** | 0.08*** | 22.1*** |
| **Inoculation (I)** | 1 | ns | 1.6*** | 0.01** | 4.7*** |
| **S × W** | 6 | ns | 0.2*** | 0.01*** | 5.08*** |
| **S × I** | 3 | ns | 0.2*** | 0.01*** | ns |
| **W × I** | 2 | ns | 0.7*** | 0* | 1.07* |
| **S × W × I** | 6 | ns | 0.2*** | 0.01*** | ns |

(*) Significant at $P \leq 0.05$, (**) Significant at $P \leq 0.01$, and (***) Significant at $P \leq 0.001$
Ns (not significant at $P \leq 0.05$).

**Table 2. Effect of salt, irrigation intervals and charcoal rot on the $Na^+$ concentration (%) in shoots of sorghum.**

| NaCl levels (mg $kg^{-1}$ soil) | Irrigation intervals (day) | | | | | |
|---|---|---|---|---|---|---|
| | 3 | | 7 | | 10 | |
| | -Mp inoculum | +Mp inoculum | - Mp inoculum | +Mp inoculum | -Mp inoculum | +Mp inoculum |
| 0 | 0.1o* | 0.3m | 0.1o | 0.2n | 0.1o | 0.2n |
| 1400 | 1f | 1.2c | 0.8h | 0.9g | 0.5l | 0.6k |
| 2100 | 1.1d | 1.3b | 1f | 1f | 0.7j | 0.7j |
| 2800 | 1.3b | 1.4a | 1f | 1.1d | 0.7j | 0.8h |

* Means followed by the same letters are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

**Table 3. Effect of irrigation intervals and charcoal rot on the $Cl^-$ concentration (%) in shoots of sorghum.**

| Irrigation intervals (day) | Inoculum | | NaCl levels (mg $kg^{-1}$ soil) | | | |
|---|---|---|---|---|---|---|
| | -Mp | +Mp | 0 | 1400 | 2100 | 2800 |
| 3 | 5.07a* | 4.2ab | 3.11ef | 2.76efg | 4.24cd | 8.43a |
| 7 | 3.36ab | 3.39b | 1.99gh | 2.59fg | 3.61de | 5.85b |
| 10 | 3.13b | 2.89b | 1.45h | 2.58fg | 3.36ef | 4.64c |

* Means followed by the same letters are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

significant ($P \leq 0.001$) decrease in shoot $K^+$ concentration with applied salt (Table 4). At any salinity level, infected sorghums accumulated more $Na^+$ and $Cl^-$ and less $K^+$ than non-infected ones.

In non-infested soil, water stress decreased the shoot $Na^+$ and $Cl^-$ concentrations (Tables 2 and 3). Conversely, the $K^+$ concentration tended to be increased with increasing irrigation intervals (Table 4).

At any irrigation intervals, a higher $Na^+$ and $Cl^-$ and a lower $K^+$ concentrations were observed in infected than in non-infected sorghums (Tables 2-4).

In non-salinized treatments, a reduction in the shoot dry weight with increasing irrigation intervals was observed, however the differences were not statistically significant (Table 5).

The results also suggest that shoot dry weight was significantly ($P \leq 0.001$) decreased with increasing NaCl levels at each irrigation interval (Table 5). Increasing irrigation intervals reduced salt injuries. The highest amount of NaCl (2800 mg NaCl $kg^{-1}$ soil) with 3 d irrigation interval caused a maximum reduction in shoot dry weight. In all the cases, the reduction of shoot dry weight was more in infected plants than in non-infected ones.

At the end of the experiment, crown and root of plants were evaluated for visible charcoal rot symptoms. No visible disease symptoms were observed in infected plants with 3 d irrigation treatment, while with 7 and 10 d irrigation intervals the typical charcoal rot symptoms were observed (data not shown). The results of the present study showed that there was less disease development in 7 d treatment, as irregular lesions being extended across the stem and root, whereas the symptoms in 10 d irrigation intervals was tissue disintegration in lower portion of stems.

The mean percentage of plants with infected crown and root (percentage of crown and root in which *M. phaseolina* was isolated) are shown in Figs 2 and 3. In non-salinized treatments, colonization by *M. phaseolina* was negatively correlated with soil water content. In other words, the percentage of colonization significantly ($P \leq 0.001$) increased by increasing irrigation intervals. However, crown had more infection than root. In our study, more incidence of infected crown and root was observed with increasing NaCl up to 1400 mg $kg^{-1}$ soil but any further increase significantly ($P \leq 0.001$) decreased colonization (Figs. 2 and 3). Our results indicate that infection by *M. phaseolina* caused plants to accumulate higher $Na^+$ and $Cl^-$ and lower $K^+$ with increasing salinity level, while increasing irrigation intervals resulted in an opposite trend. In addition, increasing irrigation intervals increased crown and root colonization.

