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RESEARCH ARTICLE

TRIAZOLE INDUCED MODIFICATION OF BIOCHEMICAL AND ANTIOXIDANT METABOLISM IN *Capsicum annuum* L. UNDER DROUGHT STRESS

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ABSTRACT

In the present study, a pot culture experiment was conducted to estimate the ameliorating effect of Triadimefon (TDM), Hexaconazole (HEX) and Propiconazole (PCZ) on drought stress in *Capsicum annuum* L. plant belongs to solanaceae family. From 30 days after sowing (DAS), the plants were subjected to 3 days interval drought (DID) stress and drought stress with TDM @ 10 mg l^{-1}, HEX @ 10 mg l^{-1} and PCZ @ 15 mg l^{-1} and one day interval irrigation was kept as control. The plant samples were collected on 40, 50 and 60 DAS. The plants were separated into root, stem and leaf for estimating the biochemical, antioxidant and antioxidant enzymes. Combined drought stress treatments increased biochemical, antioxidant contents and antioxidant enzymes activities when compared to control. The triazole treatment mitigated the adverse effects of drought stress by increasing the antioxidant potentials and thereby paved the way for overcoming drought stress in *C. annuum* L.

INTRODUCTION

Currently, drought study has been one of the main directions in global plant biology and biological breeding. So anti-drought physiology study is of importance to production and biological breeding for the sake of coping with abiotic and biotic conditions (Shao *et al.,* 2005). Water is one of the most important ecological factors determining crop growth and development; water deficit plays a very important role in inhibiting the yields of crops. Drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation (Shao *et al.,* 2007). There are many reports in the literature that underline the intimate relationship between enhanced constitutive antioxidant enzyme activities and increased resistance to environmental stresses (Vranova *et al*., 2002; Bor *et al*., 2003). Plant experiences drought stress either when the water supply to roots becomes difficult or when the transpiration rate becomes very high. These two conditions often coincide under arid and semiarid climates. Water stress tolerance is seen in almost all plant species but its extent varies from species to species (Lin *et al*., 2006). Triazole compounds such as triadimefon (TDM), Hexaconazole (HEX), propiconazole (PCZ), tebuconazole (TBZ) and paclobutrazole (PBZ) etc. are widely used as fungicides and they also posses varying degrees of plant growth regulating properties (Fletcher *et al*., 2000). Triazoles have been called plant multiprotectants because of their ability to induce tolerance in plants to environmental and chemical stresses (Kraus and Fletcher, 1994). Protection of plants from apparently unrelated stress by triazole is mediated by a reduction in free radical damage and increase in antioxidant potential (Kraus *et al*., 1995). Triazoles affect the isoprenoid pathway and alter the levels of certain plant hormones by inhibiting gibberellin synthesis, reducing ethylene evolution and increasing cytokinin levels (Kamountsis and Chronopoulou-Sereli, 1999). Triazole treated plants have a more efficient free-radical scavenging system that enables them to detoxify active oxygen (Kopyra and Gwozdz, 2003). Morphological and physiological changes associated with triazole treatment in various plants, include the inhibition of plant growth, decreased internodal elongation, increased chlorophyll levels, enlarged chloroplasts, thicker leaf tissue, increased root to shoot ratio, increased antioxidant potentials and an enhancement in alkaloid production (Muthukumarasamy and Panneerselvam, 1997; Muthukumarasamy *et al*., 2000; Jaleel *et al*., 2006). The drought stress amelioration by triazole compounds is of major research interest, because, these compounds have innate potentiality for increasing antioxidant enzymes and molecules in oxidative stressed plants (Fletcher *et al*., 2000). So in the present investigation, an attempt has been made to evaluate the drought stress ameliorating ability of triazole fungicide, with special emphasis to antioxidant molecules and antioxidant enzymes in drought stressed C. *annuum* L. plants.

