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Article

Alfalfa responses to drought, salinity, and herbivory by *Tetranychus urticae* (Acari: Tetranychidae) and performance of the pest on water-stressed plants

Samira Khodayari[1*] and Fatemeh Abedini[2]

1. *Department of Plant Protection, Faculty of Agriculture, University of Maragheh, P.O. Box: 55181-83111, Maragheh, Iran; E-mail: khodayari@maragheh.ac.ir*
2. *Department of Horticultural Sciences, Faculty of Agriculture, University of Maragheh, P.O. Box: 55181-83111, Maragheh, Iran; E-mail: abedini_5883@yahoo.com*

[*] Corresponding author

ABSTRACT

Stress is hypothesized to be one of the primary abiotic factors that influence the structuring of food webs. Drought and salinity stresses are the most serious induced problems of agriculture due to climatic changes. Considering the global warming and drying of Urmia salt lake in northwestern Iran which can result in salinization of agricultural lands nearby, the focus of this study is to examine the effects of the two above-mentioned stresses on a host plant (*Medicago sativa* L.) and its pest (*Tetranychus urticae* Koch) performance. Two levels of water deficit (kept dry for 5 and 10 days and received 50% and 25% of field capacity respectively) and three levels of salinity (100, 200 and 300 mM NaCl) were added to alfalfa plants as stress factors and the following characteristics were measured before and after herbivore infestation: relative water, chlorophyll, carotenoid, protein, proline, malondialdehyde (MDA) and H_2O_2 contents of leaves and catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) enzyme activities. The life table parameters of two-spotted spider mite reared on stressed plants were measured and compared with control. We found that drought and salinity stresses led to a decrease of alfalfa plant growth. Morphological and physiological changes included a decrease of relative water, chlorophyll and carotenoid contents and an increase of protein, proline, MDA and H_2O_2 contents and APX enzyme activity. The net reproductive rate (R_0), mean generation time (T), intrinsic rate of increase (r) and finite rate of increase (λ) were significantly higher for mites grown on control host plants compared to mites grown on stressed ones and were 25.66 offspring/ individual/generation time, 16.38 d, 0.1971 day^{-1} and 1.2180 day^{-1} respectively. These parameters reduced significantly in mites grown on stressed host plants; the significant lowest values were recorded for mites grown on host plants subjected to high salinity (300 mM) and were 0.5 offspring/individual/generation time, 14.47 d, −0.0514 day^{-1} and 0.9502 day^{-1} respectively. In terms of this experiment, the water stress had a stronger influence on the morphological and physiological characteristics of alfalfa plant compared to spider mite infestation.

KEY WORDS: Intrinsic rate of increase; *Medicago sativa*; NaCl; two-spotted spider mite; water deficit.

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INTRODUCTION

Stress is hypothesized to be one of the primary abiotic factors that influence the structuring of food webs (Hacker and Gains 1997). The productivity of plants is greatly affected by various

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environmental stresses (Sairam and Tyagi 2004). Climate changes are happening and result in temperature increasing and drought events in the next decades (IPCC 2013). Water stress can be defined as reduced water availability, either by water scarcity (drought) or osmotic stress (high salt concentrations). When the availability of water is not sufficient to maintain plant growth, photosynthesis, and transpiration, plants become water deficit stressed (Fan *et al.* 2014); it is a serious problem that reduces world crop production (Vincent *et al.* 2005). Water deficit stressed plants often have diminished osmotic potential (Bussis and Heineke 1998), heightened oxidative stress (Knight and Knight 2001), and accumulated osmolytes such as antioxidants, amino acids, carbohydrates, and inorganic ions which alter their attractiveness and nutritional value (Showler and Castro 2010). The oxidative stress is one of the major causes of cellular damage in plants during water stress (Miller *et al.* 2010). Many antioxidant systems, both enzymatic and non-enzymatic are up-regulated in response to the increased reactive oxygen species (ROS) levels during water stress. These antioxidants scavenge the reactive oxygen species and reduce the oxidative damage (Arve *et al.* 2011).

The salinity of the soil is one of the main factors that limits the spread of plants in their natural habitats. It is an ever increasing problem in arid and semiarid regions (Shanon 1986). Salinity can be primary, i.e. due to soil parent material or shallow groundwater, or it can be secondary which could be due to application of fertilizers, irrigation with saline water, or due to changes in the hydrologic balance in soil between the amount of water applied and the amount used by crops (Bansal 2016). Salinity has so far been a limiting factor in plant productivity in most Iranian ecosystems (Bagheri *et al.* 2019). To cope with salinity stress, plants develop physiological and biochemical responses (Sandhu *et al.* 2017). During the onset and development of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis, energy production and lipid metabolism are affected (Turan *et al.* 2007). The plants usually keep a low osmotic potential in their cells with the production and accumulation of compatible solutes such as sugars, polyols and amino acids which facilitate water uptake (Arve *et al.* 2011). Salinity stress elicits similar physiological responses in plants as water-deficit stress, but its ecological consequences are poorly understood (Schile and Mopper 2006).

Alfalfa (*Medicago sativa* L.) is an important leguminous forage crop with about 32 million hectares under cultivation globally (Anower *et al.* 2013). It normally lives four to eight years, but can live more than 20 years, depending on its variety and climate (Bansal 2016). Alfalfa has very special features: first, it is drought tolerant; second, alfalfa is moderately salt tolerant; and third, it fixes nitrogen in its roots, which improves soil fertility. Alfalfa roots contain the bacterium *Sinorhizobium melliloti* which helps in converting atmospheric nitrogen to ammonia and then to amino acids for the plants to utilize (Postgate 1998).

Spider mites (family Tetranychidae) are serious pests of agricultural crops and cause a significant yield loss. Hot and dry weather favors reproduction and survival of these pests (Haile and Higley 2003). Chemical constitution of the host plant may influence fecundity, egg viability and the rate of development of Tetranychidae (van de Vrie *et al.* 1972). Studies have shown that water stress of host plants can result in an increase (Youngman *et al.* 1988; Klubertanz *et al.* 1990; Nikolova *et al.* 2014; Shibuya *et al.* 2016; Sinaie *et al.* 2019) or decrease (Specht 1965; Mellors *et al.* 1984; Oloumi-Sadeghi *et al.* 1988) in population density of spider mites. However, in some cases no impact has been observed (Ferree and Hall 1980; Sadras *et al.* 1998; Gillman *et al.* 1999). Youngman *et al.* (1988) studied the life history parameters of *Tetranychus pacificus* McGregor on almond trees exposed to different water stress regimes and concluded that variable water stress was more likely to result in mite outbreaks. Khodayari *et al.* (2021) reported that saline stress and water deficit of common bean decreased the rate of population increase of *T. urticae* Koch fed on them.