Soil moisture deficit (SMD) at the various irrigation intervals and NaCl levels is shown in Table 6. SMD is approached to zero when soil moisture content is more. The mean of soil moisture depletion for each irrigation interval was calculated using the mean of applied water (Table 8) during the 7 wk irrigation treatment (Table 7). To determine the SMD during the growth period, the mean soil moisture depletion at each irrigation interval was divided by the maximum accessible moisture for 5000 g soil /pot (709ml) (Equation 1). For example, in the 3 d irrigation interval and 1400 mg $kg^{-1}$ soil, the mean of applied water was 6000 $cm^3$. Thus, the mean of applied water and SMD at each irrigation interval during the irrigation treatments were 375 $cm^3$ and 52.8% according to $6000/(48\div3)$ and $(375/709)\times100$, respectively.

According to Table 7, at each irrigation interval the loss of soil moisture in non-infected treatments was more than that of infected ones. It indicates that in non-infected plants, roots were intact and therefore water uptake was more. Also, in each irrigation interval, the loss of soil moisture was reduced with increasing salinity showing detrimental effect of salinity on the roots as well as water uptake limitation. In addition, the higher ion concentrations caused more reduction in soil water osmotic potential.

At the end of the experiment, the electrical conductivity of saturated soil extract ($EC_e$) in both non-salinized and salinized treatments was measured at three parts of the pots (Table 9). In salinized treatments, at 3 d irrigation interval, the highest $EC_e$ was

**Table 4. Effect of salt, irrigation intervals and charcoal rot on the $K^+$ concentration (%) in shoots of sorghum.**

| NaCl levels (mg $kg^{-1}$ soil) | Irrigation intervals (day) | | | | | |
|---|---|---|---|---|---|---|
| | 3 | | 7 | | 10 | |
| | - Mp inoculum | + Mp inoculum | - Mp inoculum | + Mp inoculum | - Mp inoculum | + Mp inoculum |
| 0 | 0.65bc* | 0.6cd | 0.71ab | 0.67bc | 0.73a | 0.7ab |
| 1400 | 0.56def | 0.56defg | 0.61cd | 0.57def | 0.72ab | 0.65bc |
| 2100 | 0.5efgh | 0.4k | 0.53efgh | 0.45ijk | 0.5efgh | 0.5fgh |
| 2800 | 0.39k | 0.4k | 0.44ijk | 0.42jk | 0.49ghij | 0.44ijk |

* Means followed by the same letters are not significantly different at $P \leq 0.05$

**Table 5. Effect of salt, irrigation intervals and charcoal rot on shoot dry weight (g/pot) of sorghum.**

| NaCl levels (mg $kg^{-1}$ soil) | Irrigation intervals (day) | | | | | |
|---|---|---|---|---|---|---|
| | 3 | | 7 | | 10 | |
| | -Mp inoculum | +Mp inoculum | - Mp inoculum | +Mp inoculum | -Mp inoculum | +Mp inoculum |
| 0 | 4.1a* | 3.87ab | 3.77ab | 3.57abc | 3.37abc | 3abcd |
| 1400 | 2.64abcde | 2.07cdefgh | 2.95abcd | 2.6abcdef | 3.07abcd | 2.65abcde |
| 2100 | 1.8defgh | 1.77defgh | 2.45bcdefg | 2.07cdefgh | 3abcd | 2.52bcdefg |
| 2800 | 1.05gh | 0.8h | 1.27efgh | 1.07fgh | 1.62defgh | 1.62defgh |

* Means followed by the same letters are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

**Table 6. Depletion of soil water holding capacity (%) at the various irrigation intervals and NaCl levels.**

| NaCl levels (mg $kg^{-1}$ soil) | Irrigation intervals (day) | | | | | |
|---|---|---|---|---|---|---|
| | 3 | | 7 | | 10 | |
| | - Mp inoculum | + Mp inoculum | - Mp inoculum | + Mp inoculum | - Mp inoculum | + Mp inoculum |
| 0 | 66.9 | 65.5 | 83.2 | 77.5 | 95.2 | 91.6 |
| 1400 | 54.3 | 52.8 | 77.5 | 71.9 | 90.9 | 86 |
| 2100 | 51.4 | 47.9 | 76.8 | 66.9 | ۸۷,۴ | 76.1 |
| 2800 | 45.8 | 36.6 | 64.8 | 59.9 | 79.5 | 71.9 |

