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Effects of Water Stress and *Fusarium oxysporum* f. sp. *lycoperseci* on Growth (leaf area, plant height, shoot dry matter) and Shoot Nitrogen Content of Tomatoes Under Greenhouse Conditions

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ABSTRACT- Effects of water stress and *Fusarium oxysporum* f. sp. *lycoperseci* (*Fol*) on the growth of tomatoes were studied in a greenhouse experiment. Treatments consisted of five levels of water stress (1, 3, 5, 7 and 9 day irrigation intervals). Infested soil consisting of 400 chlamydospores g^1 of *Fol* and non infested soil were used. Experiments arranged in a completely randomized design with 8 replications (4 infested and 4 non infested soil) under greenhouse conditions (18-35 ^{0}C). Six week old tomato seedlings, cultivar Porimo, were exposed to water stress after transferring to infested and non infested soils. During the experiment, leaf area, final plant height, shoot dry weight and shoot nitrogen content were measured. Disease symptoms appeared earlier in treatments with high water stress than the other t reatments. Results showed that leaf area, final plant height, shoot dry weight and shoot nitrogen content were reduced both with increasing irrigation intervals and in infested soils. Root colonization by *Fol* increased with increasing irrigation intervals, but differences were not significant.

Keywords: ***Fusarium oxysporum*, Growth, Nitrogen Content, Tomatoes seedlings, Water stress**

INTRODUCTION

Drought induces a reduction in plant tissue water content with subsequent reduction in water potential, leaf elongation, leaf photosynthesis and changes in protein synthesis, and nitrogen metabolism and also a change in cell membrane properties (7, 32). The extent of these changes depends on interactions among various environmental factors. With regard to the relation between plant water status and plant diseases, it is easier to find examples of the effects of the pathogen on plant water status than to find examples of the effects of plant water stress on the susceptibility to attack by pathogens (22).Water stress results in injury to the root systems, blockage of the xylem (34), disturbance of normal stomatal functioning, and

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damage to the cuticle (2). It is difficult to separate the direct effects of an excess or water deficit in the environment on susceptibility to disease from the indirect effects caused by change in the soil water status (22). It is generally believed that water-stressed plants are more susceptible than unstressed plants to attacks by pathogens (30). The nature of the defense mechanism in stressed plants would explain more clearly the effect of water deficit on the susceptibility to the pathogens.

Control of water potential in soil either alone or in combination with other control measures (pesticide, insecticide, fertilizer, etc.) could provide a means for disease control. Under unfavorable environmental factors to the host, various physiological processes in plants including disease resistance are interrupted (3, 14, 10). Water stress enhances both the establishment of the pathogen in the host and the development of infections. The pathogenicity of *Macrophomina phaseolina*, causing charcoal rot on many plant species, is apparently dependent on plant water status. In sorghum (12) and cotton (15), production of typical severe diseases in the greenhouse was possible only if plants were grown in heated beds, subjected to water stress. *Fomes annosus (Heterobasidium annosum)* (37) and possibly *Fusarium solani* f. sp. *phaseoli* (9) are also favored by host water stress.

Fusarium and possibly Verticillium wilts are apparently favored by wet soils but illustrate some special problems in interpretation. Fusarium wilt of peas (23) and celery (29) were shown to be more severe in wet than in dry soil. These reports apparently contradict earlier ones by Gilman (16) and Humbert (18) for Fusarium wilt of tomato, who observed severe disease following hot dry weather and little or non during cool moist weather.

Leaf is an important plant organ, which has a major role in photosynthesis and transpiration. Therefore, leaf area measurements are required in most physiological studies involving plant growth (17). Many methods have been developed for leaf area measurements. Direct methods for determining leaf area are restricted to the use of an automatic area-integrating meter. Tracing, shadowgraphing or the use of a planimeter to measure the leaf area attached to shoots is time consuming and tedious; also, in some experiments time is not enough to make measurements directly. Estimation of leaf area from mathematical models involving linear measurements of leaves is relatively precise and non-destructive (24).

