Journal of Scientific Agriculture 2017, 1: 146-157
doi: 10.25081/jsa.2017.v1.52
http://updatepublishing.com/journals/index.php/jsa

REGULAR ARTICLE

Alteration of photosynthetic pigments and antioxidant systems in tomato under drought with Tebuconazole and Hexaconazole applications

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Abstract

Present investigation was focused on the response and regulation of the antioxidant defense system and photosynthetic pigment variation effect of two important fungicides or plant growth regulators Hexaconazole (HEX) and Tebuconazole (TBZ) on drought stressed tomato (*Lycopersicon esculentum* Mill.) plants. Drought stress was imposed for 30 Days after sowing (DAS) of tomato plant. The water was irrigated by 4 Days Interval Drought (DID) and the control plants were regularly irrigated. Triazole treatment like HEX at 15 mg L^{-1} and TBZ at 10 mg L^{-1} imposed on 30, 40 and 50 DAS. The plant samples were collected on 40, 50 and 60 DAS. The photosynthetic pigments like chlorophyll – a, chlorophyll – b and total chlorophyll were estimated. The drought stress reduced the photosynthetic pigments and increased the antioxidant contents and antioxidant enzymes activities. The combined drought stress with triazole treatments increased the photosynthetic pigments then reduced the ascorbic acid (AA), α-tocopherol, catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) activities, when compared to drought stressed plants. It can be concluded that the triazole treatment partially mitigated the adverse effects of drought stress in *L. esculentum*.

Key words: Drought, triazole, photosynthetic pigments, Hexaconazole, Tebuconazole

Introduction

Tomato (*Lycopersicon esculentum* Mill) is an important, popular and nutritious vegetable grown all over the world in outdoor fields, greenhouses and net houses (Sharma et al., 2017). They contribute to a healthy, well-balanced diet in rich minerals, vitamins, essential amino acids, sugars and dietary fibres, iron and phosphorus. Tomato contains good source of vitamins A and C (Farooq et al., 2005). Recently, Zanfini et al. (2017) reported phenolic compounds, carotenoids and antioxidant activities in tomato. The overall increase in tomato consumption in recent time is mainly associated with the benefits of some phytochemicals including carotenoids (Zewdie, 2017).

Water limited conditions in agricultural fields is the main hindering factor for tomato cultivation in countries like India. Drought is a major abiotic stress not only for tomato but for almost all the agricultural crops around the world (Yordanov et al., 2003). Zhou et al. (2017) recently reported effect of drought in producing over heat stress on three tomato varieties.

There are traditional and nontraditional plant growth regulators. Triazole compounds are one among the nontraditional growth

Received 21 April 2017; Accepted 10 June 2017; Published 13 June 2017

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regulators. In the same time, they possess stress protection effects on plants under various abiotic stresses (Fletcher et al., 2000). The present study was aimed to evaluate the drought stress protection action of two important fungicides or plant growth regulators Hexaconazole (HEX) and Tebuconazole (TBZ) by estimating the changes in photosynthetic pigments, antioxidants and antioxidant enzyme activities of *L. esculentum* under drought with triazole treatments.

Materials and methods

Plant cultivation and treatment induction

The seeds of Tomato (*Lycopersicon esculentum* Mill) belonging to the family Solanaceae was selected present investigation. The Tomato variety PKM-1 was obtained from Horticulture College and Research Institute, Periyakulam at Theni district, Tamilnadu, India. The triazole compound Tebuconazole, Hexaconazole was obtained from syngenta India ltd., Mumbai.

The experimental part of this work was carried out in Botanical Garden and Plant Growth Regulation Lab, Department of Botany, Annamalai University, Tamil Nadu, India. The mud pots filled with homogenous mixture of the garden soil containing red soil, sand along with farmyard manure ratio of (1:1:1). The pots were arranged in Completely Randomized Block Design (CRBD). The experimental seeds were surface sterilized with 0.2% Mercuric chloride solution for five minutes with frequent shaking and thoroughly washed with tap water. The plants were allowed to grow up to 30 days with regular water irrigation.

After 30days, well established plants were selected for treatments. The drought stress given on 4 DID (Days Interval Drought) and drought stress with TEBU @ 15 mg L^{-1}, HEX @ 10 mg L^{-1} treatments are given on 30, 40, and 50 DAS plant. One-day interval irrigation on ground water was kept as control. The plant samples were collected on 40, 50 and 60 DAS.

