

EFFECT OF WATER DEFICIENCY ON PHYSIOLOGICAL AND CHEMICAL PROPERTIES OF PEPPER GROWN IN GREENHOUSE

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(Received 28th Feb 2016; accepted 30th May 2016)

Abstract. The cultivar Jalapeno pepper (*Capsicum annuum* var. *annuum*) was used in this research. The experimental design was split plot with five replications and four water restriction levels (control, 0%, 25%, 50%) were used. The research was made in a cold plastic greenhouse at Namik Kemal University, Agricultural Faculty, Department of Horticulture. After planting, plants were grown in normal growing conditions (hoeing, watering and fertilization) for a month and water restriction treatments were applied from the second month onwards. The plants development and other parameters were recorded. During the experiment leaf water potential (MPa), leaf relative water content (%), membrane damage index (%), total chlorophyll content (SPAD) and leaf temperature (°C) were measured. Artificial draught stresses made by water restrictions affected the growth and development of plants badly. The normal growth was observed in control (100% water). The plants watered with 50% were affected less than 25% and 0% water treatments. The treatment (25% water) gave noticeable stress symptoms, leaf wilting, reduced growth and development and yield reduction. The treatment (0% water) caused reduced growth, small leaf, wilting, drying, reduced yield and gradually the death of plants. As a result, leaf water potential, leaf relative water content and total chlorophyll content were the highest in 100% water treatment. The lowest leaf damage, membrane damage and leaf temperature were determined in 100% water treatment as well.

Keywords: *water stress, leaf water potential, relative water content, total chlorophyll content, membrane damage index*

Introduction

The problem of water deficit is becoming more serious and United Nation Organization is ringing the bell of danger from the looming crisis of drought. Water is one of the principle environmental limiting factors for crop production and distribution throughout the world. Water deficit, which is a consequence of either intermittent or terminal period of drought, causes significant yield reduction on presently cultivated lands. It was found that in fertile soil, plant growth and yield are reduced more often by water deficits than any other cause. These restrictions on yield potential are of great concern in terms of meeting food demand for accelerated increasing world population (Mohd et al., 2004).

In order to produce sufficient food for the world's growing population, control of these problems, increasing productivity of water use in agriculture and also finding new ways to utilize the extensive sodic and saline soils and water resources are vital and urgent. (Pessarakli and Szabolcs, 1999).

Especially drought stress during early growth stages might result in reduced plant number of blossoms and fruits (Rylski and Spigelman, 1982). However, despite that water management in pepper is extremely important at all stages of plant development

due to its influence on stand establishment, fruit set and quality, there are very limited data on mechanisms of drought avoidance or defence in pepper (Sziderics et al., 2010)

Pepper (*Capsicum spp.*) is a member of the Solanaceae family (Parson et al. 2013). *Capsicum annuum* is one of the most diverse species among the Solanaceae, and continues to be the primary subject of selection to this day, mainly focused on increasing fruit yield and quality (Paran and Van der Knaap, 2007).

Pepper cultivation is confined to warm and semi-arid countries where water is often a limiting factor for production. This necessitates optimisation of water management (Dorji et al., 2005). Pepper is cultivated nearly all over the world and 29601 Mt produced from 1837704 ha area in 2011. Turkey is the third of largest pepper producer (1975 Mt) after China (15520 Mt) and Mexico (2131 Mt) (Unlukara et al., 2015).

The motherland of Jalapeno pepper which has an important place in global industrial pepper production is Mexico and it is not prevalently cultivated in Turkey (Oguz et al., 2012).

Jalapeno cultivation is especially made in moist areas. However there is not enough information about its cultivation in highlands and especially in dry areas. For this reason cultivation of this species in dry areas and determination of its resistance against drought will be very useful for cultivators.

In our research the aim was to determine the physiological and chemical changes occurring in pepper due to various water deficits.

Materials And Methods

Plant material and experimental design

The research was carried out in the polyethylene greenhouse condition of the Horticulture Department, Faculty of Agriculture, Namik Kemal University, Tekirdag, Turkey (40°59' N, 27°29' E and 4 m altitude) in the summer season of 2012.