In association with climatic warming and drying of Urmia Lake in northwestern Iran which can result in salinization of agricultural lands nearby, the primary focus of this study is to examine the effect of increased salinity and water restriction on the plant physiological responses of alfalfa and then to evaluate the performance of the phytophagous mite, *T. urticae* (Acari: Tetranychidae), on alfalfa plants experiencing water deficit and salinity stresses.

MATERIALS AND METHODS

Plant material

Alfalfa plants (*M. sativa*, Hamedani cultivar) were grown in a greenhouse from May to August 2021 (25 ± 2 °C, 65 ± 10% RH, 16:8 h L:D) at the University of Maragheh, Iran. Seeds were sown in six Styrofoam plug trays with 48 cells (6 × 8 cells) maintained in the greenhouse. Three seeds of alfalfa were sown in each cell of the plug tray (5 × 5 × 7 cm) which contained a potting mix consisting of vermiculite, perlite and field soil. Nutrient solution (Amino Fe6 which contained 6% Fe, 10% amino acid, 5% K_2O and 3% N) was used once a week to meet the nutritional needs of plants. The seedlings were grown for 21 days and then treatments were imposed. The treatments consisted of two drought levels, three salinity levels and mite infestation.

Mite culture

The stock culture of *T. urticae* was obtained from bean cultures of Maragheh (Iran, N 37° 28.709′, E 046° 13.475′), northwestern Iran, in summer 2020. The mites were reared on bean under optimal controlled conditions at the University of Maragheh (Iran) (Temperature: 25 ± 2 °C; RH: 60 ± 5%; 16:8 h L:D) for 10 generations before being used for the experiments. More than 30 mated females were selected and transferred to a leaf disc for 24h for oviposition. Then, 50 freshly laid eggs were kept individually in an experimental arena consisting of a plastic dish (diameter: 3.5 cm) containing one leaf disc (diameter: 3 cm) from one of the six treatments (control, low, intermediate and high salinity concentrations, and two levels of drought received 50% and 25% of field capacity (FC)). Leaf discs were made from young leaves that were collected from the top of the plants. Edges of leaf discs were covered by wet cotton wool to keep them fresh, and leaf discs were replaced every week to avoid their degradation over time. Experiments were conducted at 25 ± 1 °C, 65 ± 10% R.H. and 16:8h L:D. The lids of Petri dishes were covered by a fine plastic mesh to provide ventilation. This experimental procedure was used for the determination of the effects of plant quality on the life cycle of mites. The developmental stage and longevity of the mites were recorded every 24h until the death of all individuals. The presence of an exuvium ensured the successful molting, and the occurrence of this molting was set at the midpoint of these two successive observations. When an individual developed to the adult stage, it was paired with an individual of the opposite sex that had received the same treatment. Whenever more females than males emerged, additional young males from the mass rearing colony were used for mating. Data from these males were not included in the analyses. Eggs were counted daily and destroyed after that.

Induction of drought and salinity stresses and herbivore infestation

To induce drought stresses one of the plug trays was kept dry for 5 days (received 50% of FC) and the other one for 10 days (received 25% of FC) after the start of the experiment and received 50% and 25% the amount of water given to control plants, respectively. Control plants received water at least every 2–3 days to ensure sufficient soil moisture throughout the experiment (received 100% FC). Salinity stress was induced by adding three levels of NaCl, 100 (low), 200 (intermediate) and 300 (high) mM to the irrigation water which is equivalent to 7.87, 15.74 and 23.62 ds/m. In order to avoid ion accumulation, flushing was performed once a week. Five adult females of *T. urticae* were added to each plant for herbivore infestation. The experiments started three weeks after the seeds were planted. Leaves were collected three weeks after application of the treatments.

Biochemical analysis

Leaf area, relative water content as well as chlorophyll, carotenoid, proline, total protein, MDA and H_2O_2 contents and catalase, ascorbate peroxidase and guaiacol peroxidase enzyme activities were measured for 0.5 g randomly selected leaves for each experiment. The leaves were cut at the petiole level and were immediately flash-frozen in liquid nitrogen and stored at −80 ℃ until analysis.

Leaf relative water content

Leaf relative water content (RWC) was calcualted using the following equation (Barrs and Weatherley 1962):

$$RWC= [(FW - DW)/(TW \times DW)] \times 100$$

FW = leaf fresh weight, DW = leaf dry weight and TW refer = turgid weight of leaf

Chlorophyll and carotenoid contents of leaves

For measurement of chlorophyll and carotenoid content, one leaf was collected from each of three different plants in each treatment group. Chlorophyll and carotenoid contents were measured with the methods described by Arnon (1949) and Lichtenthaler (1987), respectively. Briefly, fresh leaf samples were homogenized in 80% acetone for 3 minutes, then the samples were centrifuged at 12000 rpm for 5 minutes and absorption was read at 663 and 645 nm for chlorophyll and at 470 nm for the determination of carotenoid content with UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan).

Proline and protein contents and enzyme activities

Proline content was measured using methods by Bates *et al.* (1973). Briefly, leaf samples (0.5 g FW) were homogenized with 10 ml of 3% sulfosalicylic acid at 4 ℃ and were centrifuged at 15000 rpm for 15 minutes. Afterward, 2 ml of the supernatant of each sample, 2 ml of ninhydrin acid 2.5% and 2 ml of glacial acetic acid 100% were mixed and transmitted to the boiling water for 1 hour. Subsequently the samples were cooled on ice for 30 minutes; absorption was read at 520 nm with UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan). Leaf samples (0.5 g) were extracted in 100 mM phosphate buffer containing 2% polyvinylpyrrolidone (PVP), and the homogenate was centrifuged at 12,000 g for 15 min at 4 ℃, then the supernatant was used for total protein amount and antioxidant enzyme activity assays. The total protein amount was determined using the method of Bradford (1976) (absorbance read at 595 nm). Catalase peroxidase activity was measured by the methods in Aebi (1984). The reaction mixture contained 50 mM phosphate buffer (pH 7.0) and 9 mM H_2O_2. Decrease in the absorbance was taken at 240 nm. Ascorbate peroxidase activity was measured by the method of Nakano and Asada (1987). The reaction mixture contained 0.5 mM ascorbate dissolved in 100 mM potassium phosphate buffer, 2 mM hydrogen peroxide, and double-distilled water. Ascorbic acid oxidation rate was determined by recording the decrease in absorbance at 290 nm for 3 min. Guaicol peroxidase was calculated by the procedure of Milosevic and Slusarenko (1996). The assay mixture comprises of 25 mM phosphate buffer (pH 7.0), 10 mM guaicol and 1.5 mM H_2O_2. Absorbance was recorded at 436 nm.