Chlorophyll content

The chlorophyll content of young and mature leaves was determined by following method (Arnon, 1949). The plant material was homogenized in mortar with pestle and extracted in 80% acetone. After that centrifuged the supernatant at 800 rpm for 15 mts. To make up the supernatant 10ml with 80% acetone and absorbance OD at 645, 663, and 480nm. The values are expressed in mg/g fresh weight.

Antioxidant analysis

Non enzymatic antioxidants such as α-Tocopherol (Backer et al., 1980), ascorbic acid (Omaye et al., 1979) were analysed by standard methods. *Antioxidant enzyme activities such as Superoxide dismutase activity* (Beauchamp and Fridovich, 1971), *Catalase activity* (Chandlee and Scandalios, 1984) and peroxidase activity (Reddy et al., 1995) were determined by following previously established methods.

Statistical analysis

Statistical analysis are performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The values are mean ± S.D. for three samples in each group. *P*-values ≤ 0.05 was considered as significant.

Results

Effect of triazole treatments on chlorophyll content of Tomato under drought condition

A significant reduction was observed in chlorophyll contents of drought stressed plants and they were 64.84, 72.48 and 68.38 percent over control respectively in chl-a, chl-b and total chlorophyll content at 60 DAS plants. Drought with triazole treatments to increase the photosynthetic pigments when compared to drought stressed plants and it was 80.03, 83.14 and 81.58 in TBZ treated plants then 90.08, 92.05 and 91.23 percent over control in chl-a, chl-b and total chlorophyll content respectively at 60 DAS plants (Table 1).

Effect of Triazole treatments on Ascorbic acid content of Tomato under drought condition

Drought stress caused an increase in the ascorbic acid content and it was 147.22, 147.83 and 137.64 percent over control on root, stem and leaves on 60 DAS plants respectively. Triazole to the drought stressed plants decreased the ascorbic acid content when compared to drought stress, but it was higher than that of control and they were 122.36, 122.36 and 101.29 in TBZ treated plants and 125.18, 127.74 and 107.56 percent over control respectively on root, stem and leaves of 60 DAS plants (Fig. 1).

Table 1. Effect of triazole treatments on chlorophyll content of tomato under drought condition.

	Control	Drought	Drought+TBZ	Drought+HEX
Chlorophyll - a ($mg^{-1}g$ f.w)				
40 DAS	0.320 ± 0.007[a]	0.173 ± 0.010[d]	0.254 ± 0.005[c]	0.288 ± 0.003[b]
50 DAS	0.506 ± 0.007[a]	0.357 ± 0.004[d]	0.434 ± 0.010[c]	0.417 ± 0.012[b]
60 DAS	0.635 ± 0.008[a]	0.412 ± 0.006[d]	0.510 ± 0.007[c]	0.572 ± 0.008[b]
Chlorophyll - b ($mg^{-1}g$ f.w)				
40 DAS	0.232 ± 0.008[a]	0.124 ± 0.005[d]	0.146 ± 0.009[c]	0.168 ± 0.005[b]
50 DAS	0.456 ± 0.008[a]	0.294 ± 0.008[d]	0.353 ± 0.011[c]	0.400 ± 0.011[b]
60 DAS	0.516 ± 0.007[a]	0.374 ± 0.011[d]	0.429 ± 0.070[c]	0.475 ± 0.012[b]
Total Chlorophyll m($mg^{-1}g$ f.w)				
40 DAS	0.552 ± 0.013[a]	0.276 ± 0.014[d]	0.400 ± 0.005[c]	0.451 ± 0.004[b]
50 DAS	0.926 ± 0.006[a]	0.651 ± 0.009[d]	0.786 ± 0.021[c]	0.871 ± 0.017[b]
60 DAS	1.151 ± 0.015[a]	0.787 ± 0.011[d]	0.939 ± 0.003[c]	1.050 ± 0.010[b]

Values are mean ± S.D. of three replicates are given a common superscript (a, b, c, d) according to Duncan's multiple range test (DMRT) at $p < 0.05$ level of probability.

Effect of Triazole treatments on α-tocopherol content of Tomato under drought condition

The higher accumulation of α-tocopherol content was found in all parts of plants and it was183.65, 169.83 and 175.59 percent over control at 60 DAS drought stressed plants. Drought with triazole treatments lowered the α-tocopherol content when compared to drought stressed plants and it was 132.39, 127.09 and 130.81 in TBZ and 137.11, 132.96 and 144.76 percent over control on HEX treatment plant root, stem and leaves respectively at 60 DAS (Fig. 2).

