

Influence of foliarly applied different triazole compounds on growth, nutrition, and antioxidant enzyme activities in tomato (*Solanum lycopersicum* L.) under salt stress

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Abstract

A pot experiment was conducted to investigate the effects of five different triazole compounds, triadimenol, tebuconazole, bitertanol, triadimefon, and paclobutrazol on the growth, macro-nutrition, antioxidative enzyme activities and other stress related parameters in *Solanum lycopersicum* L. (tomato) plants grown in greenhouse conditions under salt stress. Plants were treated with 30 mg L⁻¹ triadimenol, 250 mg L⁻¹ tebuconazole, 250 mg L⁻¹ bitertanol, 40 mg L⁻¹ triadimefon, and 40 mg L⁻¹ paclobutrazol in separate treatments using foliar spray. NaCl treatment at 125 mM decreased overall growth and fruit yield; reduced chlorophyll, carotenoid, and relative water content; but increased proline accumulation, superoxide dismutase, peroxidase and polyphenol oxidase enzyme activities, soluble protein content, and electrolyte leakage. In addition, NaCl stress resulted in high accumulation of Na⁺ and decreased the contents of Ca²⁺, K⁺, and P in the leaf and roots. With different triazoles, compound treatments overcame, to different extents, the adverse effects of NaCl stress on the above physiological and biochemical parameters. Triazole compounds treatment significantly enhanced the fresh and dry weight of shoots and roots as well as macro element contents of plant organs. Among the treatments, TRI, TEB, and TDM improved these parameters to a greater extent compared to other compounds. NaCl treatments remarkably increased the antioxidative enzyme activities at 5% probability level when compared to control plants. In addition, exogenous application of different triazole compounds promoted this status. Triazol treatment increased the total chlorophyll, carotenoid, and relative water contents to a greater extent compared to salt-stressed plants. Compared to other triazoles, TDM and TEB treatments increased the above-mentioned parameters to a greater extent. Thus, the results of the present study indicate that the application of different triazole compounds reduced the detrimental effects of salinity and increased resistance to salinity in tomato plant.

Keywords: Salinity, *Solanum lycopersicum*, Stress, Triazoles.

Abbreviations: TRI_Triadimenol, TEB_Tebuconazole, BIT_Bitertanol, TDM_Triadimefon, PBZ_Paclobutrazol, SOD_Superoxide dismutase, POX_Peroxidase, PPO_Polyphenol oxidase, RWC_Relative water content, EC_Electrolyte leakage, ROS_Reactive oxygen species.

Introduction

Soil salinity can inhibit plant growth and agricultural productivity. Excessive sodium (Na⁺) causes plant cell dehydration, and reduced cellular growth inhibits growth of many salt-sensitive plants, which includes most crop plants, and possibly causes death in less tolerant plants (Ashraf, 2009). Tomato, like most crop plants, is sensitive to severe levels of salinity. All stages of tomato plant development, including seed germination, vegetative growth, and reproduction, show sensitivity to salt; hence, economic yield is reduced when plants experience severe salt stress (Cuartero and Munoz, 1999). Tomato is a major crop in Turkey and worldwide, and its cultivation is concentrated in semi-arid regions where saline waters are frequently used for irrigation. Furthermore, ion homeostasis is an essential factor of the mechanism of salt tolerance in tomato. Salinity raises Na⁺ concentration in roots and leaves of tomato plants while it reduces Ca²⁺ and K⁺ concentrations in leaves rather than roots of salinized tomato plants. While K⁺ and Ca²⁺ play key roles in several physiological processes, Na⁺ does not function as a macronutrient, and thus the substitution of K⁺ by Na⁺ and decrease in Ca²⁺ concentration may lead to nutritional imbalances (Cramer, 1997). The control of Na⁺

accumulation by an exclusion strategy and high shoot K/Na and Ca/Na ratios may enhance salt tolerance or resistance in tomato crops (Dasgan et al., 2002). One biochemical change that can occur when plants are subjected to biotic or abiotic stresses is the production of reactive oxygen species (ROS). The main sites of ROS production in the plant cell during abiotic stress are chloroplasts, mitochondria, and microbodies. ROS are highly reactive and in the absence of any protective mechanism, they can seriously disrupt normal metabolism through oxidative damage to lipids, proteins, and nucleic acids (Ashraf, 2009; Meloni et al., 2003). Plants possess a number of antioxidant systems that protect them from potential cytotoxic effects. Antioxidant enzymes are important components of the scavenging system of ROS (Meloni et al., 2003). Triazole compounds are used mostly as systemic fungicides and have plant growth regulating properties (Fletcher et al., 2000). Triazole compounds, like triadimefon, triadimenol, propiconazole, uniconazole, and hexaconazole, have growth regulating properties and can induce many morphological and biochemical changes, like reduction in shoot elongation; stimulation of rooting; changes in stem length and weight of seedlings; inhibition of

Table 1. Some plant growth parameters and fruit yield of tomato plants grown under salt stress in the presence of different triazole compounds.