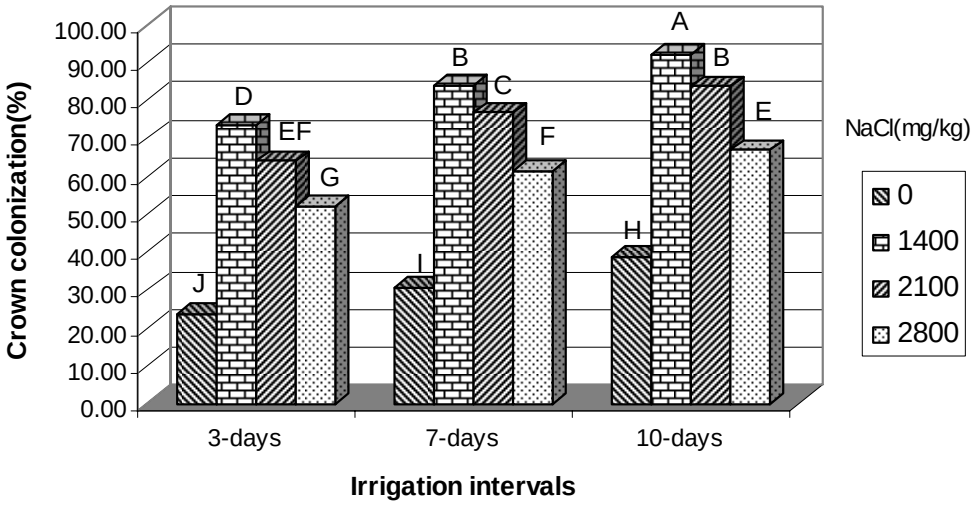


**Fig. 2. Effect of salt and irrigation intervals on crown colonization by *Macrophomina phaseolina*. Columns followed by the same letters are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.**

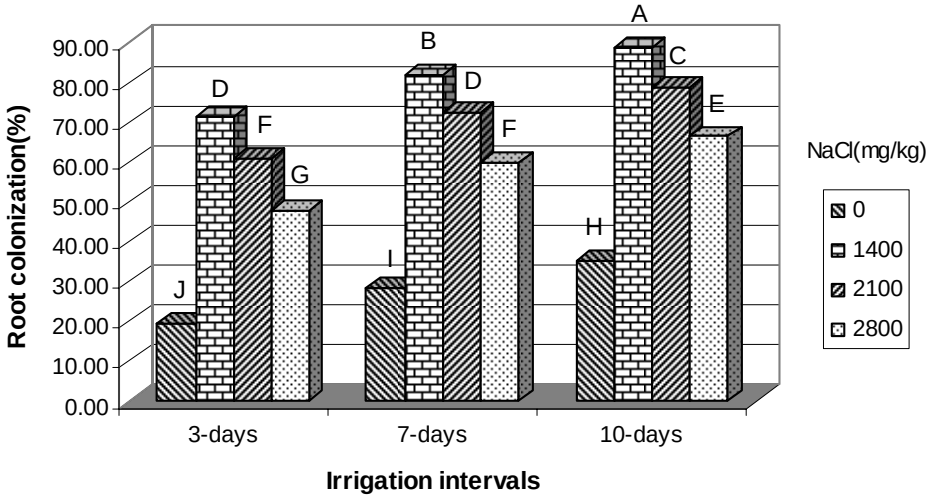


**Fig. 3. Effect of salt and irrigation intervals on root colonization by *Macrophomina phaseolina*. Columns followed by the same letters are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.**

**Table 7. The mean of soil water loss ($cm^3$) during the irrigation treatments.**

| NaCl levels (mg $kg^{-1}$ soil) | Irrigation intervals (day) | | | | | |
|---|---|---|---|---|---|---|
| | 3 | | 7 | | 10 | |
| | - Mp inoculum | + Mp inoculum | - Mp inoculum | + Mp inoculum | - Mp inoculum | + Mp inoculum |
| **0** | 475 | 465 | 590 | 550 | 675 | 650 |
| **1400** | 385 | 375 | 550 | 510 | 645 | 610 |
| **2100** | 365 | 340 | 545 | 475 | 620 | 540 |
| **2800** | 325 | 260 | 460 | 425 | 565 | 510 |