Effect of drought, Triazole and their combination on Super Oxide Dismutase activity

Drought stress increased the SOD content. The highest SOD content was recorded and it was 155.56, 146.78 and 123.62 percent over control on 60 DAS plants root, stem and leaves respectively. TBZ and HEX to the drought stressed plants decreased the SOD content when compared to drought stressed plant and it was 121.81, 123.98 and 105.39 in TBZ and 130.86, 129.43 and 112.02 percent over control in HEX treated 60 DAS plants of root, stem and leaves respectively (Fig. 5).

Effect of drought, Triazole and their combination on Catalase activity

The highest level of increase in catalase content was observed in drought stressed plants and it was 153.20, 157.40 and 147.13 percent over control on root, stem and leaves at 60 DAS plants respectively. Drought with triazole treatment reduced the catalase content when compared to drought stressed plants and it was 127.77, 133.03 and 125.79 in TBZ then 128.93, 134.48 and 129.72 percent over control in HEX treated root, stem and leaves respectively at 60 DAS tomato plants (Fig. 3).

Effect of drought, Triazole and their combination on Peroxidase activity

In all the sampling days peroxidase content accumulation was high in tomato plants under drought stress when compared to control and it was 146.28, 122.06 and 139.00 percent over control at root, stem and leaves respectively at 60 DAS. Drought with triazole treatments showed a decreased peroxidase content when compared to drought stressed plants but higher than that of control and it was 124.79, 108.77 and 113.49 on TBZ and 128.93, 111.28 and 115.59 percent over control at root, stem and leaves respectively on 60 DAS (Fig. 4).