The experiment was conducted on clayey loam soil. Some of the physical and chemical properties of soil determined in the laboratory are given in *Tables 1* and *2*.

In this study, Mexican origin Jalapeno species pepper (Lee et al., 2006) which is grown was used as material (*Capsicum annuum* var. *Annuum*). The seedlings were transplanted into the greenhouse on the 30th May 2012. Some of the climate data results of experiment area are shown in *Table 3*. Seedlings were planted with 100x40 cm distance in greenhouse borders. It was grown in normal maintenance and watering conditions (Crosby, 2008). The plants were irrigated through pressure compensated drip irrigation system. Dripper spacing was 40 cm and having 2 Lh⁻¹.

The plants, normal water requirements of which was fulfilled by drip irrigation until flowering period (first month), were later subject to artificial drought stress. Criteria measurements initiated starting from flowering.

The experiment was carried out according to split plot design with 5 replications and 4 different irrigation applications (100% irrigation (control), 50% irrigation, 25% irrigation and 0% irrigation). In all the experiments; 20 parcels in total and 20 plants for each parcel were used. In order to find out differences between treatment, LSD multiple comparison test was made using the SPSS 18.0 for Windows statistical software (SPSS Inc., Chicago, USA) and the difference degrees were stated at 1% probability. No analysis of variance was made for leaf water potential and measurement of leaf temperature. The results were given as observations and for additional information.

Four different irrigation programs were tested in polyethylene greenhouse:

- 100% irrigation (control): Irrigation was applied in 7 days intervals starting from flowering period (2nd July 2012). Climate data of Tekirdag city were examined for long years and the average of the sum of 7 days long evaporation was taken as a basis in determination of irrigation water amounts.
- 50% irrigation: 50% of the control application.
- 25% irrigation: 25% of the control application.
- 0% irrigation: No irrigation was applied.

Table 1. Some physical properties of soil*

| Soil depth (cm) | Texture class | EC (dS m ⁻¹) | Field capacity | | Wilting point | | Bulk density (gcm ⁻³) | Usable water retention capacity (mm) |
|-----------------|---------------|--------------------------|----------------|-------|---------------|------|-----------------------------------|--------------------------------------|
| | | | % | mm | % | mm | | |
| 0-30 | CL | 0.6 | 28 | 157.1 | 16 | 89.8 | 1.87 | 67.2 |
| 30-60 | SCL | 0.5 | 26 | 134.9 | 17 | 88.2 | 1.73 | 46.7 |
| 60-90 | SC | 0.4 | 27 | 137.7 | 17 | 86.7 | 1.70 | 51.0 |

*Source: Agricultural soil analysis report of T.R. Tekirdag commercial exchange

Table 2. Some chemical properties of soil*

| Parameter | Unit | Result | Method |
|--------------------|-------|----------|----------------|
| pH | | 7.64 | Saturation |
| Salt | (%) | 0.06 | Saturation |
| Lime | (%) | 2.46 | Calcimetry |
| Structure | (%) | 59 | Saturation |
| Organic matter | (%) | 1.06 | Walkey-Black |
| Total nitrogen (N) | (%) | 0.05 | Kjeldahl |
| Phosphorus (P) | (ppm) | 73.9 | Olsen-ICP |
| Potassium (K) | (ppm) | 290.36 | A. acetate-ICP |
| Calcium (Ca) | (ppm) | 5.194.97 | A. acetate-ICP |
| Magnesium (Mg) | (ppm) | 432.07 | A. acetate-ICP |
| Iron (Fe) | (ppm) | 8.05 | DTPA-ICP |
| Copper (Cu) | (ppm) | 1.45 | DTPA-ICP |
| Zinc (Zn) | (ppm) | 1.33 | DTPA-ICP |
| Manganese (Mn) | (ppm) | 4.05 | DTPA-ICP |

*Source: Agricultural soil analysis report of T.R. Tekirdag commercial exchange.