**Table 8. The mean of applied water ($cm^3$) during the irrigation treatments at the various irrigation intervals and NaCl levels.**

| NaCl levels (mg $kg^{-1}$ soil) | Irrigation intervals (day) | | | | | |
|---|---|---|---|---|---|---|
| | 3 | | 7 | | 10 | |
| | - Mp inoculum | + Mp inoculum | - Mp inoculum | + Mp inoculum | - Mp inoculum | + Mp inoculum |
| **0** | 7600 | 7440 | 4012 | 3740 | 3240 | 3120 |
| **1400** | 6160 | 6000 | 3740 | 3468 | 3096 | 2928 |
| **2100** | 5840 | 5440 | 3706 | 3230 | 2976 | 2592 |
| **2800** | 5200 | 4160 | 3128 | 2890 | 2712 | 2448 |

**Table 9. Electrical conductivity ($EC_e$) (dS $m^{-1}$) of saturated soil extract in control various salinity treatments.**

| NaCl levels (mg $kg^{-1}$ soil) | Irrigation intervals (day) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 3 | | | 7 | | | 10 | | |
| | upper | middle | bottom | upper | middle | bottom | upper | middle | bottom |
| **0** | 0.83 | 0.94 | 0.97 | 0.9 | 0.95 | 0.805 | 0.89 | 0.98 | 0.8 |
| **1400** | 2.73 | 5.45 | 9.5 | 6.41 | 6.62 | 4.91 | 6.76 | 8.97 | 3.51 |
| **2100** | 3.79 | 8.17 | 11.72 | 7.38 | 7.23 | 6.61 | 7.21 | 13.42 | 12.15 |
| **2800** | 4.85 | 10.88 | 13.93 | 8.34 | 7.84 | 8.3 | 7.64 | 17.87 | 15.78 |

obtained at the bottom third of the pots. It indicates that during irrigation, salts had accumulated in the bottom of the pots, where the roots were active. While in 7 and 10 d irrigation intervals the salt amount in middle part of the pots was more where the end of nylon tubing was placed that shows inadequate of irrigation water to wash the salt.

## Discussion

As demonstrated in this work, the percentage of plants that developed charcoal rot was negatively correlated with the soil water content during the experiment. In other words, the predisposing of plants to the disease was greater at low than high soil water content. These results indicate the inhibitory effect of higher soil moisture on infection of crown and root and are in agreement with the previously published reports (Ma *et al*. 2001, Mayek-Prez *et al*. 2002, Triky-Dotan *et al*. 2005). Defense mechanisms may be slowed down more in water-stressed than in non-stressed plants (Blodgett *et al*. 1997). Water stress cause many complex changes in plant metabolism. For instance, stress can affect cell growth, cell division, cell wall synthesis, and plant hormonal balance, and these may affect plant resistance to diseases (Schoeneweiss, 1981). In sorghum, resistance to *M. phaseolina* depends on the maintenance of physiological vigor caused by stable transpiration rates (Edmunds 1964).

In addition, drought stress increases the negative effects caused by *M. phaseolina* in plants (Mayek-Perez *et al*. 2002). More infection under water stress may result from the effect of matric potential on this fungus. Sclerotial germination and mycelial growth of

*M. phaseolina* increase with decreasing matric potential up to -1.2 MPa (Goudarzi *et al*. 2008). There is a rapid loss of viability of *M. phaseolina* sclerotia at high soil water levels (Shokes *et al*. 1977). Saturated conditions may also affect the sclerotial survival of other plant-pathogenic fungi, including *Sclerotinia minor* (Abawi *et al*. 1985), *Verticillium dahliae* (Ioannou *et al*. 1977), and *Rhizoctonia solani* (Ploetz and Mitchell 1985). Reduction of the survival of sclerotia at higher soil water potentials is related to the inability of sclerotia to regulate their water content and the absence of constitutive dormancy (Cook and Al-Hamdani 1986).

Similar charcoal rot symptoms that were observed in this work, were found in other susceptible hosts (Mayek-Perez *et al*. 2002). In our view, crown and root charcoal rot that were not expressed in 3 d irrigation interval suggesting the possibility of a latent or quiescent infection. This pathogen apparently infects early, but remains latent until the host is stressed, at which time the disease progresses rapidly. Subjecting sorghum plants to water stress after inoculation and during reproductive growth may ensure that any latent infections develop scorable regions. Drought stress (high soil temperatures and low soil moistures) caused a marked reduction in total stalk sugars in sorghums which correlated with increased development of charcoal rot. The infected plants show early maturity, reduced head size, and less number of grains setting. Visibility of the symptoms depends upon the severity of infection (Khan 2007).