A mathematical model can be obtained by correlating the leaf length (L), width (W) or length multiplied by width (LW) to the actual leaf area (LA) of leaf samples using regression analysis. Non-destructive methods based on linear measurements are faster and easier to be executed in several crops like cucumber (28), tomato, strawberry (36), lettuce (17), and pumpkin (33). Non-destructive methods of the leaf area measurement are useful for small plant populations and allow the measurement of the same plant several times during the growing period.

The objective of the present study is to investigate the effect of water stress and *Fusarium oxysporum* f. sp. *lycoperseci* (*Fol*) infection on disease severity, growth (leaf area, plant height, shoot dry matter) and shoot nitrogen content of tomatoes under greenhouse conditions.

MATERIALS AND METHODS

Soil Characteristics

The soil used in this study was collected from non-cultivated soil in Bajgah 15 km north of Shiraz. It had sandy clay texture, pH=7.9, EC=0.83 ds/m, with 2.2 percent organic matter and was sieved by a 3-mm mesh screen.

Inoculum Production

Mycelium from a 4-day-old, single spore, sporodochial culture of *Fol* race 1 was transferred into a 250-ml flask containing 50 ml of potato dextrose broth (extract of fresh potato 300 g, dextrose 20 g, and distilled water 1000 ml) at pH 6.5. Cultures were incubated at room temperature on a reciprocal shaker (60 strokes/min) for 3-4 days. The conidia were centrifuged down at low speed and washed three times with sterile distilled water. The inoculum suspension consisted mainly of microconidia with a few mycelial fragments and hyaline chlamydospores. The inoculum was mixed with sterile sand and incubated at 20 ^{o}C for 4-6 weeks and at 4 ^{o}C to reach stable population (5) and checked periodically by soil dilution method (4). The initial population of *Fol* was 2.44×10^{8} CFU/g sand.

Soil Infestation and Transplanting

A proportion of sand inoculum was mixed with 120 kg of field soil to obtain about 400 CFU of *Fol*/g soil. Seven liter plastic pots were used. The lower halves of the pots were filled with 3500 g infested and the rest with non-infested soil (6). Six-week-old tomato seedlings, cultivar Porimo, grown in small pots were transferred with the soil block to infested and non- infested pots and filled with 3500 g non-infested soil. Equal amounts of dry soils were used in all treatments.

Treatments

For root establishment, plants were irrigated similarly for five days prior to water stress treatments. The experiments were arranged in a completely randomized design with 4 replications under greenhouse conditions (18- 35 ^{0}C day and night). Five levels of stress (1, 3, 5, 7 and 9 day irrigation intervals) were imposed. The soil water content at field capacity was determined using a pressure cell at 0.33 bar suction. The weight of each pot at field capacity was determined. Then at each irrigation interval, pots were weighed and water was added to the soil to reach the field capacity.

At the end of the study final plant height, shoot dry weight and nitrogen content and root colonization by *Fol* were determined.

Measuremnts:

Leaf area

Leaf area of tomato plants was determined weekly (Fig. 1) by measuring the plants lengths (L) and widths (W).

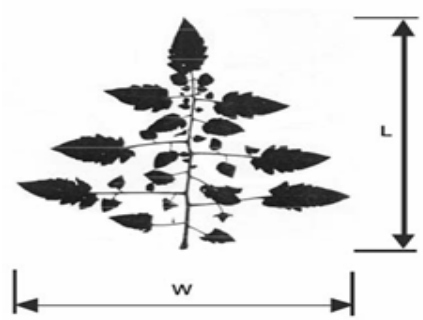

Fig.1. Diagram of tomato leaves showing length (L) and width (W).

Shoot Dry Matter

At the end of the experiment, shoots were separated from roots, dried at 70 ^{o}C for 48 hours and weighed.