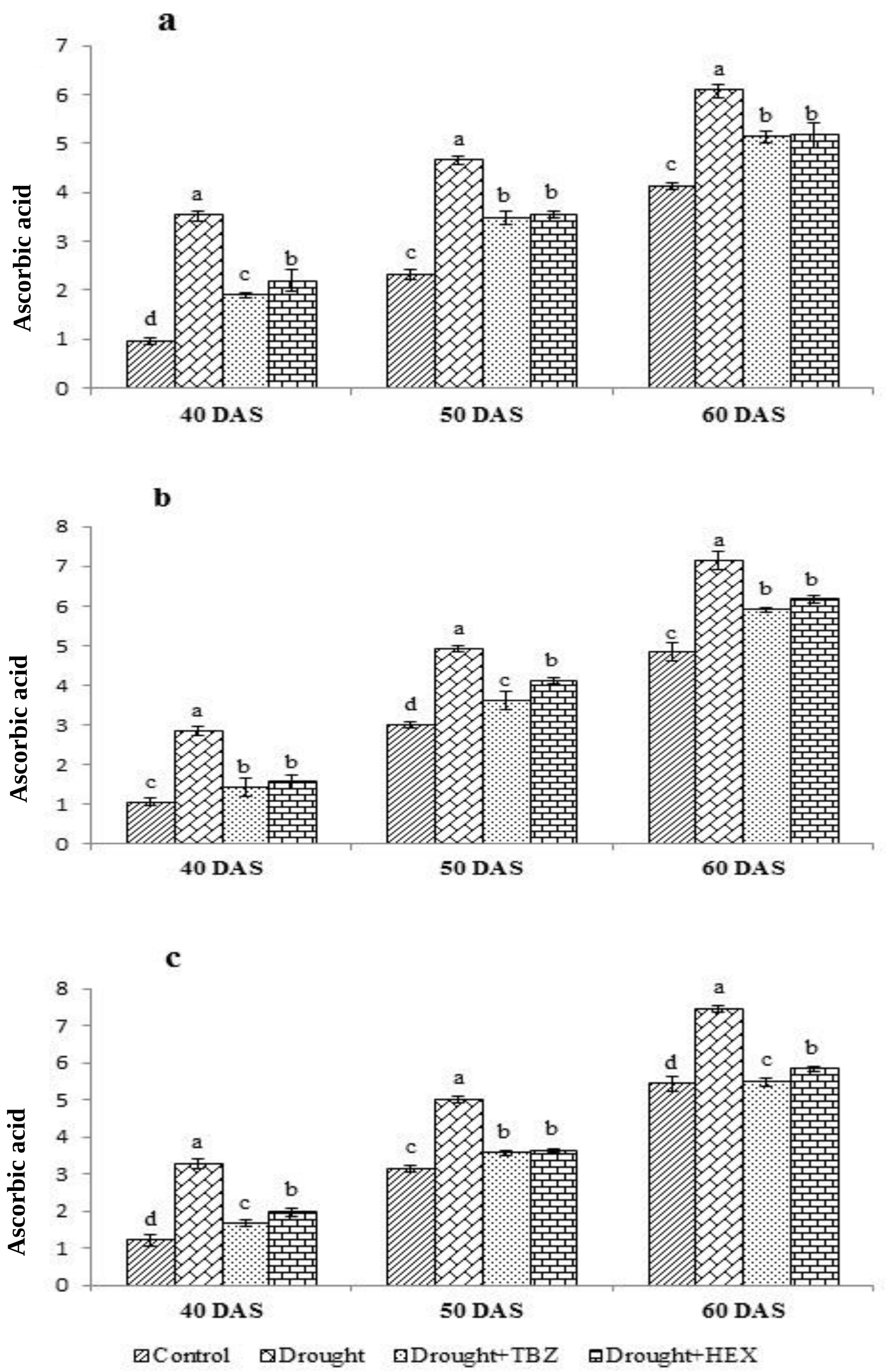

Fig. 1. Effect of drought with triazole treatments on Ascorbic acid content (a) Root (b) Stem and (c) Leaves of tomato plants. Values are given as mean ± SD. of three samples in each group.
Bar values are significantly at ≤ 0.05 (DMRT).