Treatments	Plant shoot length (cm plant ⁻¹)	Stem diameter (mm plant ⁻¹)	Stomatal density (number mm ⁻²)	Fruit yield (g plant ⁻¹)
Control	178±9.6 ^a	12.8±0.71 ^a	172±18.7 ^a	1288±114 ^a
NaCl	135±6.9 ^{cd}	9.2±0.82 ^c	130±14.4 ^d	861±98 ^c
NaCl+TRI	161±11.1 ^{ab}	11.7±1.11 ^{ab}	140±14.1 ^{bc}	914±88 ^b
NaCl+TEB	141±8.4 ^{bc}	11.8±0.66 ^{ab}	143±16.9 ^b	945±101 ^b
NaCl+BIT	143±10.7 ^{bc}	11.4±1.07 ^{abc}	133±9.7 ^{cd}	841±125 ^{cd}
NaCl+TDM	150±13.5 ^{bc}	10.9±0.77 ^{abc}	136±12.5 ^{bcd}	905±109 ^{bc}
NaCl+PBZ	118±12.8 ^d	10.2±0.82 ^{bc}	131±13.6 ^d	834±90 ^{cd}

Each column with the different letters indicate significant differences by LSD's . multiple range test at ($p < 0.05$). The values are the means of four replicates ± SD

gibberellin synthesis; increase in chlorophyll and carotenoid content; improvement of the carbohydrate status; lipid peroxidation; changes in membrane permeability, ascorbic acid, alpha tocopherol, total phenols and nucleic acid contents; increase in cytokinin synthesis; improvement of photosynthetic activity and water balance; increase in the content of proline and soluble sugars; stimulation of antioxidative enzyme systems; and enhancement in carbohydrate metabolism (Berova and Zlatev, 2000; Gomathinayagam et al., 2007; Zhang et al., 2007; Jaleel et al., 2007a; Jaleel et al., 2007c; Houshyarfard and Aalishah, 2008; Kishorekumar et al., 2008; Rajalekshmi et al., 2008; Rajalekshmi et al., 2009; Sridharan et al., 2009; Sivakumar and Panneerselvam, 2011). Triazole compounds influence hormone balance, photosynthetic rate, enzyme activities, lipid peroxidation, and yield components in various crop plants (Zhou and Ye, 1996). Triazole-treated plants present a more efficient free-radical scavenging system that enables them to detoxify active oxygen (Jaleel et al., 2008a). Application of uniconazole and paclobutrazol increases the total lipid soluble antioxidants' alpha tocopherol and ascorbic acid levels in bean leaves. These triazole compounds may protect membrane components from oxidative damage and lipid peroxidation during abiotic stress conditions by increasing the defense mechanisms of the local tissues against free radicals (Fletcher and Hofstra, 1990; Fletcher et al., 2000). Moreover, exogenous application of the growth regulator uniconazole was partially effective in overcoming the adverse effects of salinity by restoring the metabolic alterations, and it improved the nutrient status imposed by salt stress in *Ammi majus* L. plants (Kandil and Eleiwa, 2008). A perusal of the literature offers very little information about the comparative effect of many different triazoles in a single study on the growth and other stress related parameters in *S. lycopersicum* plants under salt stress. In light of this information, this study aimed to investigate whether different triazole compounds are involved in the regulation of antioxidant enzymes, plant growth, fruit yield, macronutrient content, and other parameters under salt stress and therefore to elucidate the physiological mechanism(s) of salt stress mitigated by different triazole compounds in tomato plants.

Results

Plant growth parameters and fruit yield

In this study, salt stress reduced the shoot and root fresh and dry weights, shoot length, stem diameter, stomata number (per observed area), and fruit yield per tomato plant. Tomato plants treated with 125 mM NaCl (salt stress) resulted in shoot and root dry weights that decreased significantly by 50% and 37%, respectively, compared to the control treatment (Figs. 1a-b). Exogenously applied triazole compounds improved the plant growth parameters under saline conditions. Shoot and root fresh and dry weights

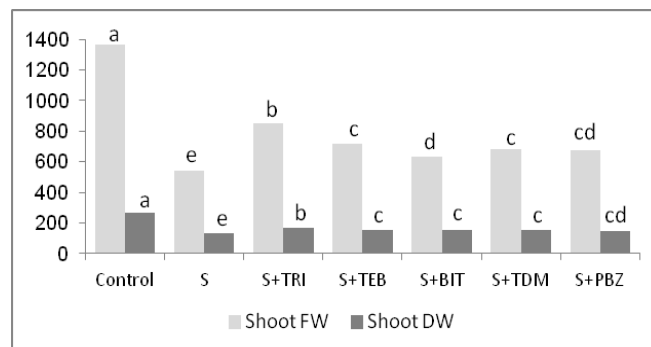


Fig 1a. Fresh and dry weights (g plant⁻¹) of shoots of tomato plants grown under salt stress in the presence of different triazole compounds. Bars with different letters are significantly different at ($p \leq 0.05$) S: NaCl.

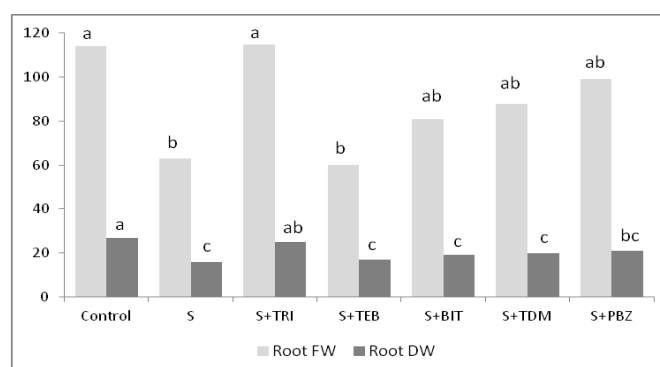


Fig 1b. Fresh and dry weights (g plant⁻¹) of roots of tomato plants grown under salt stress in the presence of different triazole compounds. Bars with different letters are significantly different at ($p \leq 0.05$) S: NaCl.

decreased in the tomato plants grown under salt stress compared to the control (unstressed) plants; however, supplementary triazoles partly ameliorated this status. TRI was consistently the most effective. In addition, although shoot length, stem diameter, and stomatal density of tomato plants increased with TRI and TEB treatments, other triazole compounds had little effect compared to the NaCl treatment (Table 1, Figs. 1a-b). In addition, fruit yield increased highly using the TRI and TEB treatment, with rates equivalent to those produced by plants in NaCl conditions. Application of TRI and TEB alleviated salt stress on plant growth parameters and fruit yield in tomato plants.

