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Effect of Triamidefon fungicide on some growth parameters and antioxidant enzymes activity in tomato (*Lycopersicom esculentum* Mill.) plant under drought stress

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Abstract

Triamidefon is a triazole derivative, which have both fungicidal and plant growth regulator (PGR) properties, and also protect plants from several types of abiotic stresses. The influential mechanism of Triamidefon on plants is not much studied. In this study effect of this compound on the drought stressed in tomato plants was investigated. The plants were subjected to 5 day interval drought stress and drought stress with TDM ($15mgl^{-1}$) and TDM alone from 30 days after sowing. The plant samples were collected for estimating the related analysis. Individual and combined drought stress and TDM treatment increased dry weight, Chlorophyll content, SOD, CAT, PPO activities and decreased leaf area and prolin content. From the result of this investigation it can be concluded that the application of TDM caused a partial recovery of the damaging effect of drought stress by its influence on antioxidant system.

Key words: Fungicide, Triamidofon, Drought stress, *Lycopersicom esculentum* Mill.

INTRODUCTION

Stress as an external factor that usually have adverse effects on plant leaves, is defined (Kramer, 1983). Drought is the most common environmental stress. Dehydration occurs when lack of adequate moisture prevents the growth and completion of the life cycle plant. Lack of water can occur as a result of reduced rainfall; coarse textured soils that prevent keeping water around plant roots or drying winds. Depending on the duration and intensity of stress, a wide range of processes at different levels of cellular molecular and biochemical happen (Vamerali *et al*, 2003). Drought stress stops photosynthetic activities. This is the result of an imbalance between light capture and utilization of light (Foyer *et al*., 2000). Stomatal closure occurs as a result of water shortages, followed by decreasing of CO2 concentration in mesophile leaves and accumulation of NADP in chloroplasts. In this condition NADP acts as limiting factor and O_2 acts as electron acceptor, as a result superoxide radicals are formed. SOD through a series of mono valence reducing reactions produce Hydrogen peroxide (H_2O_2) and hydroxyl radical (OH•) (Thomson, 1987). On the other hands, down regulation of PSII causes imbalance between production and consumption of electrons. Reactive oxygen species damage biological systems. For example DNA cleavage, oxidation of proteins and amino acids and peroxidation of lipids. Triazols are a

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group of growth inhibitor chemical compounds that widely used as fungicides. The compounds contain three nitrogen atoms and a pentagonal rings. It can be noted to many types of triazol such as uniconazol, paclobutrizol and triamidefon. Triadimefon is a triazole compound having fungicidal as well as plant growth regulating properties.Triazole make changes in plant by preventing the enzyme activity of CytP450 (Zhu, 2004). These enzymes catalyze the oxygenation of fatty acids and also are responsible for the synthesis of phenylpropanoids. The primary effect of these compounds inhibits gibberellic acid biosynthesis through affecting kaurene oxidase and stimulates the accumulation of ABA. This effect is similar to the effect of drought stress on plants. Prevent degradation of ABA and convert it to phaseic acid (PA), decrease in ethylene concentration and increase in concentration of cytokinin are the other effects of triazoles in plants (Jaleel *et al*., 2006). Increased tolerance to drought stress affected uniconazole, paclobutrizole in wheat (Berova, 2003), corn (Marshal, 2000) and tomatoes (Still, 2004) has been seen. Secondary effects of these compounds included morphological changes in plant such as reduced shoot growth and physiological changes such as increased ABA, proline and the antioxidant system, has been reported (Marshal *et al*., 2008). This study investigated the use of triamidefon (TDM) as one of the triazole less has been tested on tolerance to the drought stress in *Lycopersicom esculentum* Mill.