Shoot Nitrogen Content

In each plant, 0.2 g of crushed matter of shoot tissues was mixed with 5 ml of H_2SO_4 and 2.5 g Kjeldahl Catalyst (Cu- Se) and placed in digestion flasks for two hours. Twenty ml H_2O was added to each sample and flasks were shaken on a shaker. Samples were placed in Kjeltec Auto 1030 Analyzer and the percent of nitrogen content in shoot dry matter was read.

Root Colonization By Fol

Acidified PDA consisted of potato-dextrose agar (extract of 300 g potato, 16 g agar, 20 g dextrose and 1000 mL distilled water) with 500 ppm of the surfactant (TMN) added prior to autoclaving. The medium was acidified to pH 4-4.2 with 50% lactic acid (4). Roots were washed and surface desinfested 1-2 min in 0.5 % sodium hypochlorite. Root pieces of 2-3 mm were randomly selected and placed on the medium using 15 segments per plate and five plates were used for each plant. Plates were incubated at 25^{o}C for 4-7 days and colonized segments in each plate were counted.

RESULTS AND DISCUSSION

Leaf Area Equation

Based on LxW, the equation (using Leaf Area Meter, Win Dias, and Excell software programs) used to calculate the plants leaf area is shown below and the correlation between LxW and leaf area is shown in Figure 2**.**

$$LA=0.334(L\acute{}W)-5.389 \quad (1)$$

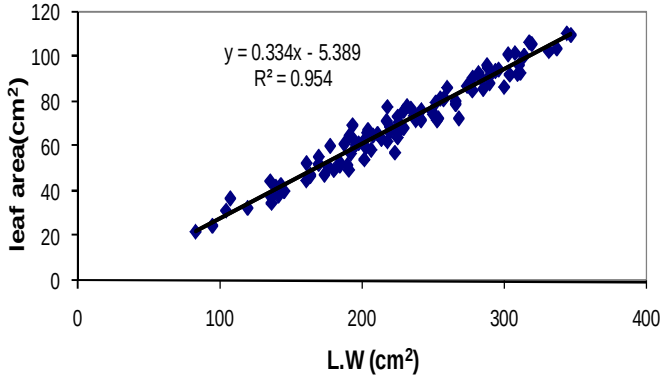

Fig. 2. Plot showing the regression correlation between measured and estimated leaves using the formula (LA=0.334(L´W)-5.389).

To verify the validity of the above equation, the area of some leaves in the treatments was measured by a leaf area meter at the end of the study and compared with the estimated values by an F-Test (5%). There was no significant difference between the calculated and measured values (Fig. 3).

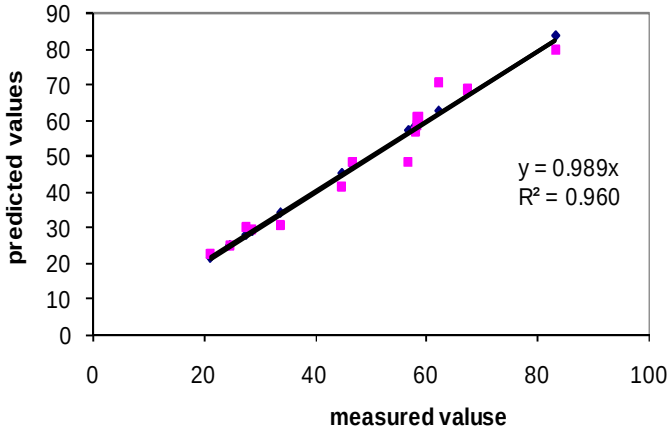

Fig. 3. Estimated and measured leaf area (LA)

Symptoms

The disease symptoms appeared first as vein clearing and drooping of the petioles and yellowing of the lower leaves. One or more branches would be affected while others remained symptomless. Plant growth was reduced roughly in proportion to the severity of the symptoms.