Electrolyte leakage, leaf RWC, proline, chlorophyll, and carotenoid contents

Electrolyte leakage of tomato leaves increased following the NaCl treatment, and it was higher in the plants treated with BIT than in plant treated with other compounds. The highest electrolyte leakage was found in BIT applied plants (59.6%).

Treatment with BIT increased the electrolyte leakage by 150% relative to the control. Moreover, compared to NaCl treatment, TRI and TEB treatment resulted in a decrease in electrolyte leakage by 17% and 20%, respectively (Table 2). The RWC in the leaves decreased with salinity treatment. Leaf RWC of the NaCl-stressed plants showed significantly lower values compared to the control plants. Under the NaCl stress, RWC content decreased significantly by 23% compared to the control. Furthermore, application of TRI and TEB significantly increased RWC levels; however, this increase was under the control group (Table 2). Proline showed a sharp increase of up to 600% in NaCl group compared to control plants. Although foliarly applied TDM reduced the accumulation of proline (27%), TRI application induced the accumulation of proline (20%) compared to NaCl-stressed plants. NaCl treatment caused a major decline in the total chlorophyll content, whereas TDM treatment of the NaCl-stressed tomato plant significantly increased not only the total chlorophyll content, but also the chlorophyll a and b contents compared to controls. A similar increase was also seen for the carotenoid content. TDM and TEB treatment increased the total chlorophyll and carotenoid contents to a greater extent in the NaCl-stressed tomato plant compared to control plants. Additionally, TDM treatment increased these contents more than did TEB.

Shoot and root macro element contents

Significant differences were observed for macro elements among treatments in the concentrations of Ca^{2+} , K^+ , P and Na^+ in shoot and root. Na^+ concentrations increased significantly in both shoot and root in the presence of NaCl, and they remained significantly higher compared to the control or when different triazole compounds were applied foliarly. Concentrations of Ca^{2+} , K^+ and P in the shoot decreased in the presence of NaCl (Table 3). Similar results were observed for K^+ and P concentrations in the root (Table 4). Ca^{2+} , K^+ , and P declined dramatically in all tissues of tomato plant, especially in shoot after the NaCl treatment. At the level of 125 mM NaCl, shoot concentrations of Ca^{2+} , K^+ , and P dropped to 22%, 30%, and 10% for controls, respectively. The data presented in Table 3 show that Na^+ , Ca^{2+} , K^+ , and P concentration in shoot increased after the beginning of treatments from 28.3 to 35.5, 29.5 to 44.7, 26.5 to 50.7, and 5.7 to 8.7 (as g kg^{-1} dry weight), respectively. Although accumulation of Ca^{2+} , K^+ , and P in the shoot and root of tomato plant was significantly reduced due to salt stress, exogenous foliar application of different triazole compounds enhanced the accumulation of Ca^{2+} , K^+ , and P in the shoot and root of the NaCl-stressed tomato plants. Treatment with TDM resulted in increases of 19%, 34%, and 37%, in Ca^{2+} , K^+ , and P contents in shoot compared to the control, respectively. Treatment with TDM inhibited the plant Na uptake by 29% relative to the NaCl treatment. Thus, the effect of TDM was more pronounced compared to other triazole treatments on macro element content of shoots. A similar trend was also seen in root macro element contents (Table 4). Salinity caused a significant increase in shoot and root Na^+/K^+ and $\text{Na}^+/\text{Ca}^{2+}$ ratios in tomato plant. However, Na^+/K^+ and $\text{Na}^+/\text{Ca}^{2+}$ ratios improved through the application of different triazole compounds, although TDM was consistently the most effective.

Antioxidative enzyme activities

The effects of exogenously applied triazoles on SOD, PPO, POX, and soluble protein content in the leaves of tomato

plant are shown in Table 5. NaCl-stressed tomato plants showed higher activities of SOD, POX, and soluble protein compared to non-stressed control plants. Plants receiving TRI, TEB, and TDM have significantly higher antioxidative system levels when compared to plants in control conditions. In the NaCl-stressed plants, the activities of SOD, POX, and soluble protein content increased significantly by 180%, 150%, and 55%, respectively, compared to the control. The PPO activity, on the other hand, showed a slight and non-significant increase compared to the control. The highest antioxidative enzyme activities (SOD, PPO, and POX) were obtained from leaves of the TEB, TRI, and TRI treated plants, which were 255%, 265%, and 170% higher compared to the control, respectively (Table 5). It can be concluded that the application of the triazoles caused an important alleviation of the adverse effects of salinity stress by its antioxidant potentials in tomato plants.