H_2O_2 and MDA

In order to determine H_2O_2 content, half a gram of fresh leaf in an ice bath was homogenized with 5 ml of 1% (w/v) trichloroacetic acid. The mixture was centrifuged at 4 ℃ for 10 min at a speed of 10,000 g. The reaction mixture consisted of 1 ml of supernatant, 0.5 ml of 100 mM potassium phosphate buffer, and 1 ml of 1 mM potassium iodide. Finally, the adsorption of the solution was measured at 390 nm. The concentration of MDA was calculated using thiobarbituric acid as the reactive material following the method of Stewart and Bewley (1980). Leaf samples (0.5 g) were homogenized in 1.5 ml of 5% (w/v) trichloroacetic acid (TCA), and the mixture was centrifuged at 12,000 g for 20 min at 4 ℃. Then 1 ml of 0.5% thiobarbituric acid solution containing 20%

trichloroacetic acid was added. The mixture was centrifuged at 15,000 g for 15 min. The samples were then placed in a hot water bath at 95 °C for 30 min. Then quickly cooled on ice and centrifuged at 11,000 g for 10 min. The absorbance of the supernatant was determined at 532 and 600 nm by spectrophotometer.

Statistical analysis

Statistical analyses were performed using the statistical program SPSS v. 16. Variables were tested for normality using Kolmogorov-Smirnov test. The effects of the three concentrations of salinity, two drought conditions and mite infestation on each measured plant variable (relative water content, chlorophyll, carotenoid, proline, protein, H_2O_2 and MDA contents, CAT, APX and GPX enzyme activities) were assessed with one-way analysis of variance (ANOVA) with post hoc Tukey's tests ($P < 0.05$). Life cycle data and population growth parameters of *T. urticae* were calculated according to Chi (1988). Data were analyzed with TWO SEX-MS Chart (Chi 2013), available at http://nhsbig.inhs.uiuc.edu/wes/chi.html (Illinois Natural History Survey, Champaign-Urbana, IL). For each parameter, means and standard errors were calculated with the bootstrap method with 100,000 permutations (Huang and Chi 2013).

RESULTS

Plant responses to stresses and mite feeding

The effect of stresses on protein content of host plants was significant but mite feeding had no significant effect. The interaction between two mentioned factors was not significant either. This parameter increased significantly with increasing stress severity. Leaf relative water content decreased significantly in all of the host plants under stress. This parameter decreased significantly after mite feeding too (Tables 1–3) and there was a significant interaction between mite feeding and drought stress (Table 1). Mite feeding decreased relative water content of host plants in all of the drought treatments. Chlorophyll and carotenoid content of host plants decreased significantly under both stresses. These parameters did not change significantly after mite feeding (Tables 1–3). The interaction between stress and mite feeding on plant chlorophyll and carotenoid contents was not significant. Proline, an indicator of drought stress was induced by both stresses. This metabolite increased significantly in control plants after mite feeding (Figs. 1, 2). The interaction between stress and mite feeding on plant proline content was significant and complex. In drought treatments, interaction of drought stress and mite feeding decreased proline content of plant significantly while it was not like that in salinity treatments (Fig. 2).

The activity level of the three analyzed enzymes showed that the effect of drought and salinity stresses was significant. Mite feeding effect was significant in salinity treatments and drought treatments except for GPX activity. There was a significant interaction between mite feeding and stresses except for GPX activity. CAT and GPX activities decreased significantly after drought and salinity stresses. There was a significant decrease in APX activity in all of the treatment groups after mite feeding (Tables 1–3). MDA content increased significantly after stress induction and decreased significantly after mite feeding. The interaction between mite feeding and drought stress was significant but between mite feeding and salinity was not significant for this parameter. The effect of salinity and drought stresses and mite feeding on H_2O_2 content of plants was significant but the interaction between them was significant only in salinity treatments (Table 1). Mite feeding increased H_2O_2 content of host plants nearly 4 folds in all of the salinity treatments.

Mite biology

According to Table 4, duration of egg stage decreased significantly in all of the treatment groups except for intermediate salinity. Duration of larval stage increased significantly in all of the salinity treatments. Duration of protonymph stage increased significantly in all of the treatment groups compared to control except for high salinity. Duration of deutonymph stage increased significantly in all of the treatment groups compared to control except for drought level 2. Longevity of males were significantly lower in all of the treatment groups compared to control. The longevity and fecundity of females and their oviposition period had a significant decrease in all of the treatment groups and the least values were recorded in high salinity. APOP (adult pre-oviposition period) was significantly shortened in drought level 2 and high salinity and TPOP (total pre-oviposition period) was significantly shortened in drought level 1 and 2 (Table 5). The net reproductive rate (R_0), mean generation time (T), intrinsic rate of increase (r) and finite rate of increase (λ) were significantly higher for mites grown on control host plants. These parameters reduced significantly in mites grown on stressed host plants; the significant lowest values were recorded for mites grown on host plants subjected to high salinity (Table 6).

Table 1. Mean square values and coefficient of variation (CV) of GLM procedure for relative water content, chlorophyll, carotenoid, proline, protein, MDA and H_2O_2 contents, GPX, APX and CAT enzyme activities of leaves of alfalfa plants before and after *Tetranychus urticae* feeding.