Table 3. 2012 climate data of experiment area*

| Months | Average temperature (°C) | Max. temperature (°C) | Min. temperature (°C) | Average relative humidity (%) |
|-----------|--------------------------|-----------------------|-----------------------|-------------------------------|
| May | 20.1 | 27.2 | 10.1 | 91.2 |
| June | 26.2 | 31.4 | 19.1 | 78.2 |
| July | 30.4 | 36.7 | 24.4 | 68.7 |
| August | 29.0 | 35.0 | 23.7 | 62.7 |
| September | 24.6 | 29.6 | 16.8 | 73.6 |

*Source: Tekirdag directorate of meteorology (2012)

Leaf water potential measurement (MPa)

Leaf water potential was measured by Scholander Pressure Chamber. The measurements were made 2 hours before (Ψ_{pd} : Pre-dawn leaf water potential) and 6 hours after (Ψ_{md} : Midday leaf water potential) sun rise respectively. The measurements were made on most developed leaves of plant (Scholander et al., 1965). The measurements were repeated with 1 week intervals after flowering.

Leaf relative water content (RWC) (%)

In trials related with resistance to drought. Studies of researchers working on various plants about leaf relative water content (RWC) (Sanchez et al., 2004). At the end of stress fresh weights of the plants (FW) were taken in order to determine the relative water contents. After that the leaves were kept in pure water for 4 hours and during this period their turgid weights (TUW) were calculated. The weight samples weights of which were determined were dried in stove at 65 °C for 48 hours and then dry weight (DW) was measured in grams. The ratios of obtained fresh and dry weights were calculated by below Equation (1) in order to find leaf relative water contents (%).

$$RCW = \frac{100(FW-DW)}{TW-DW} \quad (\text{Eq.1})$$

Membrane damage index (%)

Membrane damage index (MDI) was calculated by measuring the electrolyte released from the cell (Fan and Blake, 1994). In each vegetation period disks with diameter of 17 mm were taken from leaves of stress and control plants were kept in ionized water for 5 hours and then their electricity conductivities (EC) were measured. Same disks were kept in autoclave at 100 °C for 10 minutes and then the EC value of the solution was measured again. From the obtained value the membrane damage in leaf cells (%) was calculated with the help of the below equation (2).

$$MDI = \frac{100(Lt-Lc)}{1-Lc} \quad (\text{Eq.2})$$

Lt: EC before autoclaving / EC after autoclaving of the leaf which is under drought stress

Lc: EC before autoclaving / EC after autoclaving of the control leaf.

Determination of total chlorophyll content (SPAD)

In the research the total chlorophyll content of the pepper leaves was measured by “Konica Minolta SPAD-502” portable chlorophyll-meter. In each period same readings was made from two regions of the leaf (close to midrib) and from five plants in each parcel (Geravandi et al., 2011).

Measurement of Leaf Temperature (°C)

Infrared thermometer technique which is based on measuring plant surface temperature allows faster and more precise measurement without touching the plant. The mentioned technique is based on transpiration which decreases the leaf surface temperature. In the measurements infrared thermometer having filters which detects rays at a wavelength of 7-18 nm (IRT) (Raynger ST8 model) were used (Erdem et al., 2010).

Results And Discussion

Leaf water potential (MPa)

In leaf water potential examination the effect of different water deficits on pepper at pre-dawn (Ψ_{pd}) and in the midday (Ψ_{md}) leaf water potential were shown in *Table 4* and *Figure 1*. The measurements were made in 6 different dates and significant differences occurred between 0% application and control applications in both pre-dawn and midday measurements. At the same time as vegetation periods advanced plants could not get over the stress.

One week after starting first water restriction application, leaf water potential measurements were initiated. After this period each week measurements were made in pre-dawn and in the midday. As harvest period was long, measurements were continued during fruiting period. First measurements were made one week after water restriction and the measurements were -1.20 MPa in 0% and -0.25 MPa in 100% application pre-dawn while the measurements were -2.20 MPa in 0% and -0.90 MPa in 100% application in the midday. Kaya and Dasgan (2013) stated that the mean and % of in plants increased compared to control as a result of salt and drought stress.