MATERIALS AND METHODS

Tomato seeds were sterilized with sodium hypochlorite 0.2% for 5 min. After washing with deionised water the seeds were transferred in plastic pots (300 mm diameter) filled with sand, clay and leaf soil. Four seeds were planted in each pot. The pots were transferred to growth chamber with day/night temperature of 25/20 ºC and a 16 h light/8 h dark photoperiod, with a relative humidity of 70%. Before treatment, all plants were irrigated regularly every day. 30 days after germination, the plants were divided into four groups:1) control, 2) The plants were irrigated once every 5 days and treated with 15 mgL^{-1} of TDM, 3) The plants were treated with 15 mgL^{-1} of TDM 4) The plants were irrigated once every 5 days without TDM treatment. Three plants were selected and rinsed with distilled water. After drying the surface water, root and shoot dry weight, root and shoot fresh weight was measured. Chlorophyll and carotenoid were extracted from the leaves and estimated by the method of Lichtenthaler (1987). Leaves samples (0.1 g) were homogenized in acetone (80 %). Absorbance was recorded at wavelengths of 646.8 and 663.2 nm for chlorophyll assay and 470 nm for carotenoids assay by UV-Vis spectrophotometer.

Chl. a = (12.25 A663.2- 2.79 A646.8),

Chl. B = (21.21 A646.4- 5.1 A663.2),

Car = [(1000A470-1.8 Chl.a-85.02 Chl.b)/198]

The proline content was estimated by the method of Bates et al (1973). The plant material was homogenized in 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 12,000 rpm for15 min. The reaction mixture consisted of 2 ml of acid ninhydrin and 2 ml of glacial acetic acid, which was boiled at 100 ◦C for 1 h. 4 ml of toluene use for extraction. Absorbance was read at 520 nm.

Enzyme extraction and assay of enzyme activity

0.5 g frozen samples was homogenized in 2.5 ml of 50 mM phosphate buffer (pH 7.2) containing 1 mM ethylenediamine tetraacetic acid (EDTA), 1 mM phenylmethanesulfonyl fluoride (PMSF) and 1% polyvinyl pyrrolidone (PVP). The homogenate was centrifuged at 14,000×g for 15 min at 4ºC. Activity of enzyme was determined by spectrophotometer.

Polyphenol oxidase (PPO) assay: Polyphenol oxidase (PPO) activity was assayed by the method of Ghanati *et al*.(2002). Oxidation of atechol in the presence of hydrogen peroxide was determined. The absorbance was read at 495 nm. PPO activity is expressed in Umg^{-1} protein.

Superoxide dismutase (SOD) assay :(SOD) was measured according to Giannopolitis & Ries (1977) method. The reaction mixture was contained: 2.5 ml of 50 mM potassium phosphate(pH=7.8), 0.1 ml of methionine 13 mM, , 0.1 ml Nitro Blue Tetrazolium (٧٥μ), 0.1 ml riboflavin (٢μ M) and 0.2 mL enzyme extract. The unit of SOD activity was defined as the amount of enzyme that inhibits the NBT photoreduction by 50 %. SOD activity values are given in units per mg of protein.

Catalase activity: Catalase (CAT) was measured according to Cakmak & Horst(1991). The assay mixture contained: sodium phosphate buffer 25mM(ph= 6.8), H_2O_2 and enzyme extract. Unit of activity was taken as the amount of enzyme which decomposes 1 mM of H_2O_2 in one min.

Statistical Analysis

Statistical analysis was carried out according to a completely randomized design. The data were expressed in mean±S.D. of three replicates. The data were subjected to an analysis of variance (two ways). Different means were compared using LSD.

RESULTS AND DISCUSSION

Effect of drought, TDM and their combination on growth parameters:

Drought stress caused an increased in root length and a decreased in shoot length, dry and fresh weight and also leaf area when compared with the control. Treatment with TDM in combination with drought increased the root length, dry and fresh weight compared to the control. Root and shoot length, dry and fresh weight and leaf area were higher when compared with plants under drought stress. TDM treated plants showed a decrease in shoot length, leaf area, dry and fresh weight and an increase in root length compare with the control (Fig 1,2,3).

Effect of drought, TDM and their combination on photosynthetic pigments

Drought stress decreased chlorophyll a, chlorophyll b and total chlorophyll and carotenoid content. The TDM treatment alone significantly enhanced the amount of photosynthetic pigments. The enhancement in chlorophyll a was more than chlorophyll b content. The combined treatments of drought and TDM had no significant effect on pigment content (Fig. 4, 5).

Effect of drought, TDM and their combination on prolin content

Prolin content increased in both drought stress and with TDM treatments when compared to control.TDM treatments decreased prolin content in plants under drought stress compared with the plants had received only water stress treatment (Fig. 6).