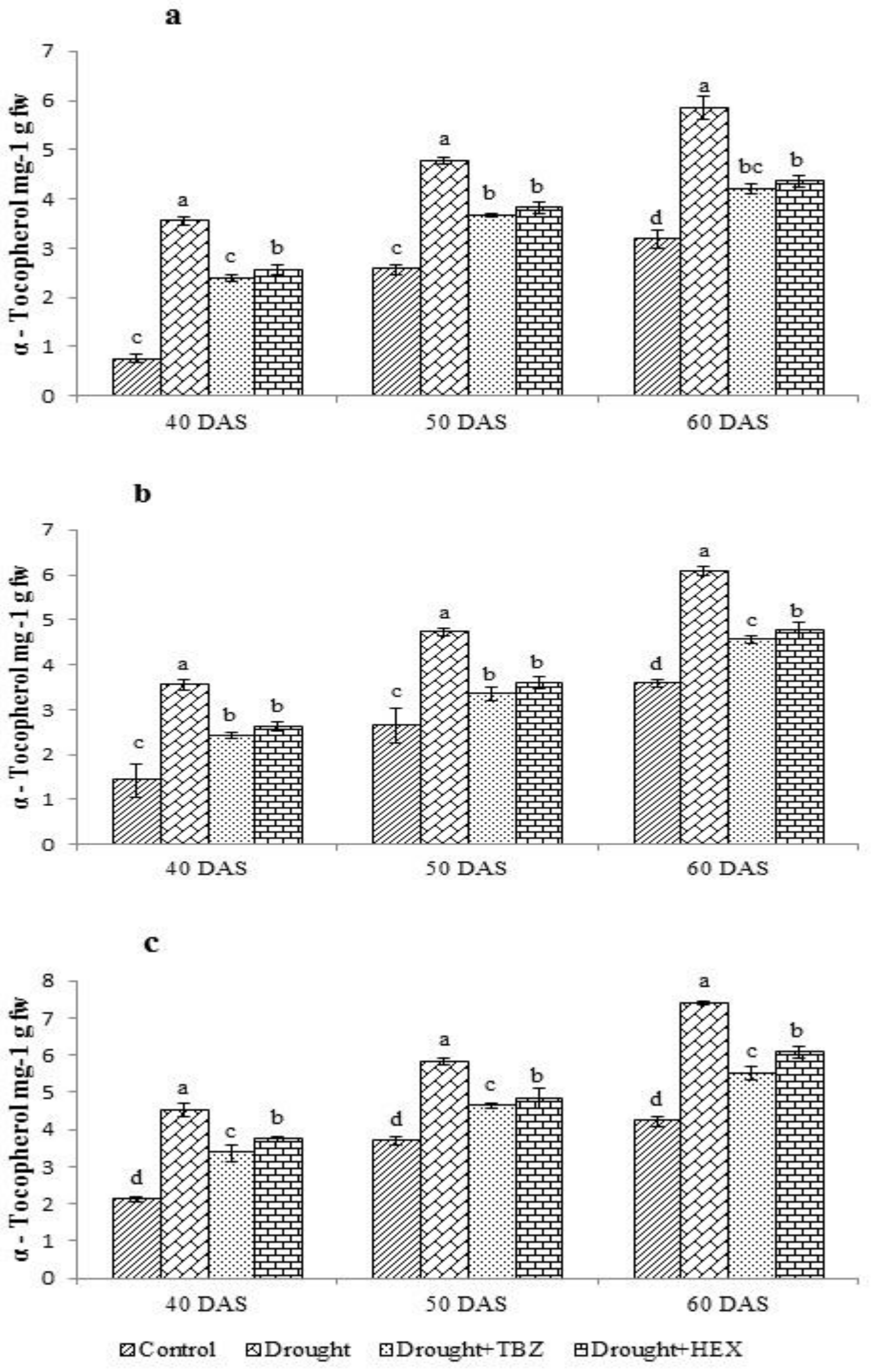

Fig. 2. Effect of drought with triazole treatments on α - Tocopherol content (a) Root (b) Stem and (c) Leaves of tomato plants. Values are given as mean ± SD. of three samples in each group.
Bar values are significantly at ≤ 0.05 (DMRT).