Leaf relative water content (%)

The effects of different water restrictions on leaf relative water content can be observed in *Table 5*. There is a direct proportion between irrigation increase applied to plants and leaf proportional water content and greatest decrease in leaf proportional water content was found to be 43.4% in 0% application. Due to increase in irrigation ratios of 55.6% was reached in 25% application and 65.2% in 50% application. Highest increase was observed in the control plant in which 100% irrigation was made (95.2%). Witting occurred in leaves with 0% and 25% applications due to low leaf proportional water content.

As the osmotic pressure increases in salty soils the water intake of the plant becomes more difficult and accordingly the leaf proportional water content also decreases (Topaloglu, 2010).

Table 4. Effect of different water deficiency levels on average pre-dawn (ψ_{pd}) and midday (ψ_{md}) leaf water potentials* (MPa)

| Leaf water potentials | Water deficiency | The number of weeks after flowering | | | | | |
|---|------------------|-------------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| | | 1 (27.07.2012) | 2 (04.08.2012) | 3 (11.08.2012) | 4 (18.08.2012) | 5 (25.08.2012) | 6 (01.09.2012) |
| Pre-dawn leaf water potential (Ψ_{pd}) | 0% | -1.20 | -1.35 | -1.65 | -1.75 | -1.88 | -2.05 |
| | 25% | -0.80 | -0.95 | -1.38 | -1.42 | -1.48 | -1.54 |
| | 50% | -0.35 | -0.40 | -0.55 | -0.74 | -0.85 | -1.05 |
| | 100% (control) | -0.25 | -0.30 | -0.36 | -0.43 | -0.55 | -0.63 |
| Midday leaf water potential (Ψ_{md}) | 0% | -2.20 | -2.35 | -2.46 | -2.53 | -2.62 | -2.85 |
| | 25% | -1.71 | -1.90 | -1.99 | -2.15 | -2.27 | -2.40 |
| | 50% | -0.98 | -1.04 | -1.26 | -1.36 | -1.42 | -1.45 |
| | 100% (control) | -0.90 | -0.94 | -1.02 | -1.06 | -1.15 | -1.31 |

