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Study of interaction between salinity and charcoal rot diseases of melon (*Macrophomina phaseolina*) in Semnan and Garmsar

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Abstract

Charcoal rot diseases of melon caused by *Macrophomina phaseolina* is one of the most important diseases of melon occurring in regions with variable climate. Reduction of yield of melon by this disease has been reported up to ١٠٠٪ in some of Garmsar field. Salinity which can cause the important trouble in plant metabolism and nutrition is present in Semnan and Garmsar areas. Melon is susceptible to salinity stress, therefore salinity stress may increase the susceptibility of melon to *M. phaseolina*. Different levels of salinity including, ٠, ١٠, ٢٥, ٥٠ and ٧٥ Mmol NaCl/l with and without fungal pathogen on two current cultivars of melon (Ivaneki and Susski) were investigated at the field conditions in Semnan and Garmsar areas. A factorial analysis for completely randomized design was used in these experiments with three replications. At all experiments, significant differences were observed among the different levels of any factors investigated including, cultivars, salinity and fungal pathogen presence. Interactions between investigated factors were significant too. The factors investigated in these experiments, clearly suggested the occurrence of a wide effect of salinity stress on charcoal rot development on melon cultivars and showed the importance of salinity management for decrease of disease severity.

Keywords: Melon*; Macrophomina phaseolina*; Salinity stress; Disease severity

١. Introduction

Charcoal rot of melon (*Macrophomina phaseolina* (Tassi) Goid.), a soil born, microsclerotia producing fungus is common in tropical and subtropical regions. *M. phaseolina* causes a root and stem rot on a large number of host plants including sorghum, sunflower, corn, melon and beans (Ghaffr and Erwin, ١٩٦٩; Diorte, *et al.*, ١٩٩٥; Shahid, *et al.*, ٢٠٠٣). Fungal pathogen generally infects many crops that are subject to severe stress caused by water and drought (Cook and Papendick, ١٩٧٢; Hoorn, *et al.*, ١٩٩٣; Duniway, ١٩٧٧), high temperatures and flowering (Edmonds, ١٩٦٤) and salinity (Roustaee, ٢٠٠٧). Charcoal rot causes crown decline and root rot in melon. *M. phaseolina* commonly infects the lower stem at the soil line causing water-soaked lesions that girdle the stem, but it also may cause root rot (Zitter, ١٩٩٦). It has been previously described as the most serious disease of melons in the dry and saline soils (Bruton and Heald, ١٩٨٧; Bruton and Miller ١٩٩٧; Carter, ١٩٧٩). More recently, vine decline of melon has been attributed to several soilborne pathogens including *M. phaseolina*, but pathogens included in the vine decline complex vary (Aegerter *et al*., ٢٠٠٠;; Zitter, *et al*., ١٩٩٦). Salinity stress increase Phytophthora on root and crown of rhododendron (Blaker and Mc Donald, ١٩٨١).

In the last few years, charcoal rot has become an important problem for melon growers. Disease incidence has increased in drip-irrigated fields, whereas it has remained virtually

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unknown in furrow irrigated fields. It has appeared in drip-irrigated fields that have never been planted with melons, and has become increasingly widespread in fields with short rotations back to melons. Since disease is often associated with stress factors, it was puzzling why the disease appeared in drip-irrigated melons.

Salinity can cause difficulty on plant metabolism, nutrition and water absorption and also can cause susceptibility to charcoal disease. The purpose of this study was to determine if increased salinity at field conditions contribute to increased incidence of charcoal rot of melon.

۲. Materials and Methods

Fungal pathogen was isolated from infected melon obtained in Semnan and Garmsar areas of Iran, and distinguished as *M. Phaseolina*.

Studies were established in Semnan and Garmsar desert areas of Iran. In all experiments, ۲ cultivars of melon were planted into pots situated into the field soil. All plants were irrigated daily with Morard nutrition solution treated by different level of salinity as treatments (Morard, ۱۹۹۵).