Effect of drought, TDM and their combination on antioxidant enzyme activities

Catalase activity increased in drought stress and with TDM treatments compared with control. Enzyme activity in stressed plants treated with TDM showed no significant increase compared with control.

SOD activity increased in drought treatment when compared to control. TDM treatment increased the SOD activity in drought stressed as well as in control plants.

PPO activity increased in drought treatment when compared to control. TDM treatment in combination with drought increased PPO activity when compared to control. TDM treatment increased the PPO activity in drought stressed as well as in control plants (Fig.7).

DISCUSSION

Drought Stress is characterized by reduce water content, turgor and leaf water potential, plant wilt, stomatal closure and decrease in cell growth. Quality and quantity of plant growth is related to cell division. Elongation and differentiation are also affected by drought (Sestak, 1971). Root characteristics in particular, the length and mass are important to get water (Manivannan, 2007). The results of the Fletcher and Hofstra investigation proved that treatment of plant by Triadimefon increase *Setaria italic* survival under drought stress. This research proved Plants treated with TDM showed higher net photosynthetic rates. It seems this triazole compounds alter the levels of plant hormones by inhibiting gibberellins and ergosterol synthesis as well as regulation of endogenous levels of ABA and cytokinin (Grossmann, 1987). In this research, TDM treatment decreased dry weight and leaf area. The primary effect of these compounds is inhibition of kaurene oxidase activity, the inhibition of GA biosynthesis (Graebe, 1984) and the increased ABA content. Triadimefon treatment increased root length. Changes in plant hormones such as increased the cytokinin content followed by an increase the cell division, increased the root length (Gopi et al., 2007). Similar results were obtained in *Phaseolus vulgaris* (Gomathinayagam, 2007), *Plectranthus aromaticus* and *Plectranthus vettiveroides* plants (Gopi et al., 2007) and *Catharanthus roseus (*Abdul Jaleel et al., 2008). Reduction in chlorophyll content in plants under water stress due to chloroplasts damage caused by reactive oxygen radicals (smirnof,1995). TDM treatment increase the level of cytokinin, that stimulate chlorophyll biosynthesis (Fletcher,2000). Triadimefon treatment increased the chlorophyll content in the leaves of many plants such as *Catharanthus roseus* (Abdul Jaleel et al., 2006) and *Withania somnifera* (Abdul Jaleel et al., 2008). An increase in the carotenoid content was observed in TDM treatments in this research. This increase is consistent with the Buchenauer and Rohner report in barley (Buchenauer and Rohner, 1981).A transient raise in ABA content in treated plants by TDM might be the cause for the increased Carotenoid content.

Proline accumulation is probably an adaptation mechanism to cope with stress. In this condition proline accumulates provide enough energy to plant growth and survival and increase tolerance to

stress. Prolin act as a scavenger of free-radicals of oxygen (Chandrashekar, 1996**)**. The TDM treatment also increased the proline content in the leaves. Similar results in *Catharanthus roseus* has been reported by Abdul Jaleel et al.,(2008) Similarly, paclobutrazol increased the proline content in *Eruca sativa* seedlings. Triadimefon and hexaconazole increased the amino acid and proline contents in *C. roseus* by transient raise in ABA content (Abdul Jaleel et al., 2007). Proline oxidase activity decreased under TDM treatment in *C. roseus* seedlings. This enzyme converts free prolin into glutamate. Based on the results of this study, TDM has a regulatory effect on function of plants. This compound through enhancing the rate of photosynthesis, increased osmoprotectan in plants under stress and effect on plant growth regulators, increases the resistance of plants to drought stress. According to Abdul jaleel et al. (2008) application of 10 mgL^{-1} increased SOD activity in all parts of plant. Enzyme activity of PPO and CAT increased in roots, leaves and stems. CAT and SOD converts the toxic $O^{\cdot-}{}_2$, H_2O_2 into water and molecular oxygen. These results are consistent with our findings. The report presented by Abdul Jaleel et al PPO activity decreased during salt stress. This leads to the phenol accumulation and auxin catabolism. Auxin oxidase increased and plant growth decreased. But after TDM treatment in plant and increased PPO activity, Phenol levels were reduced in plants and increased levels of auxin therefore improves plant growth (Kishorekumar, 2008). Increased catalase activity has also been reported in bananas treated by TDM (Coolbaugh *et al*.,1978). Changes in catalase activity depends on the intensity and duration of stress and and can induce new isozymes (Jaleel *et al*., 2007). From our results it can be concluded that, the triadimefon can be used as an effective compound to increase resistance to other environmental stresses.TDM improve the harmful effects of stress by its influence on antioxidant potentials in *Lycopersicom esculentum* Mill.