Disease symptoms in treatments with high water stress appeared earlier than the other treatments. The amount of applied water to reach the field capacity was discussed in the method and material section. Table 1 shows the time of initial

symptoms, disease development and initial mortality in *Fusarium* infected plants after inoculation.

Table 1. The time of initial symptoms, disease development and initial mortality in *Fusarium* infected plants after inoculation

Irrigation intervals (days)	Time of initial symptoms (days)	Time of disease development (days)	Time of initial mortality (days)
1	**60**	**73**	**-**
3	**55**	**69**	**-**
5	**46**	**60**	**72**
7	**34**	**47**	**63**
9	**23**	**43**	**58**

Leaf area

High water stress resulted in lower leaf area (Fig. 4). Significant differences ($P \leq 5\%$) in leaf area in infected and non-infected plants varied among treatments. Under high water stress this occurred earlier.

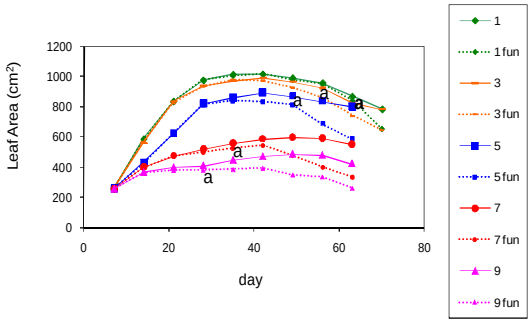

Fig. 4. Leaf area versus time (day) after treatments. Letter a indicates the time when the difference between infested and non-infested leaves became significant ($P \leq 0.5$)

Plant Height, Shoot Dry Matter and Shoot Nitrogen Content

Drought treatment significantly decreased plant height and shoot dry matter as well as nitrogen content. The effect of water stress and also *Fol* on plant height, shoot dry matter and nitrogen content are shown in Figures 5, 6 and 7 respectively.

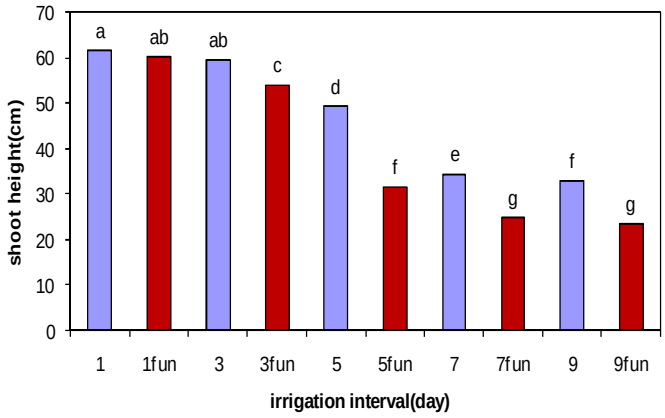

Fig. 5. Final shoot height of infected and non-infected plants under different irrigation intervals. Means with different letters are significantly different at the 0.05 level according to Duncan Multiple Range Test

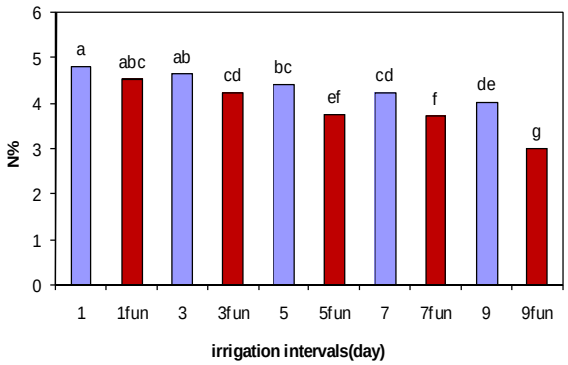

Fig. 6. Nitrogen content in infected and non-infected plants shoots under different irrigation intervals. Means with different letters are significantly different at 0.05 level according to Duncan Multiple Range Test