Standard solution include ۷ meq/l K+, ۱۰ meq/l Ca++, ۳meq/l Mg++, ۱۵meq/l NO۳-, ۲meq/l H۲PO٤- and ۳meq/l SO٤--. Microelements and ferre in nutrition solution were used according Morard (۱۹۹۵), too.

At first, pre-determined ratios of NaCl were used on melon seeds germinating to adjust salinity levels ۰, ۱۰, ۲۵, ۵۰ and ۷۵ Mmol/lit.

Different levels of salinity including, ۰, ۱۰, ۲۵, ۵۰ and ۷۵ Mmol NaCl/l with fungal pathogen and ۰ Mmol NaCl/l without fungal pathogen on two current cultivars of melon (Ivaneki and Susski) were investigated in field conditions in Semnan and Garmsar areas. A factorial analysis for completely randomized design with three replications, was used in these experiments. A factor had two levels including absence and presence of fungal pathogen, B factor were two cultivar of melon including Ivaneki and Susski and C factor were different levels of salinity including, ۰, ۱۰, ۲۵, ۵۰ and ۷۵ Mmol NaCl/l.

M. phaseoli was isolated on potato dextrose agar medium used as inoculum in all experiment. After ۱٤ days, plants inoculated by one plug of *M. phasolina* according Roustaee *et al.* ۲۰۰۷. Alive plants were numbered ۱٤ days after inoculation with *M. phaseolina*. Each replication was three pot with ۱۵ plants. To make sure that plants had died by charcoal rot disease, Koch test was done.

۳. Results and Discussion

Salinity stress increased the severity and incidence of charcoal rot significantly in all experiments (Table ۱, ۲, ۳, ٤, ۵ and ٦). The symptoms of *M. phaseolina* appeared one week after treatments.

At all experiments, significant differences were observed among the different levels of any factors investigated including, cultivars, salinity and fungal pathogen presence. Interactions between investigated factors were significant too(Table ۱, ۲, ۳, ٤, ۵ and ٦). Non-inoculated plants as control, were smaller when irrigated at the highest salinity compared to inoculated plants. The results indicated that charcoal rot of melon increase under salinity stress.

Table ۱ and ۲ showed that, there was significant differences between absence and presence of fungal pathogen and interaction between this factor (A) and different levels of salinity including, ۰, ۱۰, ۲۵, ۵۰ and ۷۵ Mmol NaCl/l were significant too. In this table difference between cultivars and different levels of salinity was significant too.

Table ۳ showed that, there was significant differences between absence and presence of fungal pathogen and between different levels of salinity. Interaction between absence and presence of fungal pathogen and cultivars was significant in this table too. Interaction between absence and presence of fungal pathogen and different levels of salinity was significantly different too. Interaction between two factors (cultivar and salinity) and between three factors including absence and presence of fungal pathogen, cultivars and different levels of salinity including, ۰, ۱۰, ۲۵, ۵۰ and ۷۵ Mmol NaCl/l were significant too.

Effects of salinity stress on plant activities such as resistance to another stress including resistance to plant diseases were reported by different authors (Edmonds, ۱۹٦٤; Robinson, ۱۹۷۱; Bernstein, ۱۹۷۵; Edmonds, ۱۹٦٤; Roustaee *et al.*, ۲۰۰۷; Poljakoff-Mayber, ۱۹۷۵; O,Leary, ۱۹٦۹; Campbell and Pitman, ۱۹۷۱).

Role of environmental stress on incidence and development of plant diseases is very important and management of any environmental factors such as salinity, nutrition, soil and water acidity, etc… can leading to decrease of plant diseases severity (Epstein, *et al.*, ۱۹۸۰; Kylin, and Quatrano ۱۹۷۵; Robinson, ۱۹۷۱; Bernstein,

١٩٧٥; Edmonds, ١٩٦٤; Diorte *et al.*, ١٩٩٥; Roustaee *et al.*, ٢٠٠٧; Toussoum, ١٩٧٠).