MATERIALS AND METHODS

Plant material and drought stress applications

The seeds of *Capsicum annuum* L. Varity bomby F1 hybrid were obtained from syngenta pvt ltd. Seeds are surface

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sterilized with 0.2 per cent $HgCl_2$ solution for 5 minutes with frequent shaking and thoroughly washed many times with deionized water to remove $HgCl_2$. A pot culture experiment was conducted to estimate the drought stress modification and ameliorating effect of combination of drought with triazole compounds, five seeds were sown in each pot of 40 × 45 cm containing 10 kg of soil mixture composed of red soil, sand and the farmyard manure (FYM) at 1:1:1 ratio. The seedlings were thinned to 2 pot^{-1} on 20 DAS. Plants pots were arranged in Completely Randomized Block Design (CRBD). Pots were irrigated with ground water one day interval as a control and other treatment is 3 days interval drought and drought with triazole compounds TDM @ 10 mg l^{-1}, HEX @ 10 mg l^{-1} and PCZ @ 15 mg l^{-1} treatments through drenching method on 30 DAS. Plants were uprooted randomly on 40, 50, 60 DAS, washed carefully and separated into root, stem and leaf for estimating antioxidant contents and antioxidant enzyme activities.

Biochemical Constituents

Amino acid (AA)

Extraction and estimation of Amino acid (AA) content was followed by the method suggested by Moore and Stein (1948). 0.5 g of plant material was taken in a pestle and mortar and homogenized with 10 ml of 80% boiling ethanol. The extract was centrifuged at 800 *g* for 15 min and the supernatant was made up to 10 ml with 80% ethanol and used for the estimation of free AAs. 1 ml of ethanol extract was taken in a 25-ml test tube and neutralized with 0.1 N sodium hydroxide using methyl red indicator, to which 1 ml ninhydrin reagent was added. The contents were boiled in a boiling water bath for 20 min, then 5 ml of diluted reagent was added, cooled and diluted to 25 ml with distilled water. The absorbance was read at 570 nm in a spectrophotometer. The standard graph was prepared by using glycine. The AA content was calculated using the standard graph. The results were expressed in milligrams per gram of dry weight.

Proline (PRO)

The PRO 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 10,000 rpm. Supernatant was used for the estimation of the PRO content. 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. After termination of the reaction in an ice bath, the reaction mixture was extracted with 4 ml of toluene and the absorbance was read at 520 nm.

Antioxidant Content Estimations

Ascorbic acid

Ascorbic acid content was assayed as described by Omaye *et al.* (1979). The extract was prepared by grinding 1 g of fresh material with 5 ml of 10% TCA, centrifuged at 3500 rpm for 20 minutes, reextracted twice and supernatant made upto 10 ml and used for assay. To 0.5 ml of extract, 1 ml of DTC reagent (2,4-dinitrophenyl hydrazine-thiourea-$CuSO_4$ reagent) was added and incubated at 37°C for 3 hrs and 0.75 ml of ice-cold 65% H_2SO_4 was added, allowed to stand at 30°C for 30 minutes, resulting colour was read at 520 nm in spectrophotometer (U-2001-Hitachi). The ascorbic acid content was determined using a standard curve prepared with Ascorbic acid and the results were expressed in mg g^{-1} dry weight (DW).

α- Tocopherol

α-Tocopherol content was assayed as described by Backer *et al.* (1980). Five hundred milligrams of fresh tissue was homogenized with 10 ml of a mixture of petroleum ether and ethanol (2:1.6 v/v) and the extract was centrifuged at 10,000 rpm for 20 minutes and the supernatant was used for estimation of α-Tocopherol. To one ml of extract, 0.2 ml of 2 per cent 2,2-dipyridyl in ethanol was added and mixed thoroughly and kept in dark for 5 minutes. The resulting red colour was diluted with 4 ml of distilled water and mixed well. The resulting colour in the aqueous layer was measured at 520 nm. The α-Tocopherol content was calculated using a standard graph made with known amount of α-Tocopherol and expressed in mg g^{-1} fresh weight (FW).