Data showed that increasing soil salinity results in growth characteristics reduction such as shoot dry weight and a greater reduction occurred in infested soil, while increasing irrigation intervals did not statistically affected plants growth. Sorghum has been considered to be a most drought-resistant (Bhaskaran *et al*. 1985) and a moderately salinity-tolerant crop (Dashti *et al*. 2009). Possibly the extensive waxy bloom on the lower surface of sorghum leaves increases the boundary layer resistance. Although the transpiration rate of sorghum is high when well watered, it is quickly reduced when the plants are subjected to water stress. In sorghum, the upper leaf surface bears only about two-thirds as many stomata as the lower surface. The stomata on the upper surfaces of well watered plants appear more sensitive to environmental factors than those on the lower surfaces (Bhaskaran *et al*. 1985).

As stated earlier, plant responses to salinity and water deficit are closely related with several overlapping mechanisms. One of the physiological responses to drought and salinity is the accumulation of intracellular organic solutes. This increase is considerable for proline (Dashti *et al*. 2009). There is a strong correlation between increased cellular proline levels and the capacity to survive both water deficit and the effects of high environmental salinity. It may also serve as an organic nitrogen reserve that can be utilized during recovery (Sairam and Tyagi 2004). Without this mechanism, it is doubtful that the sorghum plants would have continued to add biomass (McCre 1986).

The effect of salinity on plant growth is a complex syndrome that involves osmotic stress, ion toxicity, and mineral deficiencies (Netondo *et al*. 2004). Reduction in dry weight of plant tissues reflects the increased metabolic energy cost and reduced carbon gain, which are associated with salt adaptation (Netondo *et al*, 2004; Richardson and McCree 1985). It also reflects salt impact on tissues (Greenway and Munns, 1980), reduction in photosynthetic rates per unit of leaf area (McCree 1986, Netondo *et al*. 2004), and attainment of maximum salt concentration tolerated by the fully expanded leaves (Munns 2002). Leaf growth is generally more sensitive than the root growth. Reduced leaf expansion is beneficial to plants under water deficit condition, as less leaf area is exposed resulting in reduced transpiration. In

accordance, many mature plants, for example cotton subjected to drought respond by accelerating senescence and abscission of the older leaves. This process is also known as leaf area adjustment (Mahajan and Tuteja 2005).

There was a significant correlation ($P \leq 0.001$) between colonization of crown and root and increasing salt up to 1400 mg $kg^{-1}$ soil. High salt may impose an environmental stress and affect plant physiology via morphological, anatomical, metabolical, and biochemical changes, such as water relations, ion homeostasis, salt accumulation, metabolic pathways, enzymes, and nucleic acids (Hasegawa *et al*. 2000). The above changes might be associated with increased susceptibility to the pathogen (Rasmussen and Stanghellini 1988, Triky-Dotan *et al*. 2005). The response to soil salt found in our experiment, are consistent with the previous work demonstrating enhanced charcoal rot in melon exposed to salt (Nischwitz *et al*. 2002). Thus, high salt levels may be a factor in charcoal rot development leading to higher disease incidences.

Salt alone tended to increase $Na^+$ and $Cl^-$ concentrations (Snapp *et al* 1991). Sodium is the major cation that accumulated in sorghum roots and stems as NaCl increased (Netondo *et al*. 2004). High $Na^+$ concentration strongly inhibited uptake and accumulation of $K^+$ and $Ca^{2+}$ and to a lesser extent of $Mg^{2+}$ by roots. Because $K^+$ is a macronutrient involved in turgor control, inhibition of $K^+$ uptake should stunt growth (Renault *et al*. 2001). Also, $K^+$ frequently is connected with resistance to pathogens (Munns 2002).

High $Na^+$ levels in the external medium greatly reduce the physiochemical activity of dissolved calcium and may thus displace $Ca^{2+}$ from the plasma membrane of root cells (Cramer *et al*. 1985). In turn, displacement of $Ca^{2+}$ from root membranes by $Na^+$ affects Na/K uptake selectivity in favor of sodium. A low $Ca^{2+}$ concentration under saline conditions may severely affect the functions of membranes as barriers to ion loss from cells (Boursier and Läuchli 1990). Also, the altered ions and water relations have a severe impact on the photosynthetic performance of the plant (Netondo *et al* 2004).