Discussion

Excess foliar accumulation of Na^+ inhibits plant growth and development (Ashraf and Harris, 2004; Munns, 2002). One of the most widely used agricultural indices to define stress tolerance is data for plant biomass and yield (Juan et al., 2005; Sairam et al., 2002). The results obtained from these experiments herein show that salt stress caused a significant reduction in plant growth parameters and fruit yield (Table 1; Figs. 1a-b). However, supplementary triazole compounds enhanced these parameters compared to stressed plants. Triazole growth regulators have been reported to protect plants from various environmental stresses, including drought, salinity, and heavy metals (Fletcher et al., 2000; Zhang et al., 2007). Our results demonstrate that exogenous application of triazole compounds improved the shoot length, stem diameter, and stomatal density of tomato plants under salt stress. These findings are in agreement with other published studies. Jaleel et al. (2008b) investigated the interactive effects of triadimefon and salt stress on antioxidative status in *Catharanthus roseus*, concluding that triadimefon treatment of NaCl-stressed *C. roseus* plants increased the root length, plant height, and total leaf area. NaCl treatments combined with triadimefon increased the FW and DW when compared with NaCl-stressed plants and even control plants. Scholars have different opinions regarding the mode of action of Triazoles. Characteristic effects of triazoles on plants include reduced height and stem width, along with increased compactness, the extent of which is dependent on plant species, age, as well as dose and method of application. The effects of triazoles and gibberellic acid (GA) are mutually antagonistic, as seen in examples of inhibition of triazole-induced physiological and biochemical processes. Inhibited gibberellin biosynthesis and increased cytokinin and abscisic acid content induced by these triazoles might be the cause of increased root growth and corresponding dry or fresh weight in plants (Basra, 2000; Rajalekshemi et al., 2009). In the present study, increasing salt concentration in the growth medium significantly reduced fruit yield compared to control plants (Table 1). Although the fruit yield was reduced, no physiological disorders like BER (Blossom end rot is a physiological disorder that is caused by calcium deficiency in the soil) were observed. Turhan et al. (2006) indicated that excessive Na^+ or a deficiency of soluble calcium salts caused a decrease in calcium uptake, thus favoring the development of the disorder. In our study, reduction in fruit yield could have been affected by the interactions of Na^+ with K^+ , Ca^{2+} in the root zone or direct detrimental effect of Sodium. A number of

Table 2. EC, proline, chlorophyll and carotenoid contents and RWC of tomato plants grown under salt stress in the presence of different triazole compounds. EC and RWC as %, proline as ($\mu\text{M g}^{-1}$ FW), chlorophyll and carotenoid as (mg g^{-1} FW).

Treatments	EC	Proline	Chl-a	Chl-b	Total Chl	Carotenoid	RWC
Control	23.8±3.22 ^c	2.3±0.15 ^f	1.48±0.09 ^c	0.64±0.04 ^{ab}	2.12±0.08 ^c	2.76±0.08 ^c	79.5±4.25 ^a
NaCl	42.9±4.25 ^{ab}	17.2±3.54 ^c	0.98±0.11 ^d	0.47±0.06 ^b	1.45±0.08 ^d	2.41±0.06 ^d	61.1±4.34 ^c
NaCl+TRI	35.6±3.85 ^{bc}	20.6±3.05 ^a	1.51±0.22 ^b	0.68±0.04 ^a	2.19±0.15 ^{bc}	3.10±0.11 ^b	72.9±3.24 ^b
NaCl+TEB	34.5±2.74 ^{bc}	13.9±2.11 ^d	1.60±0.21 ^{ab}	0.74±0.02 ^a	2.34±0.16 ^{ab}	3.18±0.18 ^{ab}	73.4±4.08 ^b
NaCl+BIT	59.6±4.96 ^a	17.3±3.06 ^c	1.32±0.14 ^c	0.78±0.08 ^a	2.11±0.08 ^c	3.01±0.17 ^b	67.4±4.11 ^c
NaCl+TDM	48.2±3.52 ^{ab}	12.5±2.22 ^e	1.71±0.08 ^a	0.73±0.05 ^a	2.44±0.07 ^a	3.28±0.32 ^a	67.9±2.29 ^c
NaCl+PBZ	41.1±5.55 ^{bc}	18.1±2.14 ^b	1.59±0.16 ^{ab}	0.73±0.08 ^a	2.32±0.11 ^{ab}	3.19±0.38 ^{ab}	63.0±2.81 ^d

Each column with the different letters indicate significant differences by LSD's multiple range test at ($p < 0.05$). The values are the means of four replicates \pm SD

Table 3. Shoot macro element contents (g kg^{-1} DW) of tomato plants grown under salt stress in the presence of different triazole compounds.

Treatments	Ca ²⁺	K ⁺	Na ⁺	P	Na ⁺ /K ⁺	Na ⁺ /Ca ²⁺
Control	37.6±3.8 ^b	37.8±2.9 ^c	1.3±0.49 ^f	6.3±0.48 ^{cd}	0.034±0.12 ^d	0.034±0.11 ^d
NaCl	29.5±2.7 ^c	26.5±1.8 ^c	28.3±1.8 ^c	5.7±0.44 ^e	1.067±0.06 ^a	0.959±0.18 ^a
NaCl+TRI	37.9±2.9 ^b	33.7±2.2 ^d	30.5±2.1 ^b	6.1±0.55 ^{cd}	0.905±0.11 ^{ab}	0.804±0.09 ^{ab}
NaCl+TEB	31.5±3.1 ^{cd}	41.2±3.3 ^b	23.1±2.5 ^d	7.6±0.28 ^b	0.560±0.18 ^b	0.733±0.14 ^b
NaCl+BIT	37.4±3.3 ^b	34.6±3.1 ^d	35.5±2.8 ^a	6.0±0.81 ^{de}	1.026±0.16 ^a	0.949±0.16 ^a
NaCl+TDM	44.7±4.1 ^a	50.7±4.6 ^a	20.2±1.4 ^c	8.7±0.74 ^a	0.398±0.19 ^c	0.451±0.11 ^c
NaCl+PBZ	30.0±2.2 ^c	27.3±2.5 ^e	31.6±2.0 ^b	6.4±0.55 ^c	1.157±0.13 ^a	1.053±0.10 ^a

Each column with the different letters indicate significant differences by LSD's multiple range test at ($p < 0.05$). The values are the means of four replicates \pm SD.