Environmental factors such as salinity (Both, ١٩٧١; Doudman, ١٩٧٠), acidity etc… not only cause plant trouble activities but also affect fungal biology (Besri, ١٩٩٧). In plant diseases management, management of plant nutrition is very important and salinity strongly affect plant nutrition (Roustaee *et al.*, ٢٠٠٧).

Results indicate that, high salinity levels was a factor in charcoal rot development leading to higher disease incidences. This may explain the increased incidence of charcoal rot in subsurface drip-irrigated melons where soil salinity increases near the soil surface and at the wetting front of the drip zone creating an environment conducive to disease development.

Results showed that, salinity stress increased the severity and incidence of charcoal rot significantly in all experiments (Table ١-٦). Such result were reported by Nischwitz *et al.*, (٢٠٠٢). He reported effect of salinity on melon diseases severity such as charcoal rot caused by *M. phaseolina*.

Increase of salinity stress increase tomato seedling diseases such as *Rhizoctonia solani* and *Fusarium oxysporum f. sp. lycopersici* and *Phytophthora cryptogea* on chrysanthemum root (Mc Donald, ١٩٨٢). Effect of osmotic stress on causal agent and salinity stress on host were reported as cause of disease increasing by these authors respectively. Salinity stress can affect root exudates, which can effect *Phytophthora* zoospore landing on alfalfa root surfaces (Kuan and Erwin, ١٩٨٠; Zentmyer, ١٩٨٠) and corn roots (Hinch and Clarke, ١٩٨٠).

Results of greenhouse experiments showed that increasing of salinity stress increase melon susceptibility to *M. phaseolina* significantly (Roustaee *et al.*, ٢٠٠٧). Results were confirmed in field conditions in this research.

Table ١. Variance analysis of interaction between salinity levels (٠, ١٠, ٢٥, ٥٠, ٧٥ Mmol NaCl/lit) and charcoal rot disease (*M. phaseolina*) on two melon cultivars at presence and absence of fungal pathogen in field conditions at Garmsar areas

S.O.V	df	SS	MS	F
Fungal presence (A)	١	١٠١٣٧,٩٢٠	١٠١٣٧,٩٢	٢٣٦,٠٠٠٩ **
Cultivars (B)	١	١٠١٤,٥١٣	١٠١٤,٥١٣	٢٣,٦١٦٩ **
A x B	١	٦,٦٠٠	٦,٦٠٠	٠,١٥٣٦ n.s
Salinity (C)	٤	٢٠٢١٦,٦١٤	٥٠٥٤,١٥٣	١١٧,٦٥٥٨ **
A x C	٤	٩٨٢,٤٥٨	٢٤٥,٦١٥	٥,٧١٧٧ **
B x C	٤	٤,٤١٤	١,١٠٣	٠,٠٢٥٧ n.s
A x B x C	٤	١٢٢,٩٤٩	٣٠,٧٣٧	٠,٧١٥٥ n.s
Error	٤٠	١٧١٩,٢٨٥	٤٢,٩٥٧	
Total	٥٩	٣٤٢٠٣,٧٥٢		

n.s, **: non-significan and, significant at ١% of probability, respectively, Cv=١١,٧٧

Table ٢. Variance analysis of interaction between salinity levels (٠, ١٠, ٢٥, ٥٠, ٧٥ Mmol NaCl/lit) and charcoal rot disease (*M. phaseolina*) on two melon cultivars at presence and absence of fungal pathogen in field conditions at Semnan areas (experiment ١)