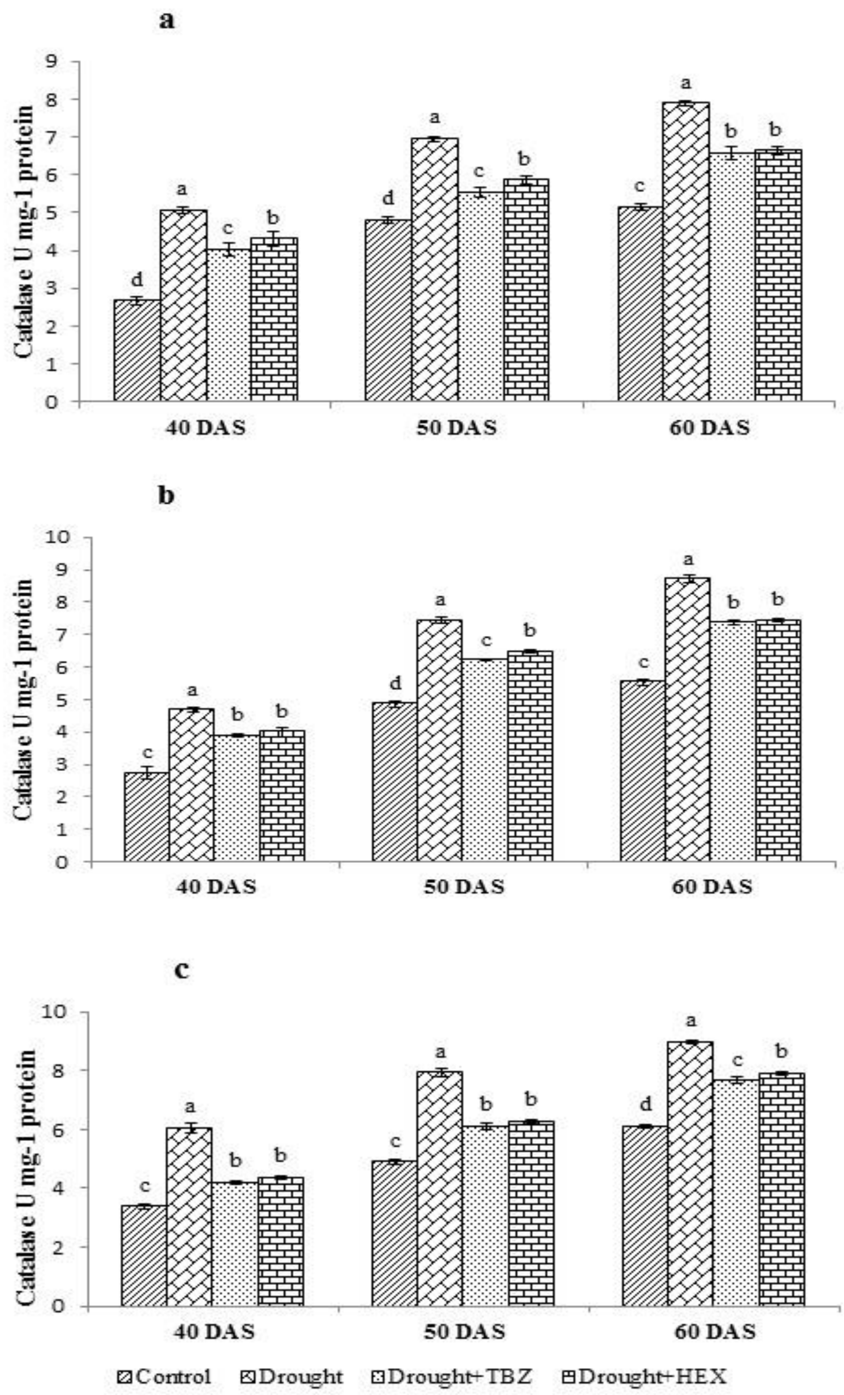

Fig. 3. Effect of drought with triazole treatments on catalase activity (a) Root (b) Stem and (c) Leaves of tomato plants. Values are given as mean ± SD. of three samples in each group. Bar values are significantly at ≤ 0.05 (DMRT).

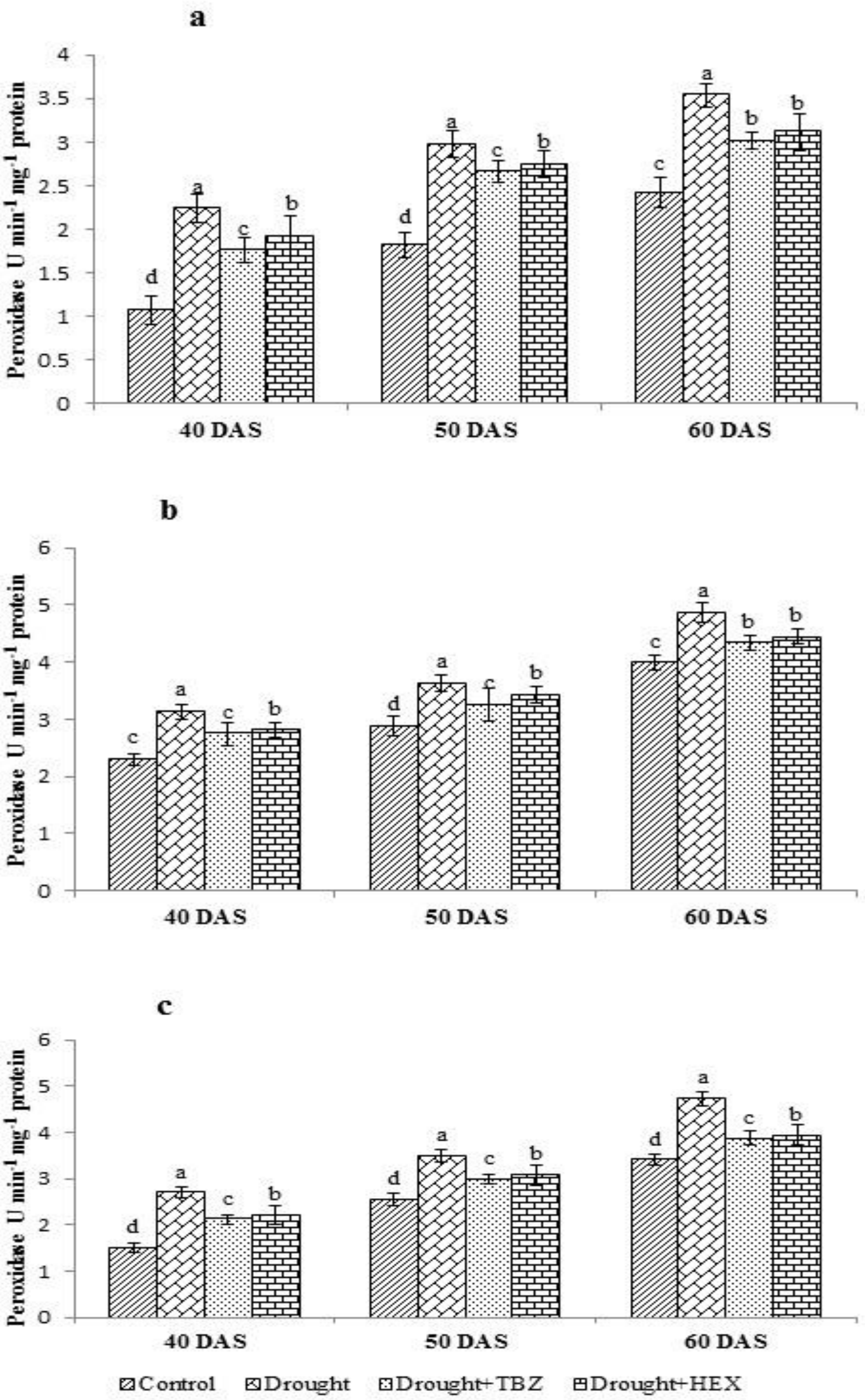

Fig. 4. Effect of drought with triazole treatments on Peroxidase activity content (a) Root (b) Stem and (c) Leaves of tomato plants. Values are given as mean ± SD. of three samples in each group. Bar values are significantly at ≤ 0.05 (DMRT).