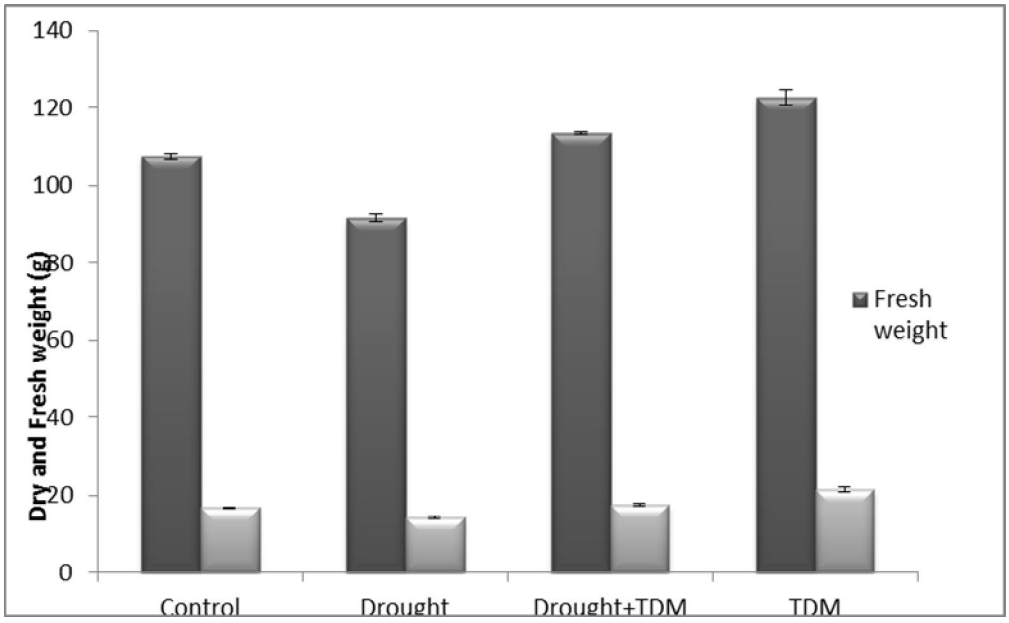

Fig. 1. Effects of drought, TDM and their combination on dry and fresh weight of *Lycopersicom esculentum* Mill.(mean ± SE)