ANOVA	Protein (mgg^{-1})	Relative water content	CAT (μMmin^{-1}g^{-1})	GPX (μMmin^{-1}g^{-1})	APX (μMmin^{-1}g^{-1})	Chl. (mgg^{-1})	Car. (mgg^{-1})	Proline (mgg^{-1})	MDA (μgg^{-1})	H_2O_2 (μMg^{-1})
Mite effect	0.007^{ns}	21.0^{**}	0.089^{**}	0.084^{ns}	5.71^{**}	196^{ns}	7.85^{ns}	2.41^{**}	1.50^{**}	21.42^{**}
Drought effect	0.160^{**}	7836^{**}	0.029^{*}	1.28^{**}	1.76^{*}	1631^{**}	165^{**}	1.33^{*}	6.11^{*}	2.34^{*}
Mite × Drought	0.032^{ns}	452^{*}	0.019^{*}	0.425^{ns}	1.74^{*}	0.41^{ns}	2.68^{ns}	2.5^{**}	4.12^{*}	0.72^{ns}
CV	32	14	33	39	45	26	43	42	20	42
Mite effect	0.072^{ns}	3407^{**}	0.283^{**}	0.451^{*}	34.41^{**}	13.60^{ns}	10.34^{ns}	0.71^{ns}	4.21^{**}	53^{**}
Salinity effect	0.374^{**}	5009^{**}	0.09^{**}	0.842^{**}	6.9^{**}	1605^{**}	111^{**}	1.94^{*}	3.14^{**}	13^{**}
Mite × Salinity	0.055^{ns}	253^{ns}	0.07^{**}	0.219^{ns}	9.2^{**}	83.38^{ns}	4.19^{ns}	2.66^{*}	0.43^{ns}	4.02^{**}
CV	22	12	45	35	46	29	39	40	27	13

Chl.: Chlorophyll, Car.: Carotenoid.
ns, * and ** means $P > 0.05$, $P < 0.05$ and $P < 0.01$ respectively.

Table 2. Effects of drought stress on mean (± SE) relative water content, chlorophyll, carotenoid, proline, protein, MDA and H_2O_2 contents, GPX, APX and CAT enzyme activities of leaves of alfalfa plants before and after *Tetranychus urticae* feeding.

Plant status	Before mite	After mite	100% FC	50% FC	25% FC
Protein (mgg^{-1})	0.47 ± 0.039^{A}	0.43 ± 0.05^{A}	0.29 ± 0.05^{B}	0.46 ± 0.04^{AB}	0.62 ± 0.07^{A}
Relative water content	84.36 ± 13.3^{A}	62.75 ± 8^{B}	112.84 ± 10.24^{A}	66.112 ± 5.25^{B}	41.72 ± 3.43^{C}
CAT (μMmin^{-1}g^{-1})	0.32 ± 0.02^{A}	0.18 ± 0.04^{B}	0.33 ± 0.04^{A}	0.23 ± 0.03^{AB}	0.19 ± 0.06^{B}
GPX (μMmin^{-1}g^{-1})	1.07 ± 0.21^{A}	0.93 ± 0.16^{A}	1.28 ± 0.22^{A}	1.25 ± 0.18^{A}	0.46 ± 0.08^{B}
APX (μMmin^{-1}g^{-1})	1.48 ± 0.39^{A}	0.35 ± 0.096^{B}	0.85 ± 0.09	1.49 ± 0.65^{A}	0.41 ± 0.15^{B}
Chlorophyll (mgg^{-1})	44.59 ± 5.6^{A}	37.99 ± 5.8^{A}	57.42 ± 3.99^{A}	42 ± 5.72^{A}	24.46 ± 2.86^{B}
Carotenoid (mgg^{-1})	9.7 ± 2.1^{A}	8.3 ± 1.77^{A}	14.77 ± 2.45^{A}	7.88 ± 1.18^{B}	4.47 ± 0.31^{B}
Proline (mgg^{-1})	1.29 ± 0.36^{A}	0.56 ± 0.12^{B}	1.41 ± 0.47^{A}	0.89 ± 0.29^{AB}	0.47 ± 0.17^{B}
MDA (μgg^{-1})	1.54 ± 0.14^{A}	0.97 ± 0.09^{B}	1 ± 0.1^{B}	1.52 ± 0.18^{A}	1.24 ± 0.21^{AB}
H_2O_2 (μMg^{-1})	2.83 ± 0.44^{A}	0.65 ± 0.12^{B}	1.17 ± 0.35^{A}	1.64 ± 0.46^{A}	2.41 ± 0.81^{A}

Different letters in the same row indicate statistical differences at the 0.05 level.

Table 3. Effects of salinity stress on mean (±SE) relative water content, chlorophyll, carotenoid, proline, protein, MDA and H_2O_2 contents, GPX, APX and CAT enzyme activities of leaves of alfalfa plants before and after *Tetranychus urticae* feeding.

Plant status	Before mite	After mite	0 mM NaCl	100 mM NaCl	200 mM NaCl	300 mM NaCl
Protein (mgg^{-1})	0.64 ± 0.09^{A}	0.53 ± 0.05^{A}	0.29 ± 0.05^{B}	0.49 ± 0.05^{B}	0.74 ± 0.05^{A}	0.85 ± 0.08^{A}
Relative water content	86.80 ± 9.2^{A}	62.96 ± 6.3^{B}	112.84 ± 10.2^{A}	73.61 ± 3.15^{B}	70.48 ± 6.96^{C}	42.59 ± 4.59^{C}
CAT ($\mu Mmin^{-1}g^{-1}$)	0.109 ±	0.32 ± 0.05^{A}	0.33 ± 0.04^{A}	0.24 ± 0.09^{A}	0.25 ± 0.10^{A}	0.043 ± 0.008^{B}
GPX ($\mu Mmin^{-1}g^{-1}$)	0.66 ± 0.12^{B}	0.93 ± 0.13^{A}	1.28 ± 0.22^{A}	0.52 ± 0.12^{B}	0.90 ± 0.12^{AB}	0.48 ± 0.02^{B}
APX ($\mu Mmin^{-1}g^{-1}$)	2.60 ± 0.66^{A}	0.20 ± 0.08^{B}	0.85 ± 0.09^{B}	1.05 ± 0.46^{B}	0.71 ± 0.30^{B}	2.99 ± 1.46^{A}
Chlorophyll (mgg^{-1})	36.14 ± 5.3^{A}	34.64 ± 4.7^{A}	57.42 ± 3.99^{A}	37.85 ± 3.93^{B}	25.52 ± 4.28^{B}	20.76 ± 4.22^{B}
Carotenoid (mgg^{-1})	9.62 ± 1.5^{A}	8.3 ± 1.2^{A}	14.77 ± 2.41^{A}	9.63 ± 0.51^{AB}	6.24 ± 0.88^{B}	5.20 ± 0.70^{B}
Proline (mgg^{-1})	1.58 ± 0.27^{A}	1.92 ± 0.29^{A}	1.41 ± 0.47^{AB}	1.31 ± 0.32^{B}	1.71 ± 0.36^{AB}	2.56 ± 0.32^{A}
MDA (μgg^{-1})	2.22 ± 0.28^{A}	1.38 ± 0.16^{B}	1 ± 0.1^{B}	1.41 ± 0.25^{B}	2.23 ± 0.33^{A}	2.58 ± 0.34^{A}
H_2O_2 (μMg^{-1})	0.97 ± 0.17^{B}	3.94 ± 0.6^{A}	1.17 ± 0.35^{D}	1.74 ± 0.48^{C}	2.36 ± 0.68^{B}	4.54 ± 1.18^{A}

Different letters in the same row indicate statistical differences at the 0.05 level.