There are various ways by which plants can keep endogenous levels of ions like $Cl^-$ and $Na^+$ low. Reduced influx at the root cell plasma membrane, efflux from roots, and retranslocation from the leaves to roots are possible mechanisms. In addition, salt tolerance during accumulation of $Na^+$ and $Cl^-$ at the cellular level can be achieved through loading in vacuoles. Sequestration of $Cl^-$ and $Na^+$ in the leaf sheath of grasses is another mechanism of salt tolerance. Exclusion of $Na^+$ from leaf blades protects the delicate photosynthesizing tissues as much as possible from the potentially toxic ion. Increased NaCl treatments reduced $K^+$ concentration in leaf sheaths but not in leaf blades (Netondo *et al*., 2004). According to Munns (2002), osmotic stress is effective in the beginning of exposure to salt, and ion toxicity becomes important in affecting plant growth after prolonged exposure.

Our observation showed that root infection affected ion uptake. Under salt stress, higher amount of $Na^+$ and $Cl^-$ and lower amount of $K^+$ was accumulated in plant tissue when fungus was present. Young leaves were able to maintain a low Na/K ratio even under the combination of high salt and greatest disease development. The only discernable effect of inoculation was to increase concentration of chloride in young leaves at the end of the season. Tomatoes may have developed mechanisms to block off ion uptake through infected tissue or to reduce toxic ion accumulation by ion-recirculation and root exudation. Sodium uptake and exudation rates were both accelerated in safflower plants with severe Phytophthora root rot (Snapp *et al.* 1991).

Our results showed that the interaction between soil water and salt stress affected ion

concentration in different ways. Salt increased $Na^+$ and $Cl^-$ but decreased $K^+$ accumulation, whereas increasing irrigation intervals reversed them. This data of $Na^+$ and $Cl^-$ concentration support by finding of Saadatmand *et al*. (2008). They presented evidence that effect of salt treatment in predisposing shoot and root colonization to Verticillium wilt was greater at high than at low soil water content.

At present, increasing irrigation intervals reduced salt injury, so that trend of growth reduction was decreased in low soil water content. Salinized plants have greater ability to continue leaf expansion and carbon gain under water stress that can be attributed primarily to slower development of water stress, which prolonged the osmotic adjustment. The slower water stress development in the salinized plants is due to a lower water loss rate per plant, which in turn was due to both smaller leaf area and reduced water loss per unit of leaf area. Under water stress, the water loss decreased less rapidly in the salinized plants because of the less rapid decrease in water potential, and the greater volume of water remaining in the growth medium. Thus, the combined effect of salinity and water stress may be less detrimental to plant growth and carbon gain than sum of the separate effects of salinity and water stress (Richardson and McCree 1985). Such result from interactive effects of salt and water stress was reported by Saadatmand *et al.* (2008) in pistachio. It is concluded that although irrigation of sorghum with saline water inhibits plant growth in comparison with non-saline water, it also inhibits water loss and allows a greater degree of osmotic adjustment, so that the plant are able to continue growing longer and reach lower leaf water potentials between irrigations. As shown in the results, the greatest amount of shoot $Na^+$ or $Cl^-$ and $K^+$ accumulation was obtained in 3 and 10 d irrigation intervals, respectively. The greater $Na^+$ and $Cl^-$ ions accumulation in the sorghum shoots at 3 d as compared to 7 and/or 10 d irrigation intervals was probably responsible for less salinity induced suppression with a rise in irrigation intervals.

As the results showed, the $EC_e$ of saturated soil extract depends on the irrigation intervals. Regarding the way for applying NaCl and irrigation through nylon tubing, soil salinity was greater at the bottom third of the pots and around the roots, but at the highest irrigation interval the greatest amount of NaCl accumulation was obtained at the middle third. Thus, it is the reason of reduced growth of plants under salt stress at the low irrigation interval.

In conclusion, artificially induced water stress identified as a major predisposing factor of sorghum to infection by *M. phaseolina*. Knowledge of the predisposition factors for charcoal rot development is essential for the elaboration of accurate screening methods (Olaya *et al*. 1996). Water management can have a significant effect on root colonization by *M. phaseolina.* Charcoal rot is readily controlled by irrigation despite high temperatures (Norton and Frank 1953). Given the importance of water stress for development of charcoal rot symptoms it seems essential that water stress be evaluated as a contributing variable when testing for resistance to charcoal rot (Diourt *et al*. 1995).

## References

ABAWI, G.S., GROGAN, R.G. and DUNIWAY, J. M. 1985. Effect of water potential on survival of sclerotia of *Sclerotinia minor* in two California soils. **Phytopathology** 75: 217-221.

BANIHASHEMI, Z. and deZEEUW, J.D. 1975. The behavior of *Fusarium oxysporum* f.sp. *melonis* in the presence and absence of host plants. **Phytopathology** 75: 1212-1217.