Enzyme Extractions and Assays

Ascorbate peroxidase (APX)

Ascorbate peroxidase (APX) (EC 1.11.1.1) activity was determined according to Asada and Takahashi (1987). The reaction mixture (1 ml) contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 200 µl of enzyme extract. The absorbance was read as decrease at 290 nm against the blank, correction was done for the low, non-enzymatic oxidation of ascorbic acid by H_2O_2 (extinction coefficient 2.9 mM^{-1} cm^{-1}). The enzyme activity was expressed in U mg^{-1} protein (U = change in 0.1 absorbance min^{-1} mg^{-1} protein).

Catalase (CAT)

Catalase (CAT) was measured according to Chandlee and Scandalios (1984), with modification. The assay mixture contained 2.6 ml of 50 mM potassium phosphate buffer (pH 7.0), 0.4 ml of 15 mM H2O2 and 0.04 ml of enzyme extract. The decomposition of H2O2 was followed by the decline in absorbance at 240 nm. The enzyme activity was expressed in U/mg protein.

Statistics

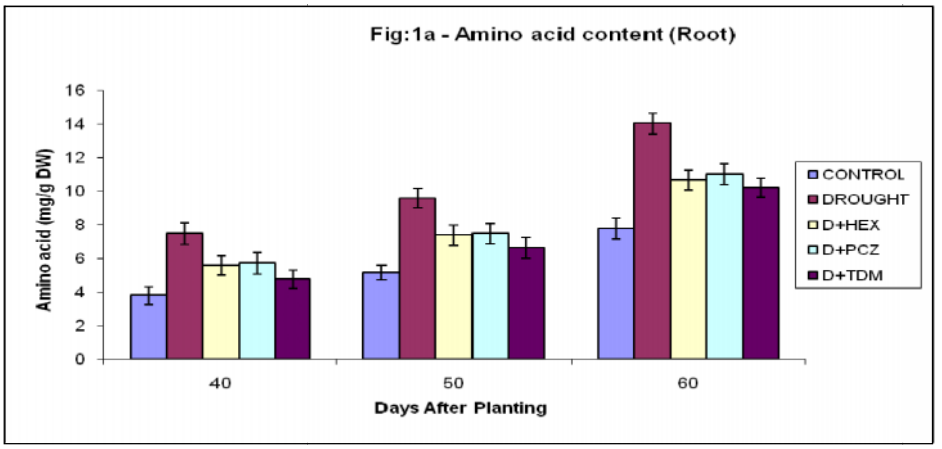
The pot culture was carried out in completely randomized design (CRBD). The data are expressed as mean ± SE for seven samples in each group.

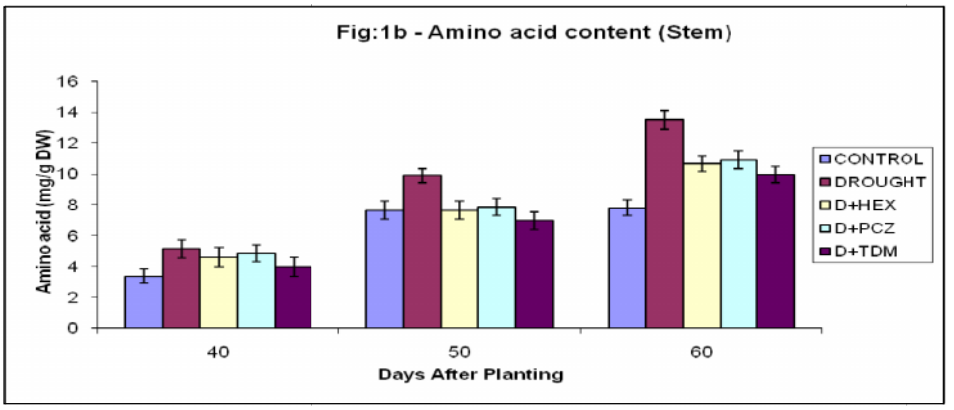
RESULTS

Amino acid (AA) contents increased in all the parts of drought stressed *C. annuum* L. when compared to control and triazole treated plants partially ameliorated drought stress (Fig.1). Proline (PRO) contents were increased in all the parts of *C. annuum* L. when compared to control (Fig.2). The ascorbic acid content increased with the age in drought stressed plants (Fig.3). α- tocopherol content of the drought stressed plants significantly increased when compared to control plants (Fig.4). Ascorbate Peroxidase (APX) activity increased in *Capsicum annuum* L (Fig.5) under drought condition and in all the treatments. Drought stress has increased the catalase (CAT) activity in all the parts of the plants to a larger extent under all the treatments in *C. annuum* L. (Fig.6).