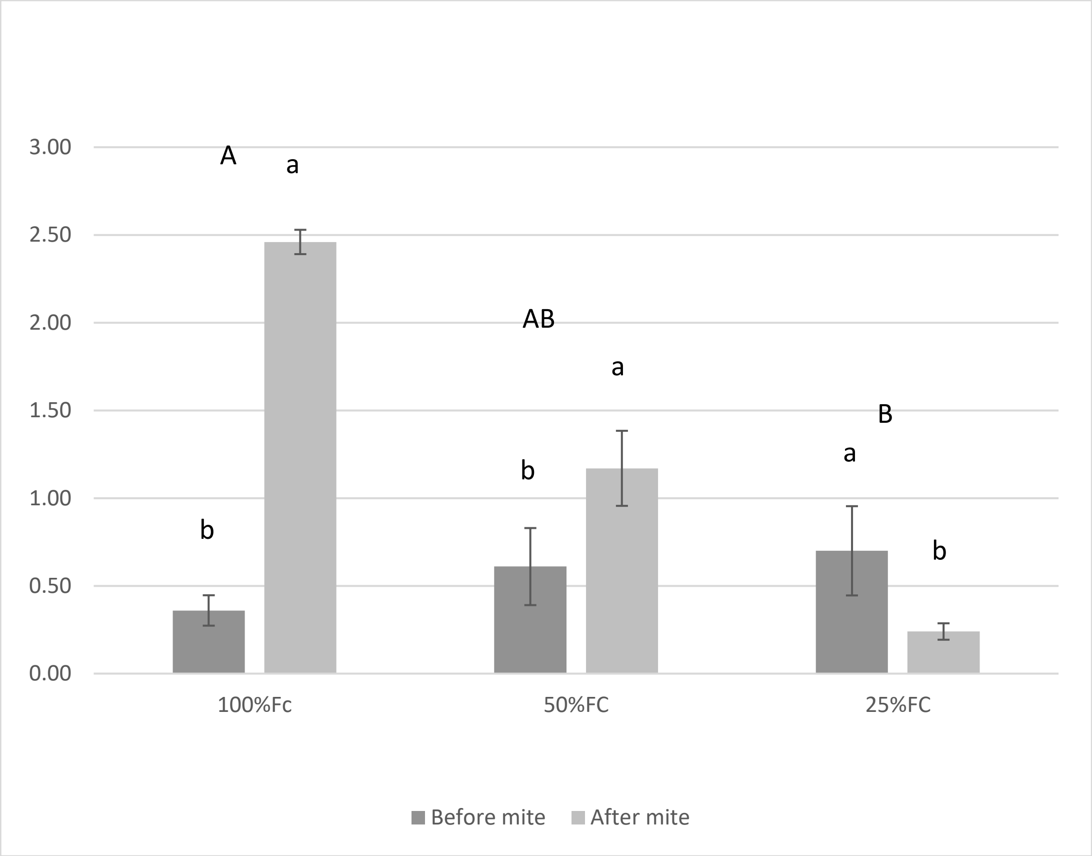

Figure 1. Effects of drought stress on proline content of alfalfa plants before and after *Tetranychus urticae* feeding. Within each column mean (± SE) followed by the same letter(s) are not significantly different. Capital letters show the effect of drought.

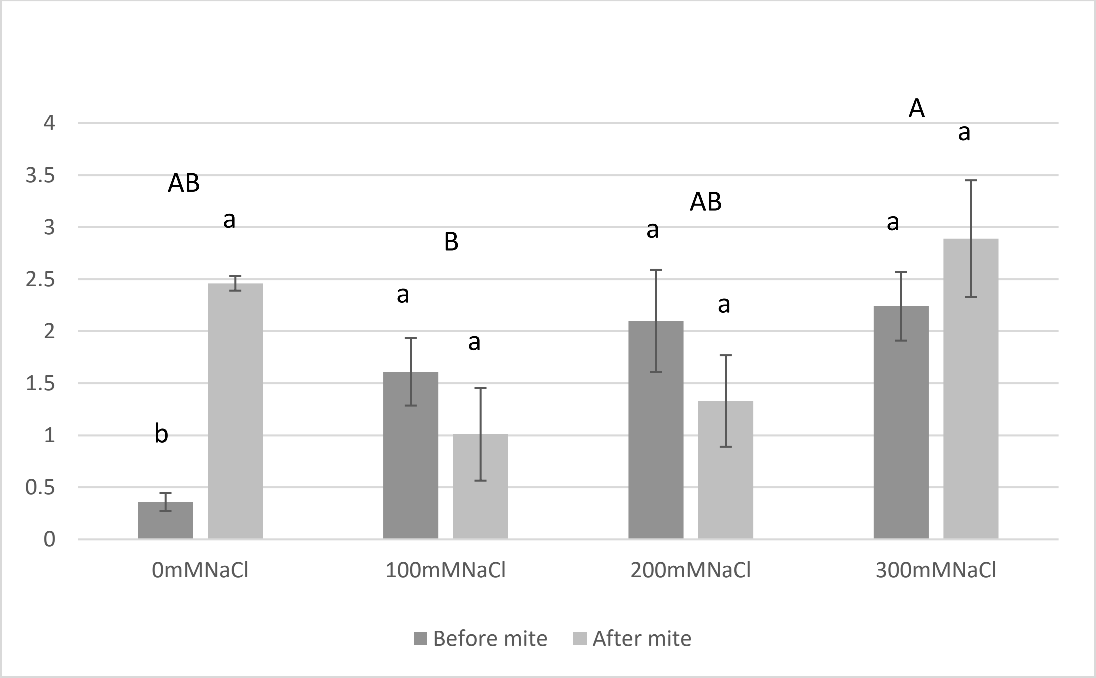

Figure 2. Effects of salinity stress on proline content of alfalfa plants before and after *Tetranychus urticae* feeding. Within each column mean (± SE) followed by the same letter(s) are not significantly different. Capital letters show the effect of salinity.