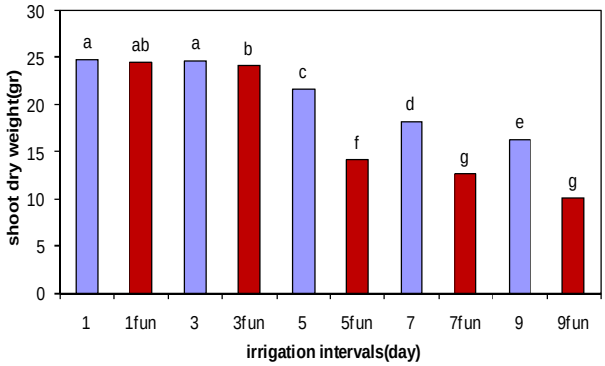

Fig. 7. Shoot dry weight of infected and non-infected plants under different irrigation intervals. Means with different letters are significantly different at the 0.05 level according to Duncan Multiple Range Test

Root colonization

Most colonies of *Fol* on root segments developed within 2-3 days at room temperature (Fig. 8). The colonization percentage of the root increased by increasing the irrigation interval but the differences were not significant (Fig. 9).

Fig. 8. Root segments of tomato colonized by *Fusarium oxysporum* f. sp. *Lycoperseci*

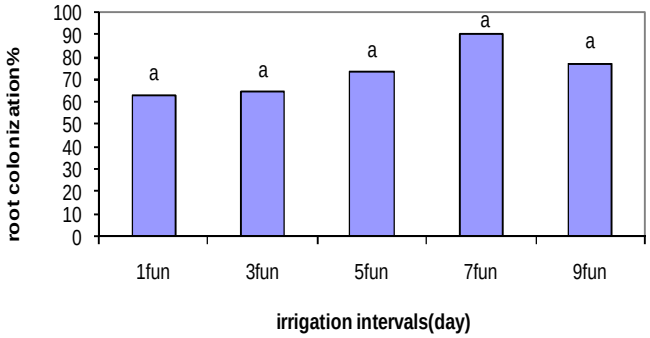

Fig. 9. colonization by *Fusarium oxysporum* f. sp. *Lycoperseci* under different irrigation intervals

Plants are usually subjected to different biotic and abiotic stress including abnormal temperature, unfavorable chemical and physical soil conditions, and various diseases and pests, but in the long run, water deficit reduces plant growth and crop yield more than all other stresses combined (22). Moisture stress in plants clearly influences such processes as water uptake, root pressure, seed germination, stomatal conductance, transpiration, photosynthesis, respiration, enzymatic activity, roots and shoots growth, shrinkage of tissues, mycorrhizal development, mineral nutrition and other processes (13, 21, 35). Plants have developed various mechanisms to withstand drought, such as developing higher root-shoot ratios, fewer and smaller leaves or increasing the concentration of compatible solutes in leaf cells (34). The particular way in which plants respond to water stress might be of special importance in understanding plants' responses to drought and also in evaluating the plants' capacity to cope with stress (25). Water deficit in plants causes premature leaves and fruits to drop, young fruits to crack or pit or internal necrosis such as bitter pits of apple, blossom end roting of tomato, and celery and head lettuce blackheart (11). Actively growing plant parts such as fruits need a constant supply of calcium which moves slowly in the plant. Therefore, adequate soil moisture should be maintained. Otherwise calcium deficiency will occur (27).