DISCUSSION

Amino acid (AA) contents increased in all the parts of drought stressed *C. annuum* L. when compared to control and triazole treated plants partially ameliorated drought stress (Fig.1).





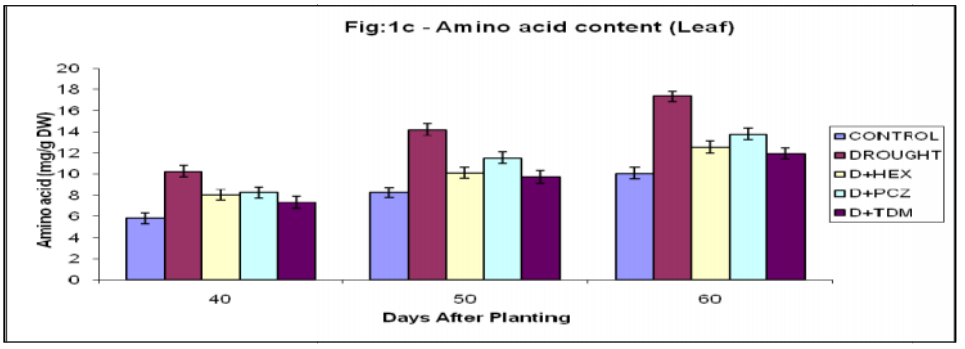
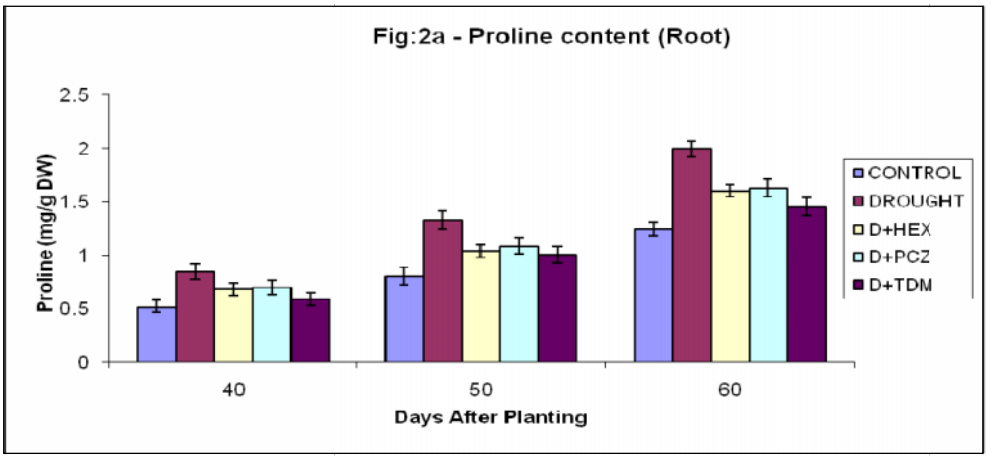
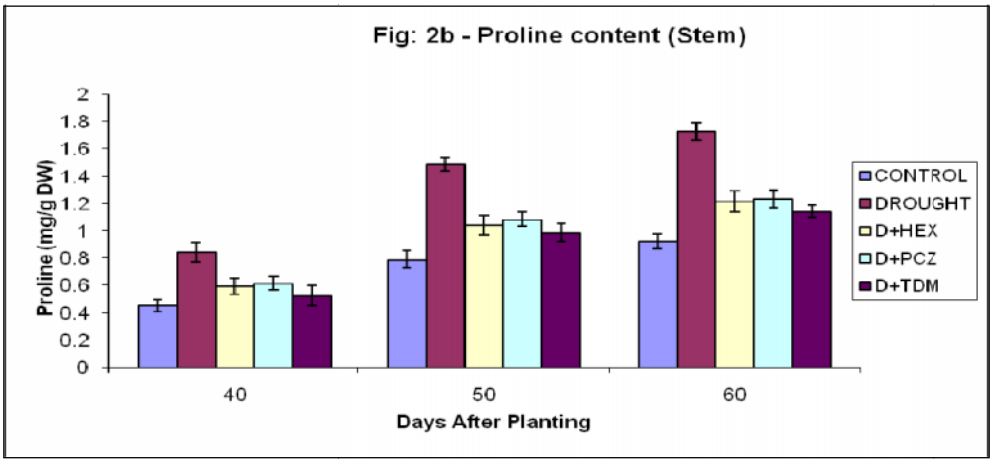