Table 4. Root macro element contents (g kg^{-1} DW) of tomato plants grown under salt stress in the presence of different triazole compounds.

Treatments	Ca ²⁺	K ⁺	Na ⁺	P	Na ⁺ /K ⁺	Na ⁺ /Ca ²⁺
Control	11.4±1.11 ^d	17.5±1.22 ^a	5.1±0.22 ^f	3.4±0.18 ^{bc}	0.291±0.08 ^e	0.447±0.07 ^e
NaCl	11.7±0.98 ^d	8.4±0.74 ^b	57.2±2.36 ^a	3.2±0.09 ^c	6.890±0.44 ^b	4.888±0.41 ^a
NaCl+TRI	15.8±1.25 ^b	6.1±0.55 ^c	51.6±2.88 ^b	4.0±0.12 ^{ab}	8.459±0.71 ^a	3.265±0.28 ^{bc}
NaCl+TEB	18.5±1.36 ^a	5.9±0.88 ^c	48.3±1.98 ^c	4.2±0.10 ^a	8.186±0.62 ^a	2.610±0.13 ^d
NaCl+BIT	15.4±1.90 ^b	8.7±0.65 ^b	47.1±3.08 ^d	4.5±0.18 ^a	5.413±0.40 ^c	3.058±0.10 ^c
NaCl+TDM	12.8±0.78 ^c	9.2±0.91 ^b	43.5±2.85 ^e	4.5±0.14 ^a	4.728±0.29 ^d	3.398±0.21 ^b
NaCl+PBZ	14.5±1.12 ^{bc}	8.7±0.87 ^b	48.8±3.01 ^c	4.2±0.17 ^a	5.609±0.36 ^c	3.365±0.14 ^b

Each column with the different letters indicate significant differences by LSD's multiple range test at ($p < 0.05$). The values are the means of four replicates \pm SD.

other researchers have reported similar effects of salinity on reducing fruit yield for a range of other agricultural and horticultural crops, including maize (Bar-Tal et al., 1991), tomato (Maggio et al., 2010; Satti and Al-Yahyai, 1995) and pepper (Kaya et al., 2009). The results obtained from the experiments herein show that high salinity reduced stem diameter and stomatal density of leaves (Table 1). We found a 25% reduction in foliar stomatal density in the NaCl group compared to control plants. Although both externally supplied TRI and TEB increased measured parameters compared to non-treated stressed plants, stomatal density and stem diameter remained much lower in treated plants compared to the control plants. The typical detrimental effects of salt stress on stomatal density have been reported in extensive reviews (Kaya et al., 2007; Romero-Aranda et al., 2001). The presence of NaCl in the rooting medium causes a disturbance in membrane permeability, as expressed by an increase in solute leakage or membrane permeability. A major effect of environmental stress (i.e., salt, drought) on plant is membrane modification, which results in cell membrane perturbed function or total dysfunction. Changes in membrane leakage and injury can be measured by the extent of EC (Electrolyte Leakage) in tissues. In the present study, membrane permeability (EC%) of tomato was measured as 42.9 in NaCl treatment and 34.5 in NaCl plus TEB treatment, which causes 20% decrease in membrane permeability (Table 2). A clear link has been found between membrane permeability and lipid peroxidation levels in plants under salt stress. Zhang et al. (2007) found a parallel increase in MDA concentration and electrical conductivity from leaves of soybean exposed to water stress, but

uniconazole reduced electrolyte leakage and MDA accumulation of stressed soybean leaves and protected soybean from stress-induced membrane damage. Triazole altered the sterol biosynthesis and changed the composition of sterol in the plasma membrane. In plants, triterpenes are precursors to sterols, a major compound of cell membrane. Any change in sterol composition may induce changes in the cell membrane, which may be reflected in a change in membrane stability and acclimatization, as observed in plants. Triazoles altered the membrane properties and facilitated the removal of damaged area in the membranes. The altered sterol composition, removal of damaged areas in the membrane and increased kinetin content induced by the triadimefon might have facilitated the increased membrane stability in plants, thereby lowering the membrane permeability (Sridharan et al., 2009). In the present study, salt stress also affected leaf RWC, and NaCl-stressed plants showed significantly lower values compared to respective control plants in response to NaCl stress (Table 2). The decrease in RWC indicated a loss of turgor, which resulted in limited water availability for the cell elongation process (Katerji et al., 1997). The decrease in RWC and chlorophyll content under salinity stress has already been reported (Srivastava et al., 1988; Veselov et al., 2008), and the latter attribute has been suggested as one of the important indicators of salt tolerance in crop plants (Sairam et al., 2002; Srivastava et al., 1988). Maggio et al. (2010), who reported that salinization of the root environment reduced plant growth and, consequently, plant water usage, obtained similar results. Subsequently, salinization gradually reduced both total and osmotic water potentials in tomato plant. Separately,

Table 5. SOD (U mg⁻¹ protein), PPO (U x 100 mg⁻¹ protein), POX activities (ΔA_{470} min⁻¹ mg⁻¹ protein) and soluble protein content (mg 0.5 g⁻¹ FW) in leaves of tomato plants grown under salt stress in the presence of different triazole compounds.