Table 4. Mean (± SE) duration (days) of the different developmental stages of *Tetranychus urticae* grown on leaves collected from alfalfa plants treated with drought and salinity stresses.

	Egg	Larva	Proton.*	Deuton.*	Preadult	Female total longevity	Male total longevity
Control	4.86 ± 0.12^{a}	2.35 ± 0.15^{b}	1.85 ± 0.08^{c}	2.15 ± 0.13^{b}	11.20 ± 0.20^{b}	20.14 ± 0.42^{a}	21.50 ± 1.12^{a}
50% FC	4.50 ± 0.11^{b}	2.37 ± 0.12^{b}	2.08 ± 0.10^{b}	2.38 ± 0.13^{a}	11.21 ± 0.21^{b}	16.13 ± 0.55^{b}	15.11 ± 1.42^{bc}
25% FC	4.65 ± 0.22^{b}	2.18 ± 0.26^{b}	2.22 ± 0.22^{a}	1.88 ± 0.39^{b}	10.25 ± 0.70^{b}	13.25 ± 0.85^{c}	13.00 ± 0.41^{d}
100 mM NaCl	4.25 ± 0.08^{b}	2.81 ± 0.20^{a}	2.06 ± 0.17^{b}	2.38 ± 0.30^{a}	11.31 ± 0.39^{b}	15.56 ± 0.99^{b}	14.29 ± 0.84^{c}
200 mM NaCl	4.88 ± 0.14^{a}	2.83 ± 0.24^{a}	2.40 ± 0.16^{a}	2.56 ± 0.24^{a}	12.89 ± 0.26^{a}	16.50 ± 1.32^{b}	17.00 ± 0.71^{b}
300 mM NaCl	4.46 ± 0.12^{b}	2.79 ± 0.18^{a}	1.73 ± 0.19^{c}	2.33 ± 0.28^{a}	11.33 ± 0.33^{b}	14.00 ± 0.71^{c}	14.25 ± 1.25^{c}

[*] Proton.: Protonymph; Deuton.: Deutonymph. The standard errors were calculated using the bootstrap procedure with 100000 bootstraps. Within each column mean (± SE) following by the same letter(s) are not significantly different (paired bootstrap test $p > 0.05$). Larval stage considered as larva + protochrysalis, protonymph considered as protonymph + deutochrysalis and deutonymph considered as deutonymph + tritochrysalis.

Table 5. Mean (± SE) fecundity, oviposition days, APOP and TPOP durations of *Tetranychus urticae* females grown on leaves collected from alfalfa plants treated with drought and salinity stresses.

Host status	APOP	TPOP	Fecundity	Oviposition days
Control	1.29 ± 0.13^{a}	12.64 ± 0.20^{bc}	38.50 ± 3.26^{a}	7.07 ± 0.37^{a}
50% FC	1.15 ± 0.10^{ab}	12.38 ± 0.21^{c}	13.67 ± 2.56^{b}	4.08 ± 0.56^{b}
25% FC	0.67 ± 0.33^{c}	10.33 ± 1.20^{d}	4.50 ± 1.85^{c}	2.33 ± 0.67^{d}
100 mM NaCl	1.71 ± 0.42^{a}	13.43 ± 1.00^{abc}	4.56 ± 1.13^{c}	2.43 ± 0.48^{c}
200 mM NaCl	1.33 ± 0.33^{a}	14.33 ± 0.67^{a}	4.75 ± 2.29^{c}	3.33 ± 0.33^{bc}
300 mM NaCl	1.00 ± 00^{b}	13.00 ± 00^{b}	2.60 ± 1.29^{d}	2.00 ± 0.58^{d}

The standard errors were calculated using the bootstrap procedure with 100000 bootstraps. Within each column mean (± SE) following by the same letter(s) are not significantly different (paired bootstrap test $p > 0.05$). APOP and TPOP mean adult pre-ovipositional period and total pre-ovipositional period respectively.

Table 6. Population growth parameters (*r*, intrinsic rate of increase, λ, finite rate of increase, R_0, net reproductive rate, *T*, mean generation time and *GRR*, gross reproductive rate) of specimens of *Tetranychus urticae* grown on leaves collected from alfalfa plants treated with drought and salinity stresses.

Host status	*r* (day^{-1})	λ (day^{-1})	R_0 (offspring/ individual)	*T* (d)	*GRR*
Control	0.1971 ± 0.0118^{a}	1.2180 ± 0.0143^{a}	25.66 ± 4.48^{a}	16.38 ± 0.29^{a}	31.73 ± 5.96^{a}
50% FC	0.1243 ± 0.0178^{b}	1.1325 ± 0.0201^{b}	6.83 ± 1.77^{b}	15.16 ± 0.30^{b}	19.64 ± 4.25^{a}
25% FC	−0.0116 ± 0.0227^{cd}	0.9887 ± 0.0223^{cd}	0.9 ± 0.23^{cd}	11.91 ± 0.56^{d}	2.42 ± 0.6^{c}
100 mM NaCl	0.0236 ± 0.0129^{c}	1.0239 ± 0.0132^{c}	1.46 ± 0.28^{c}	15.38 ± 0.72^{abc}	10.26 ± 0.6^{b}
200 mM NaCl	-0.0219 ± 0.0205^{cd}	0.9786 ± 0.0199^{cd}	0.73 ± 0.23^{d}	16.93 ± 0.31^{a}	4.45 ± 1.24^{c}
300 mM NaCl	-0.0514 ± 0.0229^{d}	0.9502 ± 0.0216^{d}	0.5 ± 0.15^{d}	14.47 ± 0.08^{c}	2.84 ± 0.7^{c}

The standard errors were calculated using the bootstrap procedure with 100000 bootstraps. Within each column, means (± SE) followed by the same letter are not significantly different (Paired bootstrap test, $P > 0.05$)