Fig. 1. Effect of drought stress and drought with triazole combination on the Amino acid content of *Capsicum annuum* L. (expressed in mg/g^{-1} dry weight)

Similar result recorded in sunflower (Manivannan *et al.,* 2007) and *Catharanthus roseus* (Jaleel *et al*., 2007). The AA content increased under drought condition in *Arachis hypogaea* (Asha and Rao, 2002); *sorghum* (Yadav *et al.,* 2005); pepper (Nath *et al.,* 2005); *Radix astragali* (Tan *et al.,* 2006); *Molus domestica* (Sircelj *et al.,* 2005) and *Marsh* grasses. Accumulated AA may be occurring in response to the change in osmotic adjustment of their cellular contents (Shao *et al*., 2007). Triazole treatments partially ameliorated drought stress in *Capsicum annuum* L. Similar results were observed in triadimefon treatment increased the AA content in radish (Muthukumarasamy *et al.,* 2000) and soybean (Panneerselvam *et al.,* 1998). Uniconazole treated *Phaseolus vulgaris* (Mackay *et al.,* 1990); penconazole induced a moderate increase in amino acid in higher plants (Radice and Pesci, 1991) and in tomato (Sharp *et al.,* 2000; Sharp and Lenoble, 2002). Proline (PRO) contents were increased in all the parts of *C. annuum* L. when compared to control (Fig.2). Water stress resulted in an increase in PRO accumulation in sorghum (Yadav *et al.,* 2005). The similar results were observed in wheat (Sawhney and Singh, 2002); in soybean (Heerden and Kruger, 2002). Triazole treatment decreased PRO content in plants under drought stress compared with the plants had received only water stress treatment. (Neda Mohamadi *et al.,* 2013). PRO content increased in both drought stress and with triazole treatments when compared to control.

The ascorbic acid content increased with the age in drought stressed plants (Fig.3). Water stress resulted in significant increase in antioxidant ascorbic acid concentration in turf grass (Zhang and Schmidt, 2000). Similar results were observed in *sorghum* seedlings (Zhang and Kirkham, 1996); *Triticum aestivum* (Carlos *et al.,* 1999); rice (Srivalli *et al.,* 2003); apple tree (Sircelj *et al.,* 2005) and in *Poncirus trifoliate* (Wu *et al.,* 2006). Triazole treatment to the drought stressed capsicum