Treatments	SOD Enzyme Activity	PPO Enzyme Activity	POX Enzyme Activity	Soluble protein
Control	72.5±5.2 ^d	89.8±6.6 ^c	7.5±1.1 ^c	0.86±0.12 ^c
NaCl	203.1±17 ^b	97.1±5.4 ^c	18.6±1.2 ^a	1.34±0.19 ^b
NaCl+TRI	244.9±19 ^a	326.5±29 ^a	20.5±2.2 ^a	1.66±0.22 ^a
NaCl+TEB	256.0±27 ^a	126.1±14 ^b	11.1±0.8 ^b	1.30±0.28 ^b
NaCl+BIT	201.4±31 ^b	86.6±11 ^c	11.3±0.6 ^b	1.28±0.15 ^b
NaCl+TDM	226.4±24 ^c	149.3±16 ^b	13.8±1.4 ^b	1.18±0.11 ^b
NaCl+PBZ	208.7±30 ^b	91.4±8 ^c	12.0±1.0 ^b	1.28±0.16 ^b

Each column with the different letters indicate significant differences by LSD's multiple range test at ($p < 0.05$). The values are the means of four replicates \pm SD

Table 6. A comparative analysis of the efficacy on some selected important parameters of triadimenol (TRI), tebuconazole (TEB) and triadimefon (TDM).

	NaCl	NaCl+TRI	% of NaCl	NaCl+TEB	% of NaCl	NaCl+TDM	% of NaCl
EC	43	35.60	83	34.50	80	48.20	112
Proline	17.25	20.65	120	13.95	81	12.51	73
Total Chlorophyll	1.45	2.19	151	2.34	161	2.44	168
Carotenoid	2.41	3.10	129	3.18	132	3.28	136
RWC	61	72.90	119	73.40	120	67.90	111
Shoot Na ⁺	28.3	30.5	108	23.1	82	20.2	71
Shoot K ⁺	26.5	33.7	127	41.2	155	50.7	191
Shoot Ca ²⁺	29.5	37.9	128	31.5	107	44.7	152
SOD	203	245	121	256	126	226	111
PPO	97	327	337	126	130	149	154
POX	19	21	111	11	58	14	74

Fruit yield and shoot DW (g plant⁻¹), EC and RWC (%), proline ($\mu\text{M g}^{-1}$ FW), total chlorophyll and carotenoid (mg g⁻¹ FW), shoot Na⁺, K⁺ and Ca²⁺ (g kg⁻¹ DW), SOD (U mg⁻¹ protein), PPO (Ux100 mg⁻¹ protein), POX (ΔA_{470} min⁻¹ mg⁻¹ protein).

it is hypothesized that protection of salinity in triazole compound-treated plants was associated with longer roots and smaller leaves for absorbing more water and losing less water, which improve salt tolerance in salt-stressed plants (Hajjhashemi et al., 2007). Proline is considered a compatible and osmoregulator solute and it accumulates in many plant species under a broad range of stress conditions, such as water shortage, salinity, extreme temperatures, and high light intensity (Abbaspour, 2012). In our study, proline content in the leaves of tomato plants grown at high salinity increased compared to the unstressed control plants. Moreover, externally supplied triazoles (especially TRI) promoted this effect and increased proline content (Table 2). The literature survey showed that biochemical effects of the triazoles include increased levels of proline. For example, the triadimefon treatment also increased the proline content in the leaves, stem, and root of *C. roseus* plants compared to the control (Jaleel et al., 2007c). Similarly, paclobutrazol increased the proline content in *Eruca sativa* seedlings (Mathur and Bohra, 1992). Consistent with these results, Baninasab and Ghobadi (2011) observed that paclobutrazol ameliorated the injuries caused by heat stress by increasing the leaf proline content and preventing an increase in leaf electrolyte leakage. Our results also confirmed these findings. The chlorophyll and total carotenoid contents of leaves generally decrease under salt stress (Karimi et al., 2005; Yokas et al., 2008). The reduction in leaf chlorophyll under salinity is attributed to the destruction of chlorophyll pigments and the instability of the pigment protein complex (Levitt, 1980). Moreover, light-scattering spectroscopy and microscopy have established that the cross-sectional areas of triazole-treated chloroplasts are significantly larger compared to those observed in untreated leaves. An increase in cytokinins by triazoles could lead to the observed enhanced chloroplast size and chlorophyll levels. For example, in maize, triazole treatment did not change the number of chloroplasts, although the findings indicated more

chlorophyll per chloroplast (Basra, 2000). In the present study, NaCl treatment caused a major decline in the chlorophyll content while TDM, TEB, and PBZ treatment of the NaCl-stressed seedlings significantly increased not only chlorophyll content, but also the carotenoids compared to control. TDM treatment increased carotenoid content to a greater level than did TEB and PBZ (Table 2). Nouriyani et al. (2012) reported that an increase in paclobutrazol concentrations increased chlorophyll content significantly in two wheat cultivars. Pinhero and Fletcher (1994) observed an increase in chlorophyll and carotenoid pigments after treatments with the triazole compound paclobutrazol in maize seedlings. Our data for the described parameters are consistent with these published studies. Most plants use K⁺ and Ca²⁺ rather than Na⁺ as an important component of osmotic adjustment, and K⁺ and Ca²⁺ are essential macronutrients for all plants (Kaya et al., 2007; Zhang et al., 2010). Consequently, crops growing in saline soils may suffer dual injury, Na⁺ toxicity and K⁺ or Ca²⁺ deficiency (Kaya et al., 2007; Schachtman, 2000; Wang et al., 2004). In the present study, our results indicated that accumulation of Na⁺ in the leaves and roots of tomato increased significantly under saline conditions (Table 3 and 4). Exogenous application of triazoles (especially TDM) inhibited the accumulation of Na⁺ in both leaves and roots of NaCl-stressed plants. Accumulation of K⁺, Ca²⁺, and P in the leaves and roots of tomato was significantly reduced due to salt stress. However, application of triazoles through foliar application enhanced the accumulation of K⁺, Ca²⁺, and P in the leaves and roots of the salt-stressed plants. According to Hajjhashemi et al. (2007), salt stress decreased the content of K⁺ and P in the leaves and roots of salt-stressed wheat plants. Furthermore, paclobutrazol treatment enhanced the K⁺ and P contents in the leaves and roots by increasing salinity. Paclobutrazol treatment reduced the accumulation of detrimental Na⁺ in plant tissues but increased the K⁺ and P contents. These observations suggest that paclobutrazol treatment may increase tolerance by diminishing ionic