DISCUSSION

The main factors, drought, salinity and mite feeding which were the subject of this study affected some morphological and physiological (relative water content, protein, proline, chlorophyll, carotenoid, etc.) characteristics of alfalfa plants. The water content of alfalfa leaves was considerably affected by water stress (drought and salinity) and mite feeding and decreased significantly. Georgieva and Nikolova (2019) reported a decrease of leaf surface area and relative water content of soybean leaves after drought stress and *T. urticae* feeding. According to Jaleel *et al.* (2009), water stress is characterized by a reduction in leaf water potential and loss of turgor, which decreases stomatal conductance and cellular expansion causing reduced plant growth. High concentrations of NaCl inhibited the growth of alfalfa plants by decreasing their leaf area. This might be due to the toxic effect of NaCl or increased crucial osmotic pressure at which the plants would not be able to uptake the required water. Additionally, salinity is known to reduce the concentrations of macronutrients (N, K, Ca and Mg) and micronutrients (Zn, Mn and Fe) in leaf tissue (Esechie and Rodriguez 1999). High salt concentrations lead to loss of K due to the depolarization of membranes and loss of Ca due to displacement by Na^{+} ions (Cramer *et al.* 1991). Transpiration rates can be affected by decreased K concentration in the leaf cells leading to stomatal closure (Hsio and Lauchli 1986).

The total pigment content (chlorophyll and carotenoid) was affected negatively by stress conditions in our experiment but mite feeding had no effects. Petolino and Leone (1980) and Turan *et al.* (2007) reported that salinity decreased the total chlorophyll concentration of bean plants, suggesting a salt-induced weakening of protein-pigment-lipid complex or increasing chlorophyll enzyme activity. Misra and Srivastava (2000) reported that the water stress reduced the chlorophyll and carotenoid content of the Japanese mint tissue compared with an unstressed control. A reduction in chlorophyll content was reported in drought-stressed cotton as well (Massacci *et al.* 2008). The decrease in chlorophyll content under stress is a commonly observed phenomenon (Reynolds *et al.* 2005). It might be due to reduced synthesis of chlorophyll pigment complexes (Allakhverdiev *et al.* 2000) or destruction of chiral macro-aggregates of light harvesting chlorophyll a or b pigment-protein complexes (ShiraniRad and Zandi 2012) or due to oxidative damage of chloroplast lipids, pigments and proteins (Tambussi *et al.* 2000). In Georgieva and Nikolova (2019), spider mites feeding reduced pigment content of soybean leaves and the reduction was greater in water-stressed plants than in plants receiving optimal water. In contrary to the present results, several other studies have demonstrated the adverse impact of spider mite herbivory to plants, which include reduced chlorophyll content (DeAngelis *et al.* 1983; Haile and Higley 2003; Bueno *et al.* 2009).

Plant total protein content was directly affected by drought and salinity stresses but not with mite feeding. Similar to our study, stressful conditions increased protein content in cucumber (Khodayari *et al.* 2018), *Batis* plant (Foust 2010) and soybean (Samarah *et al.* 2006), whereas a contrasting effect with decreased protein contents in stressed plants was reported for strawberry (Çakmak and Demiral 2007), tomato (Ximénez-Embún *et al.* 2016) and bean (Khodayari *et al.* 2021). Variations in responses to environmental stress between plant species and cultivars are common (Gutbrodt *et al.* 2011). A large number of proteins involved in ion exclusion, ion compartmentalization and detoxification are induced in alfalfa plants exposed to salinity stress (Sandhu *et al.* 2017). The levels of proline in the plants mainly designates their ability to tolerate or to adapt to saline conditions (Turan *et al.* 2007) which was increased significantly in our experiments under effect of drought stress and mite feeding. When the both factors were induced simultaneously proline content of plants decreased significantly (Fig. 1). Salinity treatments increased proline content of host plants however when both salinity and mite feeding were induced no significant effects were observed (Fig. 2). Khodayari *et al.* (2021) observed an increase and decrease in proline content of bean plant in response to drought and salinity stresses respectively compared to control. Dehydration often occurs concurrently with salinity stress, and the accumulation of solutes like proline helps plant systems adapt to saline environment by reducing water loss (Bohnert *et al.* 1995). Accumulation of proline in tissues of the plants as a response to drought and salt stresses and also mite feeding has been attributed to enzyme stabilization and osmoregulation of the plants (Madan *et al.* 1994).

One adverse effect of stress is the production of reactive oxygen species, such as hydrogen peroxide (H_2O_2), inducing oxidative stress (Ertani *et al.* 2013). MDA is also a reactive species occurring naturally and a biological marker for oxidative stress. Plants employ antioxidants and detoxifying enzymes, such as superoxide dismutase (SOD), CAT and APX to combat oxidative stress induced by salinity (Hernandez *et al.* 1995; Chinnusamy *et al.* 2005). Among the three studied antioxidant enzymes here, APX was the only one that increased significantly in high salinity treatments. GPX and CAT activities decreased significantly in some of the treatments after abiotic stresses and increased significantly after mite feeding. APX activity decreased significantly in all of the treatments after mite infestation. Khodayari *et al.* (2018) reported reduction in APX, GPX and CAT enzyme activities in cucumber after salinity stress induction. On the contrary, Khodayari *et al.* (2021) recorded an increase in the activity of the three mentioned enzymes in response to salinity and drought stresses in bean plant. In agreement with our finding, H_2O_2 and MDA increase was reported by Bagheri *et al.* (2019) in Pistachio seedlings during short term salt stresses. MDA production reflects oxidative damage to cell membrane (Farooq *et al.* 2017) as a result of induced stresses. H_2O_2 has an important role in redox signaling in regulating normal processes, including oxidative stress and enhance plant tolerance to salt stress (Li *et al.* 2011). In our studies, MDA content increased under drought stress but H_2O_2 did not change; these parameters decreased significantly after mite feeding. MDA content in salinity treatments increased significantly but decreased after mite feeding. H_2O_2 content increased significantly in all of the salinity treatments especially after mite feeding possibly as a result of oxidative stress.