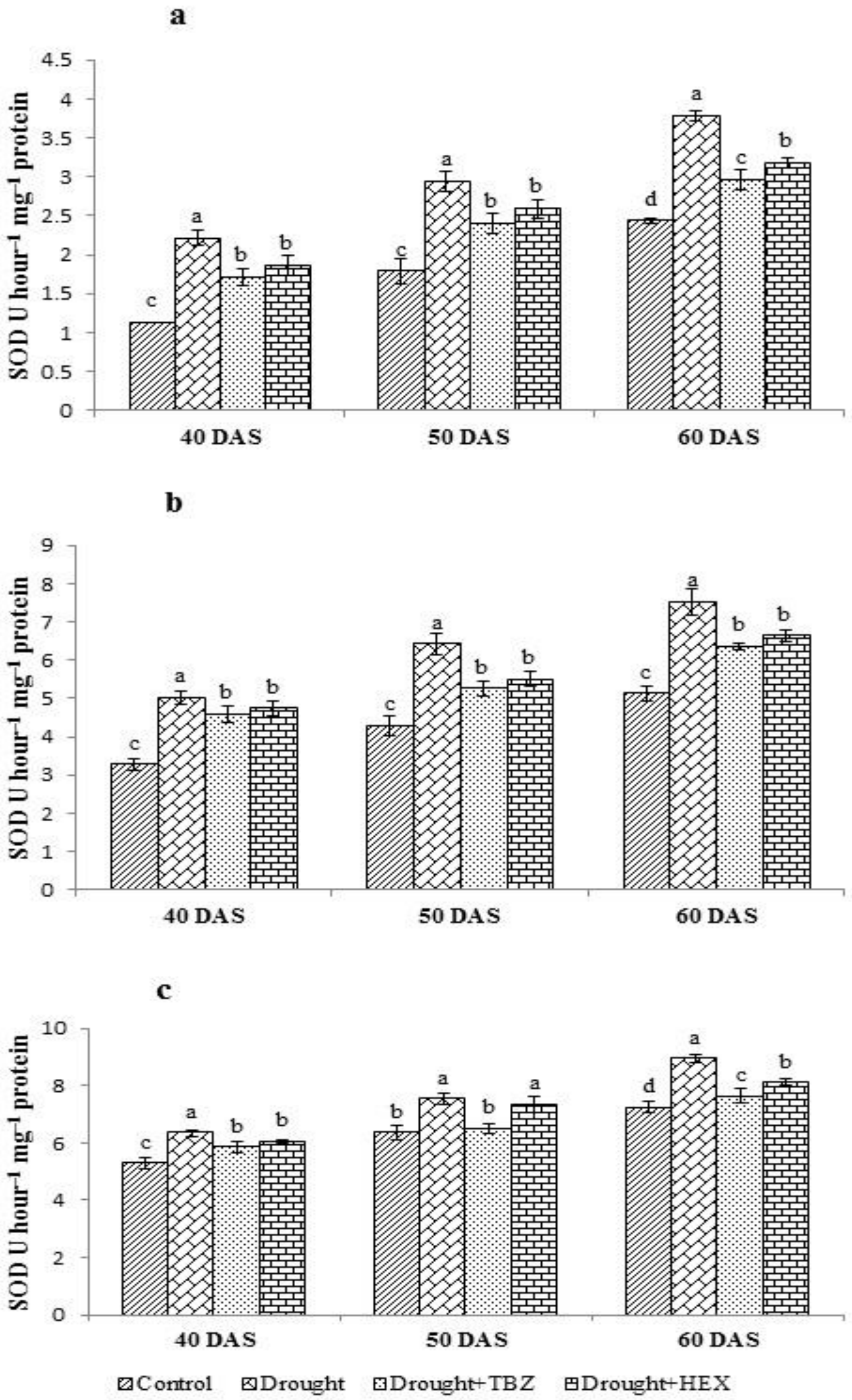

Fig. 5. Effect of drought with triazole treatments on SOD activity (a) Root (b) Stem and (c) Leaves of tomato plants. Values are given as mean ± SD. of three samples in each group.
Bar values are significantly at ≤ 0.05 (DMRT).