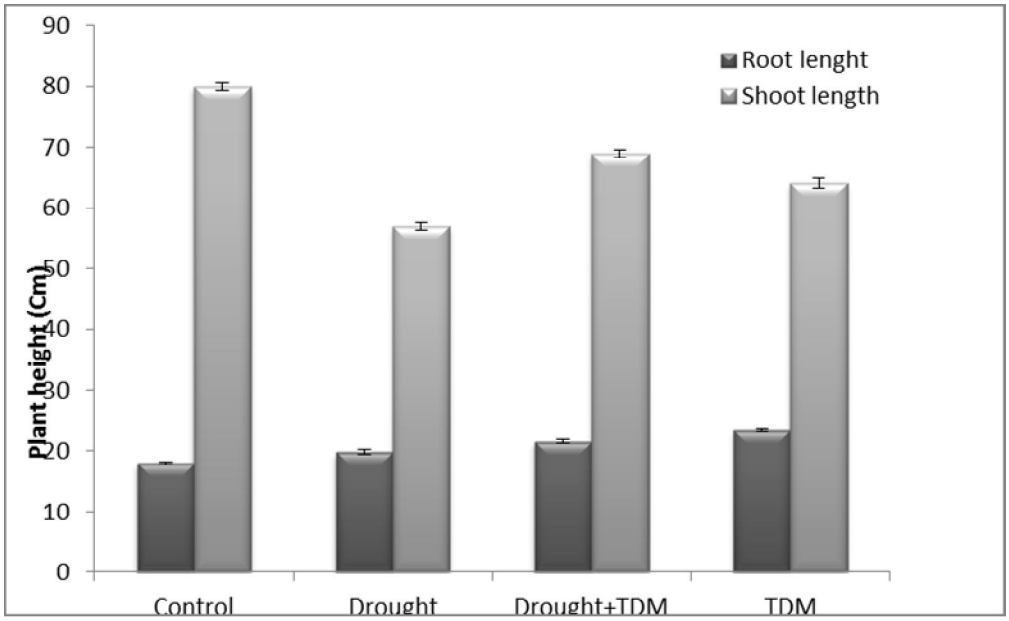

Fig. 2. Effects of drought, TDM and their combination on plant height of *Lycopersicom* esculentum Mill.(mean ± SE)

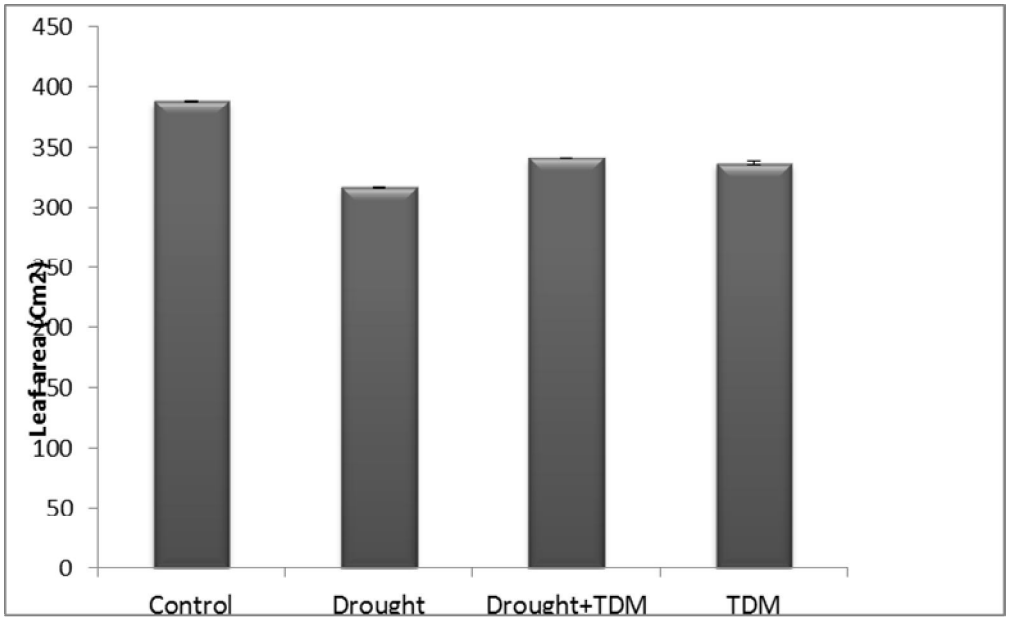

Fig. 3. Effects of drought, TDM and their combination on leaf area of Lycopersicom esculentum Mill.(mean ± SE)