BHASKARAN, S., SMITH, R.H and Newton R.J. 1985. Physiological changes in cultured sorghum cells in response to induced water stress. **Plant Physiol.** 79: 266-269.

BLODGETT, J.T., KRUGER, E. L. and STANOSZ, G.R. 1997. Effects of moderate water stress on disease development by *Sphaeropsis sapinea* on red pine. **Phytopathology** 87: 422-428.

BOURSIER, P. and LAUCHLI, A. 1990. Growth responses and mineral nutrient relations of salt-stressed sorghum. **Crop Sci**. 30:1226–1233.

CHAPMAN, H.D. and Pratt, D.F. 1961. Method of analysis for Soil, Plant and Water. **Univ. Calif., Div. Agric. Sci**. pp 60-62.

COOK, R.C. and AL-HAMADANI, A.M. 1986. Water relation of sclerotia and other infective structures. Pp: 49-63 *In*: Ayres, P.G. and Boddy L. (Eds.), **Water, Fungi and Plants**. Cambridge University Press, Cambridge.

CRAMER, G.R., LAUCHLI, A. and POLITO, V.S. 1985. Displacement of Ca by Na from the plasmalemma of root cells. **Plant Physiol**. 79: 207–211.

DASHTI, A., KHAN, A.A. and COLLINS, J.C. 2009. Effects of salinity on growth, ionic relations and solute content of *Sorghum bicolor* (L.) Monench. **Plant Nutr.** 32: 1219–1236.

DIOURT, M., STARR, J.L., JEGER M.J., STACK, J.P. and ROSENOW, D.T. 1995. Charcoal rot (*Macrophomina phaseolina*) resistance and the effects of water stress on disease development in sorghum. **Plant Pathol**. 44: 196-202.

EDMUNDS, L.K. 1964. Combined relation of plant maturity, temperature, and soil moisture to charcoal stalk rot development in grain sorghum. **Phytopathology** 54: 514-517.

GIRMA F.S. and KRIEG, D.R.1992. Osmotic adjustment in sorghum.II. Relationship to gas exchange rates. **Plant Physiol**. 99: 583-588.

GOUDARZI, A., BANIHASHEMIa, Z. and MAFTOUN, M. 2008. Effect of water potential on sclerotial germination and mycelial growth of *Macrophomina phaseolina.* **Phytopathol. Mediterr**. 47: 107-114.

GREENWAY, R. H. and MUNNS, R. 1980. Mechanisms of salt tolerance in nonhalophytes. **Aust. J. of Plant Physiol.** 31: 149–190.

HASEGAWA, P., BRESSAN, R.A., ZHU, J.K. and BOHNERT, H.J. 2000.Plant cellular and molecular responses to high salt. **Annu. Rev. Plant Physiol. Plant Mol. Biol.** 51: 463-499.

IOANNOU, N., SCHNEIDER, R.W., GROGAN, R.G. and DUNIWAY, J.M. 1977. Effect of water potential and temperature on growth, sporulation and production of microsclerotia by *Verticillium dahliae*. **Phytopathology** 67: 637-644.

KHAN, S.N. 2007. *Macrophomina phaseolina* as causal agent for charcoal rot of sunflower. **Mycopathologia** 5: 111-118

MA, Z., MORGAN, D.P. and MICHLAILIDES, T.J. 2001. Effects of water stress on Botryosphaeria blight of pistachio caused by *Botryosphaeria dothidea*. **Plant Dis**. 85: 745-749.

MAHAJAN, S. and TUTEJA, N. 2005. Cold, salinity and drought stresses: An overview. **Arch. Biochem. Biophys**. 444: 139–158

MASS, E.V. 1986. Salt tolerance of plants. **Appl. Agric. Res**. 1: 12-26.

MAYEK- PERE, N. GARCIA-ESPINOSA, R., LOPEZ-CASTANED, C., ACOSTA-GALLEGO, J.A. and SIMPSON, J. 2002. Water relations, histopathology and growth of common bean

(*Phaseolus vulgaris* L.) during pathogenesis of *Macrophomina phaseolina* under drought stress. **Physiol. Mol. Plant Pathol**. 60: 185-195

McCREE, J.K. 1986.Whole-plant carbon balance during osmotic adjustment to drought and salt stress. **Aust. J. Plant Physiol**. 13: 33–43.

MIHAIL, J.D. 1992. Macrophomina. Pp: 134-136. *In*: **Methods for Research on Soilborne Phytopathogenic Fungi.** Singleton L. L., Mihail, J. D. and Rush C. M. (Eds.). APS Press.