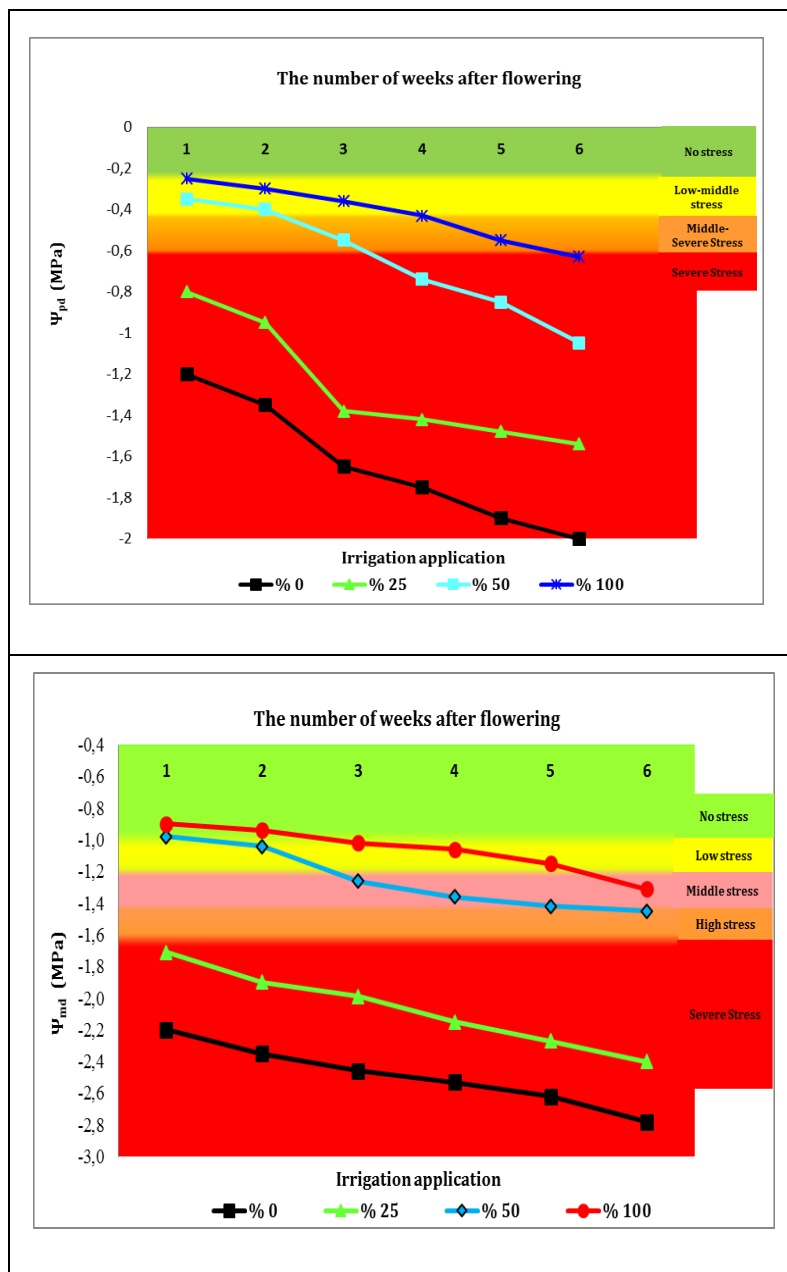


Figure 1. Effect of different water deficiency levels on average pre-dawn (ψ_{pd}) and midday (ψ_{md}) leaf water potentials (MPa).

Membrane damage index (%)

According to the measurements made and statistical analysis results the membrane damage occurred in Jalapeno pepper leaves are shown in *Table 5*. According to statistical results in terms of membrane damage in leaf cells the effect of irrigation applications remained within the 1% statistical error limits.

The values in *Table 5* varies between 6.9% and 67.3% and lowest value was in control application (6.9%) and highest value in no irrigation (67.3%). 58.4% value was obtained from 25% application while 47% value was obtained from 50% application. Based on this result we can say that there is an inverse relation between increase of

irrigation ratio and membrane damage. Stress was very obvious in no irrigation, many vital functions stopped and this situation resulted with the death of the plant. As a result of the damage occurred in the cell membranes of the plants which were subject to stress, it was emphasized that the materials dissolved in water in the cell leaked to the gaps between cells and accordingly the tissue electrical conductivity increased. In other words it was determined that there is an inverse relation between tissue electrical conductivity and membrane unity (Houimli et al., 2010).

Total chlorophyll content (SPAD)

Averages of total chlorophyll amount according to water restriction in drought stress were measured as shown in *Table 5* the chlorophyll amount averages remained within 1% statistical error limits among all applications as seen in *Table 5*.

When we emphasize the effect of water stress applications in terms of total chlorophyll amount it was observed that averages varied between 46.8 and 72.1 the lowest chlorophyll amount average was measured in 0% application (46.8) and the from control application (72.1). While total chlorophyll average was measured as 59.8 in 25% application this average was 67.4 in 50% applications. It was determined that chlorophyll amount decreased by increasing water stress.

Oliveira Neto et al., (2009), stated that drought stress negatively affected chlorophyll content and that photosynthetic pigments were damaged as a result of drought stress and chlorophyll decreased in all plants. Mishra et al., (2002) proved (by chlorophyll level) in a study they conducted in tomato that tolerant species decreased the negative effects of water restriction.