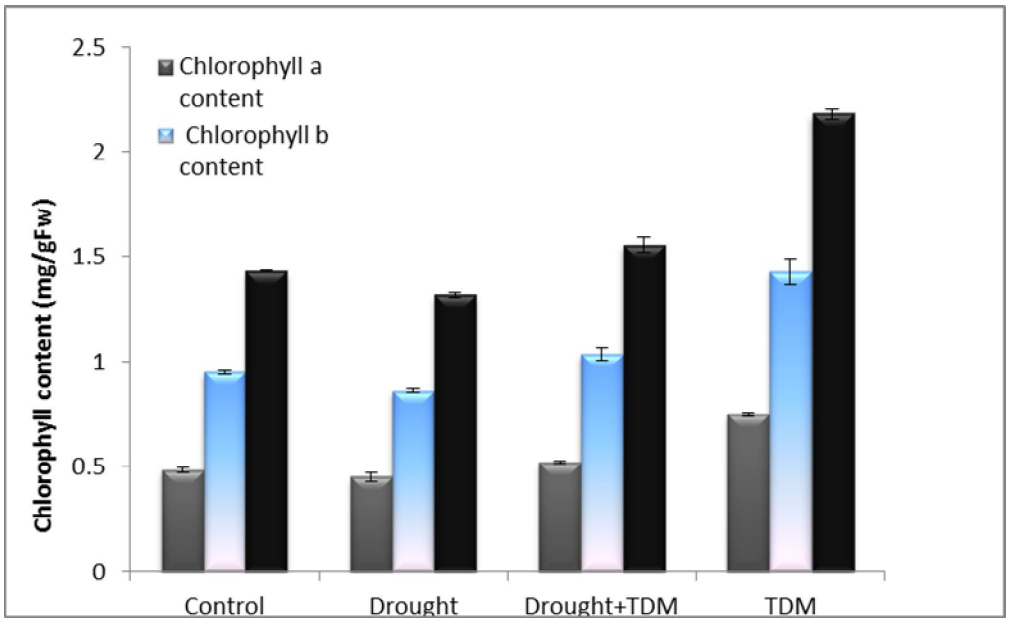

Fig. 4. Effects of drought, TDM and their combination on chlorophyll content of *Lycopersicom* esculentum Mill.(mean ± SE)

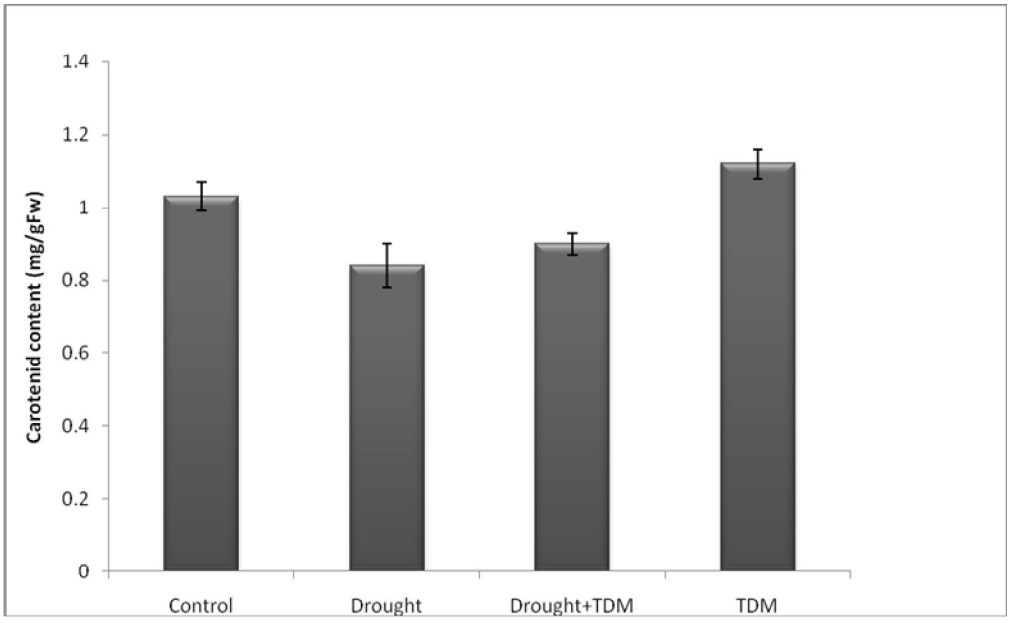

Fig. 5. Effects of drought, TDM and their combination on carotenoid content of *Lycopersicom* esculentum Mill.(mean ± SE)