The present study showed that moisture stress affected many physiological processes such as plant metabolism, nitrogen content and growth. The presence of the pathogen augmented the deleterious effects of water deficit on various aspects of growth and development. Increasing the matric potential of the root zone reduced growth, photosynthesis and nitrogen content of the plant and increased root colonization by *F. oxysporum* f.sp. *lycopersici*. Disease symptoms appeared earlier in moisture stressed plants. Water deficit in plants and infection by different *forma specialis* of *Fusarium oxysporum* have been reported in different crops, but no solution mechanisms have been suggested. Soil moisture deficiciency affects plants more than pathogen. It has clearly been shown that propagules of *F. oxysporum* f.sp.*melonis* germinate in media with a water potential of up to -10 MPa, suggesting that the fungus can grow in dry soil, restricting plant growth (19). Water stress has great influence on growth regulators (22). It has been shown that water deficiency increases both abscisic acid and ethylene and decreases cytokinine accumulation in plants (1). The increase in ABA would result in cytokinine reduction and promote the onset of scenescence (38). Temporay wilting and moisture stress of plants increases the release of amino acids from roots (20). The increase in exudation would have significant effects on chlamydospore germination of the fungus (31). In the

present study, water deficit resulted in additional colonization of the roots by the pathogen; probably as a result of root scenescence and increased root exudation and the invasion of the vascular system of susceptible plants through increasing the inoculums' potential on the root surface. Other physiological factors might have been involved in the predisposing plants to the pathogen on defence mechanisms which can not be ruled out.

Conclusion

Because of its negative effects on plant metabolism, water stress predisposed plants for infection by *Fol*. Leaf area, final plant height, shoot dry weight and shoot nitrogen content were reduced by increasing the irrigation interval and also in infested soil. Root colonization by *Fol* also increased by increasing irrigation intervals. On the other hand, water stress reduced activity of some enzymes and secondary metabolites. Decrease in the production of secondary metabolites caused the collaps of the defensive system of the plant against the aggression of pathogens (8).

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اثر تنش آبی و قارچ فوزاریوم بر رشد (سطح برگ، ارتفاع گیاه و ماده خشک اندام هوایی) و میزان نیتروژن اندام هوایی گوجه فرنگی در شرایط گلخانه

علی اصغر قائمی[1]، **آیت اله رحیمی[1]*** **و ضیاالدین بنی هاشمی[2]***

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چکیده - به منظور مطالعه اثر تنش آبی و قارچ فوزاریوم (*Fol*) بر رشد گوجه فرنگی در شرایط گلخانه ای از تیمارهای با دورهای آبیاری ۱، ۳، ۵، ۷ و 9 روز استفاده گردید. در این بررسی از خاک آلوده به قارچ فوزاریوم به میزان 400 کلامیدوسپور در هر گرم خاک و خاک بدون آلودگی به قارچ استفاده شد. تیمارها در قالب طرح بلوک های کاملا تصادفی هر یک با 8 تکرار (4تکرار خاک آلوده به قارچ و 4 تکرار خاک سالم) تحت شرایط گلخانه ای و در دمای بین 18 تا ۳۵ درجه سانتیگراد در نظر گرفته شد. گیاه گوجه فرنگی گونه پوریمو در زمان 6 هفتگی به گلدان های دارای خاک آلوده و سالم منتقل شدند. در طول دوره‌ی رشد، سطح برگ، ارتفاع گیاه، وزن ماده خشک و مقدار نیتروژن اندام هوایی اندازه گیری شد. علائم بیماری در گیاه در تیمارهای با تنش آبی بیشتر نسبت به سایر تیمارها زودتر آشکار گردید. نتایج نشان داد که سطح برگ، ارتفاع گیاه، وزن ماده خشک و میزان نیتروژن اندام هوایی گیاه در خاک آلوده به قارچ و با افزایش دور آبیاری کاهش پیدا کرده است. میزان کلونیزاسیون قارچ فوزاریوم در ریشه در تیمارهایی که تحت تنش آبی بیشتری قرار داشتند بیشتر از تیمارهای با دور آبیاری کمتر بود اما اختلاف ها معنی دار نبودند.

واژه کلیدی: **تنش آبی، قارچ فوزاریوم، گوجه فرنگی، میزان نیتروژن**

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** مکاتبه کننده