Table 5. Effect of different water deficiency levels on average leaf relative water content (%), membrane damage index (%) and total chlorophyll content (SPAD) and LSD groups*

| | 0% | 25% | 50% | 100% (control) |
|---|---------|---------|---------|----------------|
| Leaf relative water content ¹ (%) | 43.41 d | 55.63 c | 65.28 b | 95.24 a |
| Membrane damage index ² (%) | 67.31 a | 58.45 b | 47.02 c | 6.92 d |
| Total chlorophyll content ³ (SPAD) | 46.88 d | 59.86 c | 67.46 b | 72.10 a |

*There is no difference in the level of 0.01 among averages that have the same letter ($p < 0.01$).
LSD¹ = 2.421385, LSD² = 3.233776, LSD³ = 3.30512

Measurement of Leaf Temperature (°C)

Mean leaf temperature (°C) was measured in pre-dawn and midday by infrared thermometer before each irrigation application without touching the leaves from beginning of water limitation till harvest period were shown in *Table 6* and *Figure 2*.

As seen in *Table 6* while highest value in leaf temperatures were measured in 0% application both in sun rise and middle of the day measurements, lowest value was measured in both periods in control application.

In measurements made in 6 different dates in pre-dawn and midday, control application leaf temperatures had values lower than the inner temperature of greenhouse while in other applications where water limitation was applied, temperature was measured to be higher than inner greenhouse temperature. This is an indication that plants are affected by stress. When we look at the drought and salinity study values of Suyum (2011) in water melon, it was stated that leaf temperature increases in salty and drought conditions.

Table 6. Effect of different water deficiency on pre-dawn and midday leaf temperature (°C) of pepper plant

| | | The number of weeks after flowering | | | | | |
|--------------------------------|------------------|-------------------------------------|--------------|--------------|--------------|--------------|--------------|
| Water deficiency | | 1 | 2 | 3 | 4 | 5 | 6 |
| | | (27.07.2012) | (04.08.2012) | (11.08.2012) | (18.08.2012) | (25.08.2012) | (01.09.2012) |
| Pre-dawn leaf temperature (°C) | 0% | 27.4 | 25.4 | 26.9 | 24.1 | 21.1 | 23.3 |
| | 25% | 27.1 | 25 | 26.3 | 23.5 | 20.1 | 22.9 |
| | 50% | 26.9 | 24.9 | 26.0 | 22.8 | 19.6 | 21.8 |
| | 100% | 25.8 | 23.2 | 24.4 | 21.9 | 18.7 | 20.6 |
| | Greenhouse temp. | 26.3 | 24.2 | 25.2 | 22.5 | 19.6 | 21.2 |
| Midday leaf temperature (°C) | 0% | 33.9 | 34.2 | 36.8 | 34.0 | 34.5 | 30.3 |
| | 25% | 33.4 | 33.6 | 36.0 | 33.1 | 31.3 | 28.9 |
| | 50% | 33.2 | 34.0 | 35.3 | 32.2 | 30.3 | 28.1 |
| | 100% | 32.7 | 32.9 | 34.4 | 31.1 | 28.8 | 26.8 |
| | Greenhouse temp. | 33.1 | 33.8 | 35.0 | 31.8 | 29.8 | 27.6 |