imbalance caused by salt stress. Our findings are in harmony with this report. Tolerance mechanisms can be categorized as those that function to minimize abiotic stress or ion disequilibrium or those that alleviate the consequent secondary effect caused by these stresses. Na^+ – K^+ or Ca^{2+} selectivity is an important factor in salt tolerance. Increasing leaf and root levels of Na^+ could lead to a Na/Ca and Na/K imbalance. The imbalance of Na^+ , Ca^{2+} , and K^+ in plants can lead to some physiological and biochemical disturbances (Cramer, 1997). The control of Na^+ accumulation and low shoot Na/K or Na/Ca ratios may enhance salt tolerance in tomato crops (Cuartero et al., 2006; Dasgan et al., 2002). In the present study, although triazole treatment alleviated Na/Ca and Na/K rates, TDM was consistently the most effective treatment. In our experiment, the results for K^+ , Ca^{2+} , and the Na/K or Na/Ca ratios were similar to those reported by other authors (Tuna et al., 2007; Voigt et al., 2009). In the present study, we found that different triazole compounds are also involved in the protection and activation of the antioxidative system under conditions of high salinity stress because triazole (especially TRI and TEB) treated plants exhibited higher antioxidative activities after being exposed to high salt stresses compared to non-treated plants (Table 5). These data are in agreement with those of earlier studies, which showed that various triazoles-treated plants are more tolerant of salinity (Jaleel et al., 2007b; Kishorekumar et al., 2008) compared to those that are untreated. Reactive oxygen species (ROS) cause damage to lipids, proteins, and DNA. Peroxidation of membrane lipids occurs when ROS react with unsaturated fatty acids, which leads to leakage of cellular contents, rapid desiccation, and cell death. The ability of plant tissues to mobilize enzymatic defense against uncontrolled lipid peroxidation may be an important feature of the tolerance mechanism (Jaleel et al., 2007c; Velikova et al., 2000). The triazole compounds enhance different H_2O_2 scavenging enzymes, like ascorbate peroxidase and catalase, and also various other antioxidants in *C. Roseus*. This enhancement would help in scavenging of ROS, like H_2O_2 . Triazole compounds not only protect plants from stress, but also induce stress-like symptoms; this might be the reason for increasing the reported H_2O_2 content in different parts of *C. Roseus* (Jaleel et al., 2006). The stress protection offered by triazoles depends heavily on the modulation of the activity and levels of antioxidants. To summarize, triazoles seem to induce increased stress resistance by 1) increasing photosynthetic efficiency, thereby reducing the generation of free radicals; 2) increasing the levels of antioxidants involved in both the prevention of free radical generation; 3) dissipating excess light energy via xanthophyll cycle; 4) stabilizing the membrane structure even under stress conditions; and 5) increasing the root/shoot ratio and more effective transpirational cooling (Singh, 2005). A literature survey indicates that triazoles have increased the activity of antioxidant potential (Kraus and Fletcher, 1994; Nair et al., 2012; Sivakumar and Panneerselvam, 2011), which helps plants tolerate stress conditions. These results are in agreement with those of Jaleel et al. (2008c) who reported that triadimefon treatment caused an increase in the activities of antioxidant enzymes, like superoxide dismutase, peroxidase and polyphenol oxidase. It can be concluded that triadimefon can be used as a potential tool to enhance the antioxidant potential in the medicinal plant *W. somnifera*. Additionally, the non-enzymatic antioxidants ascorbic acid and α -tocopherol were increased in root, stem, and leaf parts of *W. somnifera* treated with triadimefon compared to control plants.