S.O.V	df	SS	MS	F
Fungal presence (A)	١	٢٠١٦٥,٥٦٧	٢٠١٦٥,٥٦٧	٥٧٩,١٦٢١ **
Cultivars (B)	١	٢٦٧,٤٢١	٢٦٧,٤٢١	٧,٦٧٠٤ **
A x B	١	٣٦,٢٧٠	٣٦,٢٧٠	١,٠٤١٧ n.s
Salinity (C)	٤	٢٧٠٧٢,٨٤٤	٦٧٦٨,٢١١	١٩٤,٣٨٥٤ **
A x C	٤	٢٠٢٢,٠٦٧	٥٠٥,٥١٧	١٤,٥١٨٦ **
B x C	٤	٦٩,٦٦٤	١٧,٤١٦	٠,٥٠٠٢ n.s
A x B x C	٤	٩٣,٣٧٨	٢٣,٣٤٤	٠,٦٧٠٥ n.s
Error	٤٠	١٣٩٢,٧٤١	٣٤,٨١٩	
Total	٥٩	٥١١١٩,٩٥٢		

n.s, **: non-significan and, significant at ١% of probability, respectively , Cv=١١,٢٣

Table ٣. Variance analysis of interaction between salinity levels (٠, ١٠, ٢٥, ٥٠, ٧٥ Mmol NaCl/lit) and charcoal rot disease (*M. phaseoli*)
on two melon cultivars at presence and absence of fungal pathogen in field conditions at Semnan areas (experiment ٢)

S.O.V	df	SS	MS	F
Fungal presence (A)	١	١٦٦٦٦,٣٣٤	١٦٦٦٦,٣٣٤	٢١٦,٣١٥٨ **
Cultivars (B)	١	٧٤,٠٨١	٧٤,٠٨١	٠,٩٦١٥ n.s
A x B	١	٣٥٨,٥٣٥	٣٥٨,٥٣٥	٤,٦٥٣٥ *
Salinity (C)	٤	٢٧١٥٠,٩٨٧	٦٧٨٧,٧٤٧	٨٨,٠٩٩٦ **
A x C	٤	١٢٢٢,٣٣٣	٣٠٥,٥٨٣	٣,٩٦٦٢ **
B x C	٤	٣٢٥١,٨١٥	٨١٢,٩٥٤	١٠,٥٥١٥ **
A x B x C	٤	٢٥٣٧,٧٦٩	٦٣٤,٤٤٢	٨,٢٣٤٦ **
Error	٤٠	٣٠٨١,٨٥٢	٧٧,٠٤٦	
Total	٥٩	٥٤٣٤٣,٧٠٦		

n.s, * and **: non-significant, significant at ٥٪ and ١% of probability, respectively, Cv=١٧,٧١

Decrease of salinity levels by drainage measures and using of organic amendment etc… and management of salinity, leading to charcoal rot management. Effect of environmental factors such as salinity, humidity (irrigation), nutrition, acidity etc… must to be investigated on charcoal rot development leading to *Macrophomina phaseolina* management on melon cultivars.

Table ٤. Means comparison of interaction between salinity levels (٠, ١٠, ٢٥, ٥٠, ٧٥ Mmol NaCl/lit) and charcoal rot disease (*M phaseolina*) on two melon cultivars at presence and absence of fungal pathogen in field conditions at Garmsar areas

Treatments	Means C	Means A x C
١٠ mmol NaCl	٦٨,٣٣ B	٧٧,٧٨ B
٢٥ mmol NaCl	٥٨,٣٣ C	٦٧,٧٧ BC
٥٠ mmol NaCl	٤٢,٢٢ D	٥٣,٣٣ D
٧٥ mmol NaCl	٢٨,٨٩ E	٤٨,٨٩ D
٠ mmol NaCl	٨٠,٥٥ A	٩٥,٥٥ A
١٠ mmol NaCl+fungus		٥٨,٨٩ CD
٢٥ mmol NaCl+fungus		٤٨,٨٩ D
٥٠ mmol NaCl+fungus		٣١,١١ E
٧٥ mmol NaCl+fungus		٨,٨٩ F
٠ mmol NaCl+fungus		٦٥,٥٦ C

Means followed by similar letters are not significantly different (Duncan ١٪). Means indicate percentages of alive plants ١٤ days after inoculation with *M. Phaseolina* and without fungus (A) under different salinity levels (C).