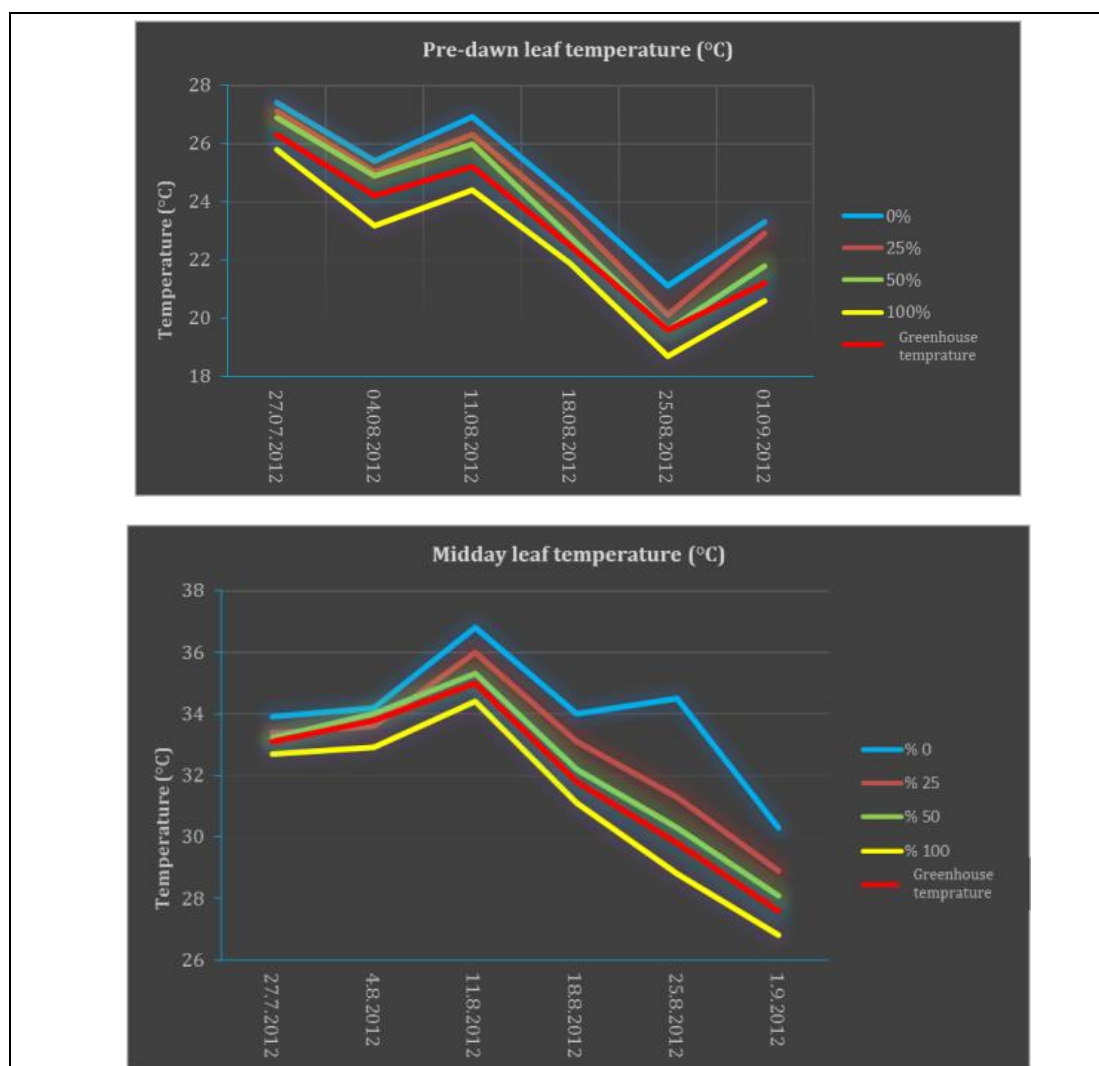


Figure 2. Effect of different water deficiency on pre-dawn and midday leaf temperature (°C) of pepper plant.

Conclusions

As a result, in case of water restriction averages decreased in criteria such as leaf proportional water content, total chlorophyll, leaf water potential, while in criteria such as membrane damage index and leaf temperature, the averages increased. In pepper cultivation in order to prevent yield and quality losses, the following results should be provided as in 100% irrigations:

- Pre-dawn leaf water potential should not decrease below -0.6 MPa after flowering and also.
- It should not decrease below -0.25 MPa in the first 15 days after flowering.
- It should not decrease below -0.40 MPa during harvest and maintain this value.
- Midday leaf water potential should not generally decrease below -1.6Mpa.
- It should not decrease below -1MPa in the first 15 days after flowering.
- It should remain over -1.3MPa during harvest.
- RWC should not decrease below 95%.
- Leaf temperatures should not exceed 35°C and these criteria should practically be taken into consideration in the irrigation activities to be conducted.

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