The effects of drought and salinity were significant for nearly all of the developmental stages of *T. urticae* with individuals feeding on stressed host plants taking longer to complete development in immature stages and shorter in adult stage. Although the differences observed between treated and control groups were in most cases not significant, when all these biological parameters were combined to calculate R_0, T and r, more remarkable differences appeared and a concentration-dependent effect was detected. The r values decreased with increasing NaCl concentrations and drought period. This may be the result of decrease of relative water content of leaves, chlorophyll, carotenoid and K or increase of APX enzyme activity, H_2O_2 and MDA contents. The population growth of mite species was positively correlated with water stress in *Oligonychus pratensis* (Banks) on maize (Perring *et al.* 1986), *T. cinnabarinus* Boisduval on sorghum and maize (Chandler *et al.* 1979), *T. pacificus* McGregor on almond trees (Oi *et al.* 1989) and *T. urticae* on soybean and corn

(Eichele-Nelson *et al.* 2017). The European red mite [*Panonychus ulmi* (Koch)] on apple (Specht 1965; Sauge and Fauvel 1994) and *T. urticae* on soybean (Mellors *et al.* 1984), apple (Wermelinger *et al.* 1985) and bean (Khodayari *et al.* 2021) are for the moment, the only examples of a negative response to water stress. When herbivorous arthropods are unable to have access to a sufficient amount of water, their population can decline (Showler 2013). For example, the reproduction and survival were negatively affected for the alfalfa aphid, *Therioaphis maculate* (Buckton) on alfalfa under water stress (McMurtry 1962). Levitt (1951) suggested that the most likely cause of the host plant's unsuitability for aphids under such conditions is low turgor, which reduces the ability of aphids to feed. The same explanation may be true for *T. urticae*.

CONCLUSION

This study contributes to the understanding of how drought and salinity stresses and a herbivore affect plants and also how a herbivore can be affected by host plant stress. We found that drought and salinity stresses led to a decrease of alfalfa plant growth by affecting its morphological and physiological characteristics such as decrease of relative water content, chlorophyll and carotenoid contents and increase of protein, proline, MDA and H_2O_2 contents and APX enzyme activity that generally cause a negative effect on *T. urticae* performance. Mite feeding caused a significant increase in proline and H_2O_2 contents and decrease of relative water content in control plants. In this experiment, the water stress had a stronger influence on the morphological and physiological characteristics of alfalfa plant compared to spider mite infestation.

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پاسخ گیاه یونجه به تنش‌های خشکی، شوری و تغذیه *Tetranychus urticae* (Acari: Tetranychidae) و بررسی عملکرد این گیاهخوار بر گیاهان تحت تنش آبی

سمیرا خدایاری[۱]* و فاطمه عابدینی[۲]

۱. *گروه گیاهپزشکی، دانشکدهٔ کشاورزی، دانشگاه مراغه، صندوق پستی ۸۳۱۱۱-۵۵۱۸۱، مراغه، ایران؛ رایانامه:* *khodayari@maragheh.ac.ir*
۲. *گروه علوم باغبانی، دانشکدهٔ کشاورزی، دانشگاه مراغه، صندوق پستی ۸۳۱۱۱-۵۵۱۸۱، مراغه، ایران؛ رایانامه:* *abedini_5883@yahoo.com*

* نویسندهٔ مسئول

چکیده

تنش یکی از مهمترین عوامل غیرزنده است که ساختار شبکهٔ غذایی را تحت تاثیر قرار می‌دهد. با توجه به تغییرات اقلیمی، تنش‌های شوری و خشکی از جمله جدی‌ترین مشکلات بخش کشاورزی هستند. به دلیل گرم شدن جهانی و خشک شدن دریاچه ارومیه در شمال غرب ایران که می‌تواند منجر به شوری زمین‌های کشاورزی اطراف آن شود، مهمترین هدف این مطالعه، بررسی تاثیر دو عامل تنش‌زای اشاره شده بر گیاه میزبان (*Medicago sativa* L.) و بررسی عملکرد آفت (*Tetranychus urticae* Koch) آن است. دو سطح از کمبود آب (۵ و ۱۰ روز عدم آبیاری و دریافت ۵۰٪ و ۲۵٪ ظرفیت مزرعه) و سه سطح شوری (۱۰۰، ۲۰۰ و ۳۰۰ میلی مولار NaCl) به عنوان عوامل تنش‌زا به گیاه یونجه اعمال شدند و ویژگی‌های زیر پیش و پس از حمله گیاهخوار مورد مطالعه قرار گرفتند: میزان آب نسبی، کلروفیل، کاروتنوئید، پروتئین، پرولین، مالونیل دی‌آلدئید (MDA) و H_2O_2 برگ‌ها و همچنین میزان فعالیت آنزیم‌های کاتالاز (CAT)، آسکوربات پراکسیداز (APX) و گایاکول پراکسیداز (GPX). فراسنجه‌های جدول زندگی کنه تارتن دولکه‌ای، که بر گیاهان تحت تنش پرورش یافته بودند برآورد و با شاهد مقایسه شد. نتایج نشان داد که تنش‌های شوری و خشکی، رشد گیاه یونجه را کاهش داد. تغییرات ریخت‌شناختی و فیزیولوژیک گیاه یونجه تحت تاثیر این تنش‌ها عبارتند از: کاهش میزان آب نسبی، محتوای کلروفیل و کاروتنوئید و افزایش میزان پروتئین، پرولین، MDA و H_2O_2 و همچنین میزان فعالیت آنزیم APX. میزان خالص تولیدمثل (R_0)، مدت زمان یک نسل (T)، میزان ذاتی افزایش جمعیت (r) و میزان متناهی افزایش جمعیت (λ) به طور معنی‌داری در کنه‌های پرورش یافته روی گیاهان میزبان شاهد بیشتر بود و به ترتیب ۲۵/٦٦ نتاج/فرد/یک نسل، ۱٦/۳۸ روز، ۰/۱۹۷۱ بر روز و ۱/۲۱۸۰ بر روز بود. این فراسنجه‌ها به طور معنی‌داری در کنه‌های پرورش یافته روی گیاهان تحت تنش کاهش یافت و کمترین مقادیر آن در تیمار شوری ۳۰۰ میلی مولار مشاهده شد که به ترتیب عبارت‌اند از: ۰/۵ نتاج/فرد/یک نسل، ۱٤/٤۷ روز، ۰/۰۵۱٤- بر روز و ۰/۹۵۰۲ بر روز. با توجه به نتایج این مطالعه تنش آبی در مقایسه با تغذیه کنهٔ تارتن اثر بیشتری بر ویژگی‌های ریخت‌شناختی و فیزیولوژیک گیاه یونجه داشت.

واژگان کلیدی: میزان ذاتی افزایش جمعیت؛ *Medicago sativa*؛ NaCl، کنهٔ تارتن دولکه‌ای، کمبود آب.

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