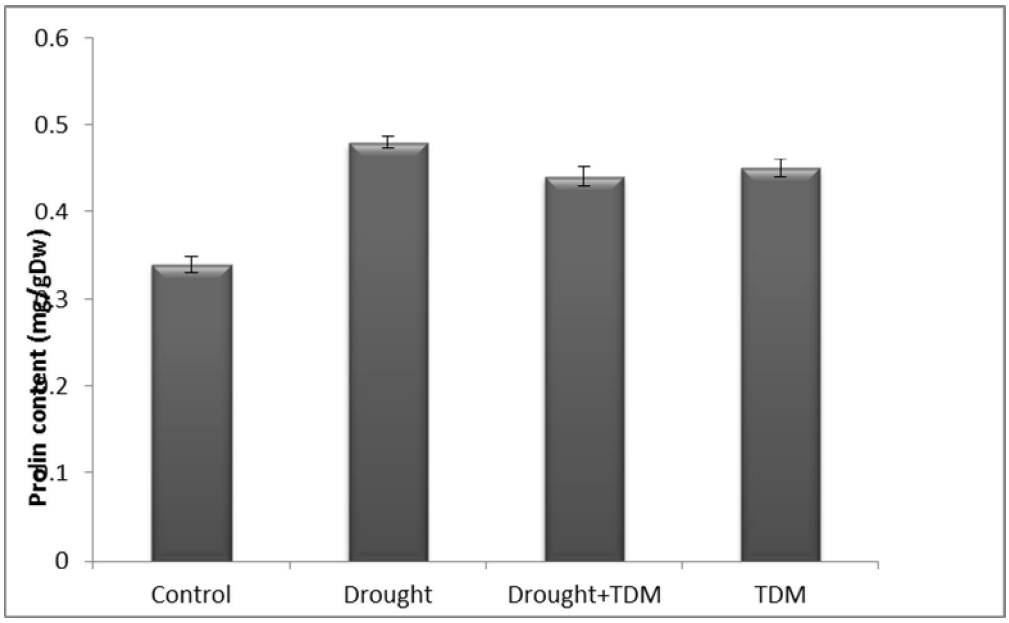

Fig. 6. Effects of drought, TDM and their combination on prolin content of *Lycopersicom* esculentum Mill.(mean ± SE)

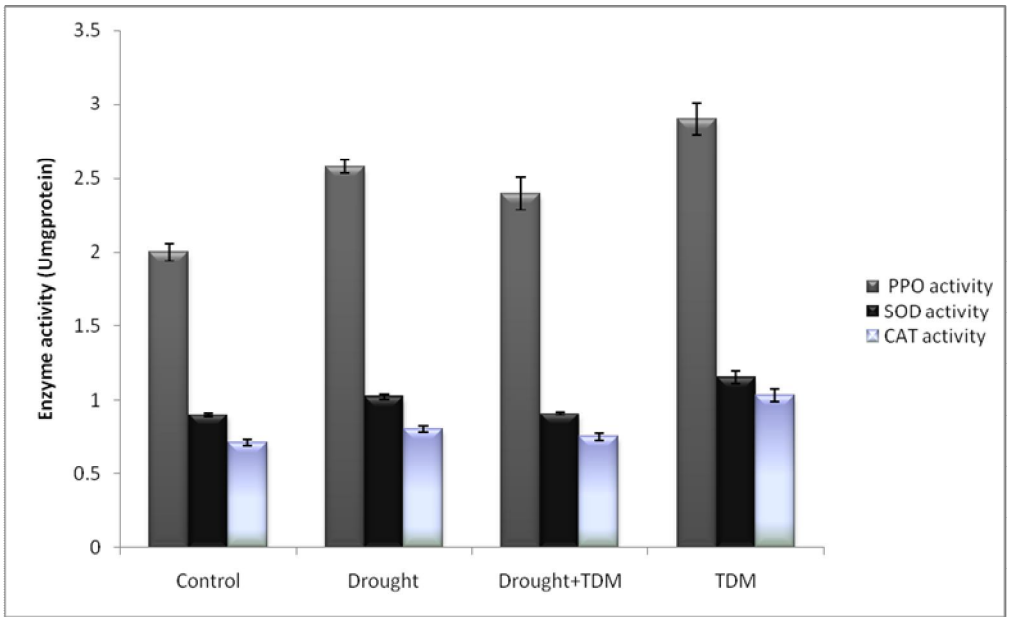

Fig. 7. Effects of drought, TDM and their combination on polyphenol oxidase (PPO), superoxide dismutase (SOD) and catalase (CAT) activity of *Lycopersicom esculentum* Mill.(mean ± SE)

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