Table ٥. Means comparison of interaction between salinity levels (٠, ١٠, ٢٥, ٥٠, ٧٥ mmol NaCl/lit) and charcoal rot disease (*M. phaseolina*) on two melon cultivars at presence and absence of fungal pathogen in field conditions at Semnan areas (experiment ١)

Treatments	Means C	Means A x C
١٠ mmol NaCl	٧٠,٥٦ B	٨٤,٤٤ B
٢٥ mmol NaCl	٥٥,٠٠ C	٦٦,٦٧ C
٥٠ mmol NaCl	٣٢,٢٢ D	٥٧,٧٨ CDE
٧٥ mmol NaCl	٢٥,٠٠ E	٥٠,٠٠ EF
٠ mmol NaCl	٨٠,٠٠ A	٩٥,٥٦ A
١٠ mmol NaCl+fungus		٥٦,٦٧ DE
٢٥ mmol NaCl+fungus		٤٣,٣٣ F
٥٠ mmol NaCl+fungus		٦,٦٦٧ G
٧٥ mmol NaCl+fungus		٠٠,٠٠ G
٠ mmol NaCl+fungus		٦٤,٤٥ CD

Means followed by similar letters are not significantly different (Duncan ١٪). Means indicate percentages of alive plants ١٤ days after inoculation with *M. Phaseolina* and without fungus (A) under different salinity levels (C)

Table ٦. Means comparison of interaction between salinity levels (٠, ١٠, ٢٥, ٥٠, ٧٥ mmol NaCl/lit) and charcoal rot disease (*M. phaseolina*) on two melon cultivars at presence and absence of fungal pathogen in field conditions at Semnan areas (experiment ٢)

Treatments	Means C	Means A x C	Means B x C	Means A x B x C
١	٦٨,٣٣ A	٨٢,٢٢ AB	٦٧,٧٨ AB	٨٠,٠٠ ABC
٢	٥٥,٥٦ B	٦٨,٨٩ BC	٥٠,٠٠ CD	٦٠,٠٠ CDE
٣	٧٢,٢٢ A	٦٢,٢٢ C	٣٧,٧٨ DE	٦٤,٤٤ BCD
٤	١٥,٠٠ D	٣٠,٠٠ E	٣٠,٠٠ E	٦٠,٠٠ CDE
٥	٣٦,٦٧ D	٨٧,٧٨ A	٦٧,٧٨ AB	٨٤,٤٤ AB
٦		٥٤,٤٤ CD	٦٨,٨٩ AB	٨٤,٤٤ AB
٧		٤٢,٢٢ DE	٦١,١١ BC	٧٧,٧٨ ABC
٨		١١,١١ F	٣٥,٥٦ E	٦٠,٠٠ CDE
٩		٥٦,٦٧ C	٧٦,٦٨ A	٠٠,٠٠ F
١٠		٠٠,٠٠ F	٠٠,٠٠ F	٩١,١١ A
١١				٥٥,٥٦ DE
١٢				٤٠,٠٠ E
١٣				١١,١١ F
١٤				٠٠,٠٠ F
١٥				٥١,١١ DE
١٦				٥٣,٣٣ DE
١٧				٤٤,٤٤ DE
١٨				١١,١١ F
١٩				٠٠,٠٠ F
٢٠				٦٢,٢٢ CD

Means followed by similar letters are not significantly different (Duncan ١٪)

Means indicate percentages of alive plants ١٤ days after inoculation with *M. Phaseolina* and without fungus (A) under different salinity levels (C) on two cultivars of melon (Susskii and Ivaneki)

For A x B x C mean comparison: ١،٢،٣،٤ and ٥ = Susskii without fungus, ٦،٧،٨،٩, and ١٠ Ivaneki without fungus,١١،١٢،١٣،١٤ and ١٥ Susskii with fungus and ١٦،١٧،١٨،١٩ and ٢٠ Ivaneki with fungus

Fig. ١. Interaction of salinity levels (٠, ١٠, ٢٥, ٥٠, ٧٥ MmolNaCl/lit, respectively, right to left) and charcoal rot disease (*M. phaseolina*) on melon seedlings, at greenhouse conditions

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