Discussion

The main objectives of the present investigation to assess the effect of drought and drought with treatments like tebuconazole and hexaconazole in tomato plants. The chl-a, chl-b and total chlorophyll content of the drought treated tomato plant was decreased when compared to control plant. Photo-oxidation may be the potential reason for reduced leaf pigments under water limited conditions (Ahmedi et al., 2009) and which in turn enables the plants to minimize the light absorption by chloroplasts (Pastenes et al., 2005). The triazole treated plant increased the photosynthetic pigments but it was lower than that of control. The same results were observed in *Solanum trilobatum* (Nivedithadevi et al., 2012) and *Sorghum bicolar* (Arivalagan and Somasundaram, 2015).

Ascorbic acid content of the drought stressed plants significantly increased when compared to control tomato plants. In plants under any type of stress the increase of scavenging enzymes is important for reducing substrate for H_2O_2 (Chen and Gallie, 2004). The increased ascorbic acid may protect the plants from stress (Reddy, 2004). Triazole compound to the drought stressed tomato plants decreased the ascorbic acid content but, it was higher than that of control. The similar results were observed (Amalan Rabert et al., 2013) in *Capsicum annuum*.

α -tocopherol of the drought stressed plants significantly increased when compared to control plants. Triazole application increased the α - tocopherol concentration which might confer stress protection (Wang and Quinn, 2000). Similar results were observed by (Somasundaram et al., 2009) in *Sesamum indicum*. The triazole treatments increased the α -Tocopherol content at all stages of growth when compared to control but, lower than that of drought stressed plants. Similar observations were made by (Sankar et al., 2007) in *Arachis hypogaea* and (Kraus et al., 1995) in wheat.

The activity of SOD increased under drought stress in tomato. SOD is one of the most important front - line enzyme in ROS defence antioxidant enzyme that remove the superoxide radical by catalysing its dismutation, one superoxide radical being reduced to hydrogen peroxide and another oxidized to oxygen (Gill and Tuteja, 2010). The highest increase of SOD activity in this study was due to the drought stressed tomato plants when compared to all other plants. The increase of SOD activity has been reported under water stress in tomato (Sánchez-Rodríguez et al., 2012) and chrysanthemum (Sun et al., 2013). Triazole treatment in combination with drought decreased the SOD activity in tomato plant but, it was higher than that of control plants. Previous study reported the increasing effect of triazoles on antioxidant enzymes (Nivedithadevi et al., 2017).

The drought stress to increase the peroxidase content when compared to control tomato plant. Drought stress is associated with increased oxidative damage to plants due to the increased production of ROS, such as H_2O_2 and O_2^-, which is further reduced to O_2 and H_2O by POD and CAT (Uzilday et al., 2012). Previous studies on the responses of antioxidant defence systems in plant to drought stress have concentrated on various commercial crops (Cakmak and Marschner, 1992; Bai et al., 2006). These reports provided substantial information about the protective mechanisms in the crops that prevented oxidative injury under dry conditions. Enzyme activities in the *F. denudata* leaves under drought conditions significantly increased, which partially confirmed results obtained by (Pompelli et al., 2010) and (Liu et al., 2014) in *Fargesia denudate*. Drought stressed plants treated with triazole showed a decreased CAT activity but, it was higher than that of control. Increased CAT activity was reported in *Phaseolus acutifolius* under drought stress (Turkan et al., 2005) and in *Solenostemon rotundifolius* (Kishorekumar et al., 2008).

The peroxidase activity was showed an increase the root of tomato under drought conditions. Under water deficit stress showed an enhancement in POX activity irrespective of different genotypes (Somasundaram et al., 2009) in *Sesamum indicum* and (Saed-Moocheshi., 2014) in maize. Drought stressed plants treated with triazole showed an increased peroxidase activity but, it was lower than that of control. Similar results were observed in rice (Pan et al., 2013).

From our results it can be concluded that, the triazole compound like tebuconazole and hexaconazile can be used as an effective compound to increase resistance to environmental stresses. The triazoles improve the harmful effects of stress by its influence on antioxidant potentials in *Lycopersicom esculentum* Mill.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The authors are acknowledging Professor Dr. K. Arumugam, Head of the Department of Botany, Annamalai University for providing laboratory and facilities for carrying out this research.

Author contributions

M.A worked under the supervision of R.S. Both the authors participated in the writing of the manuscript. Both the authors agreed the final version of manuscript for publishing in Journal of Scientific Agriculture.

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