Materials and Methods

Plant culture and treatments

The experiments were conducted with tomato plants (*Solanum lycopersicum* L.) in greenhouse conditions in Muğla-Ortaca (Turkey) from the end of August to the middle of November. Three seeds of tomato were sown in each plastic pot containing 10 kg of an equal mixture of peat, perlite, and sand. After germination, the seedlings were thinned to two plants per pot. Plants were grown for 12 weeks at average day/night temperatures of 25/15°C. Pots were covered with black plastic to block light to the roots and to prevent evaporation. The basic nutrient solution used in this experiment was a modified Hoagland formulation. The composition of the nutrient solution was (mg L^{-1}): 270 N, 31 P, 234 K, 200 Ca, 64 S, 48 Mg, 2.8 Fe, 0.5 Mn, 0.5 B, 0.02 Cu, 0.05 Zn, and 0.01 Mo. The pH of the nutrient solution was adjusted to 6.0 with 0.1 mM KOH. The volume of the nutrient solution, ranging from 200 to 500 mL, was applied to the root zone of the plants from September to November twice a week depending on plant age. Twenty-five days after germination, different treatments were initiated, as follows: (1) control (C): nutrient solution alone; (2) salt stress (S): 125 mM NaCl; (3) S + foliarly BIT: salt plus 250 mg L^{-1} Bitertanol; (4) S + foliarly PBZ: salt plus 40 mg L^{-1} Pacllobutrazol; (5) S + foliarly TEB: salt plus 250 mg L^{-1} Tebuconazole; (6) S + foliarly TDM: salt plus 40 mg L^{-1} Triadimefon; and, (7) S + foliarly TRI: salt plus 30 mg L^{-1} Triadimenol. Triazole compounds were used at different concentrations due to their active ingredients and different effectiveness. Salt stress was created by adding NaCl to nutrient solution. Plants were sprayed foliarly with different triazole compounds mixed with Tween-20 ($\text{C}_{58}\text{H}_{114}\text{O}_2$; a surfactant and spreading agent) once a week, from day 25 after germination up to harvest time. Control plants were sprayed with an equal amount of water mixed with Tween-20. Each treatment was replicated four times (two plants in per pot), and plants were harvested 90 days after seedling emergence.

Enzyme extractions and assays

Approximately 1 g of frozen (-40°C) plant leaf material was homogenized in 50 mM phosphate buffer (pH 7.0) containing 1% polyvinyl pyrrolidone (PVP). The homogenate was filtered and then centrifuged in a refrigerated centrifuge (4°C) at $12,000 \times g$ for 15 min, and the supernatant obtained was used as a source of enzyme. The activity of SOD (superoxide dismutase; EC [Enzyme Commission Number]: 1.15.1.1) was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium, as described by Beauchamp and Fridovich (1971). The activity of POX (Peroxidase; EC: 1.11.1.7) was assayed by adding an aliquot of the tissue extract (100 μL) to 3 mL of assay solution consisting of 3 mL of reaction mixture, which contained 13 mM guaiacol, 5 mM hydrogen peroxide (H_2O_2), and 50 mM Na-phosphate (pH 6.5) (Chance and Maehly, 1955). The POX activity was expressed as change in absorbance $\text{min}^{-1} \text{mg}^{-1}$ protein. The increase in A_{470} was measured for 3 min, and activity was expressed as $\Delta A_{470} \text{mg protein}^{-1} \text{min}^{-1}$. PPO (Polyphenol oxidase; EC: 1.10.3.1) activity was assayed with 4-methylcatechol substrate according to the method of Zaubermann et al. (1991).

Electrolyte leakage, leaf relative water content, proline determination, and chlorophyll and carotenoid contents

Electrolyte leakage (EC) was assessed using young leaf discs, as described by Lutts et al. (1996). Leaf relative water content (RWC) was estimated according to Wheatherley (1950) and calculated as follows: $RWC (\%) = [(fresh\ mass-dry\ mass)/(saturated\ mass-dry\ mass)] \times 100$. Proline content in the leaves was measured as described by Bates et al. (1973). Chlorophyll and carotenoid contents were estimated by extracting 0.05 g of the leaf material in 10 cm³ dimethylsulfoxide (DMSO) (Hiscox and Israelstam, 1979).

Dry weight determinations and chemical analysis

Plant material from each replicate was divided into leaves and roots and then dried in an oven at 70 °C for 2 days to determine dry weights and elemental concentrations. Chemical analyses were carried out on a dry weight basis. Ground samples were dry-ashed at 550°C for 6 h, mixed with 2M hot HCl, filtered, and then brought to a final volume of 50 mL with distilled water. Ca²⁺, K⁺, Na⁺, and P in the sample solution were determined using an ICP-AES (inductively coupled plasma-atomic emission spectroscopy), and the results were expressed in g kg⁻¹DW for all inorganic ions (Chapman and Pratt, 1982).

Stomatal density

Stomatal density was determined on the same leaves used for RWC. Leaf impressions were obtained from both surfaces of the basal leaflet of five leaves from each plant. Impressions were examined with a light microscope at 400× magnification, and stomata were counted on 10 fields taken randomly from each sample.

Statistics

This study used a randomized complete block design. Each pot was considered an experimental unit. Each treatment was replicated four times and each replicate included two plants. The data for all parameters were statistically analyzed using the Statview-ANOVA test. Statistically different groups were compared using an LSD test ($p \leq 0.05$). The mean values with the \pm SD rates are given in the tables.

Conclusion

The results of this study imply that treatment of tomato plant with triazoles may increase the shoot and root dry and fresh weight, elevate the chlorophyll and carotenoid contents, regulate the EC and RWC rates, and stimulate the antioxidative system and macronutrients. In conclusion, these findings led us to conclude that when applied foliarly, different triazole compounds ameliorate the adverse effects of salt stress on growth of tomato by improving some stress parameters and growth related parameters of tomato plant against salt-induced oxidative stress and by maintaining ion homeostasis. Hence, although all five triazoles applied in this study have the potential to be stress protectants, TRI, TEB, and TDM were consistently the most effective. Amongst the three triazoles tested, TRI was slightly more effective compared to the TEB and TDM in protecting tomato plants from salt stress. From these results, it can be concluded that suggested doses application of TRI, TEB, and TDM could be used to ameliorate salinity stress in tomato plants at least partially. Thus, according to the results of present study,

triazole compounds have a significant differential effect on some important stress-related growth parameters, as opposed to the NaCl treatment. These comparative effects are given Table 6.

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