MUNNS, R. 2002. Comparative physiology of salt and water stress. *Plant* **Cell Environ.** 20: 239-250.

NETONDO, G.W., ONYANGO, J.C. and BECK, E. 2004. Sorghum and salt: I. Response of growth, water relations, and ion accumulation to NaCl salt. **Crop Sci**. 44: 797-805.

NISCHWITS, C., OLSEN, M. and RASMUSSEN, S. 2002. Influence of salt and root-knot nematode as stress factors in charcoal rot of melon. Available Online: **http://ag.arizona.edu/pubs/crops/az1292/.**

NORTON, D.C. and FRANK, F.A. 1963. Charcoal rot (caused by *Sclerotium bataticola* Taub.) on guayule in southwest Texas in 1951 and 1952. **Plant Dis. Rep**. 37: 41-43.

OLAYA, G., ABAWI, G.S. and BARNARD, J. 1996. Influence of water potential on survival of sclerotia in soil and on colonization of bean stem segments by *Macrophomina phaseolina*. **Plant Dis**. 80: 1351-1354.

PEREZ-LPEZA´, U., ROBREDOA, A. LACUESTA, M., MENA-PETITA, A, MUNOZ – RUEDAA,u A. 2009. The impact of salt stress on the water status of barley plants is partially mitigated by elevated CO2. **Environ. Exper. Bot**. 66: 463–470.

PLOETZ, R.C. and MITCHELL, D.J. 1985. Influence of water potential on the survival and saprophytic activity of *Rhizoctonia solani* AG-4 in natural soil. **Can. J. Bot**. 63: 2364-2368.

RASMUSSEN, S.L. and STANGHELLINI, M.E. 1988. Effect of salt stress on development of Pythium blight in *Agrostis palustris*. **Phytopathology** 78: 1495-1497.

RENAULT, S., CROSER, C., FRANKLIN, J.A. and ZWIZEK, .J. 2001 Effect of NaCl and $Na_2SO_4$ on red-osier dogwood (*Cornus stolonifera* Michx). **Plant Soil** 233: 261–268.

RICHARDSON, S.G. and McCREE, K.J. 1985. Carbon balance and water relations of sorghum exposed to salt and water stress. **Plant Physiol**. 79: 1015-1020.

SAADATMAND A.R., BANIHASHEMI, Z., SEPASKHAH, A.R. and MAFTOUN, M. 2008. Soil Salinity and water stress and their effect on susceptibility to Verticillium wilt disease, ion composition and growth of pistachio. **Phytopathology** 156: 287-292.

SAIRAM, R.K. and TYAGI, A. 2004 Physiology and molecular biology of salinity stress tolerance in plants. **Curr Sci**. 86: 407-421.

SANOGO, S. 200. Response of chile pepper to *Phytophthora capsici* in relation to soil salt. **Plant Dis**. 88: 205-209.

SCHOENEWEISS, D.F. 1981. The role of environmental stress in diseases of woody plants. **Plant Dis**. 81: 308-314.

SHOKES, F.M., LYDA, S.D. and JORDAN, W.R. 1977. Effect of water potential on the growth and survival of *Macrophomina phaseolina.* **Phytopathology** 67: 239-241.

SHORT, G.E. and WYLLIE, T.D. 1978. Inoculum potential of *Macrophomina phaseolina*. **Phytopathology** 68: 742-746.

SMIRNOFF, N. 1998. Plant resistance to environmental stress. **Curr. Opin. Biotech.** 9: 214-219.

SNAPP, S.S., SHENNAN, C. and BRUGGEN, A.. 1991. Effect of salt on severity of infection by *Phytophthora parasitica* Dast., ion concentration and growth of tomato, *Lycopersicom esculentum* Mill. **New Phytol**. 119: 275-284.

TRIKY-DOTANo, S., YERMIYAHU, U., KATAN, J. and GAMLIEL, A. 2005. Development of crown and root rot disease of tomato under irrigation with saline water. **Phytopathology** 95: 1438-1444.

WALLER, J.M. 1986. Drought, irrigation and fungal diseases of tropical crops. Pp. 175-187 *In*: Ayres PG and L. Boddy. (Eds.), **Water, Fungi and Plants**. Cambridge University Press, Cambridge.

ZHANG, J., JIA, W., YANG, J. and ISMAIL, A.M. 2006. Role of ABA in integrating plant responses to drought and salt stresses. **Field Crop. Res.** 97: 111–119.