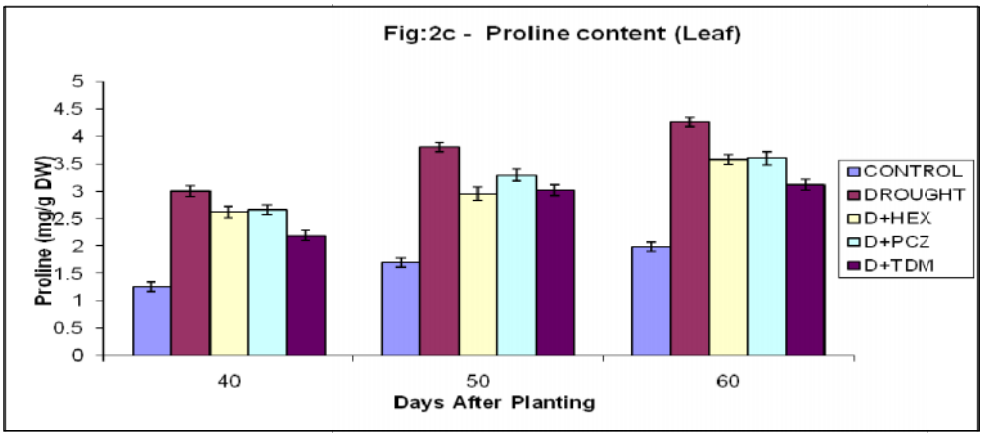
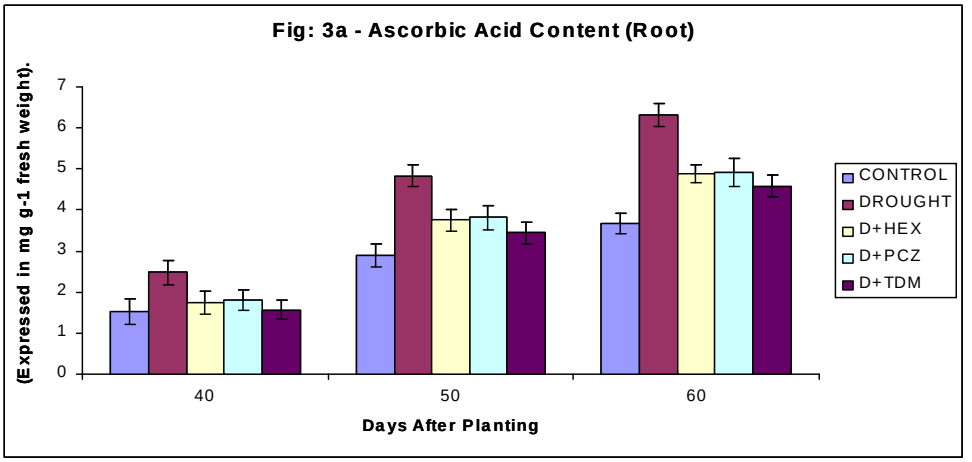
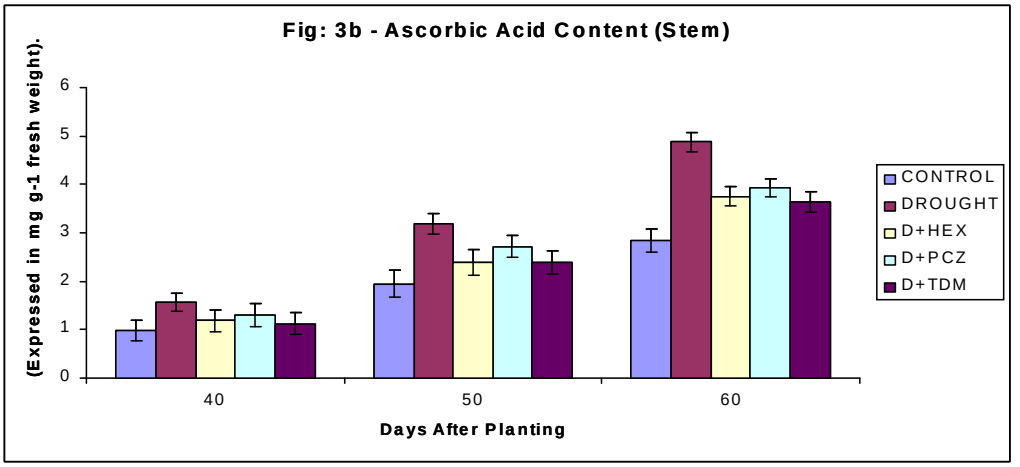


Fig. 2. Effects of drought stress and drought with triazole combination on the Proline content of *Capsicum annuum* L. (expressed in mg/g^{-1} dry weight)





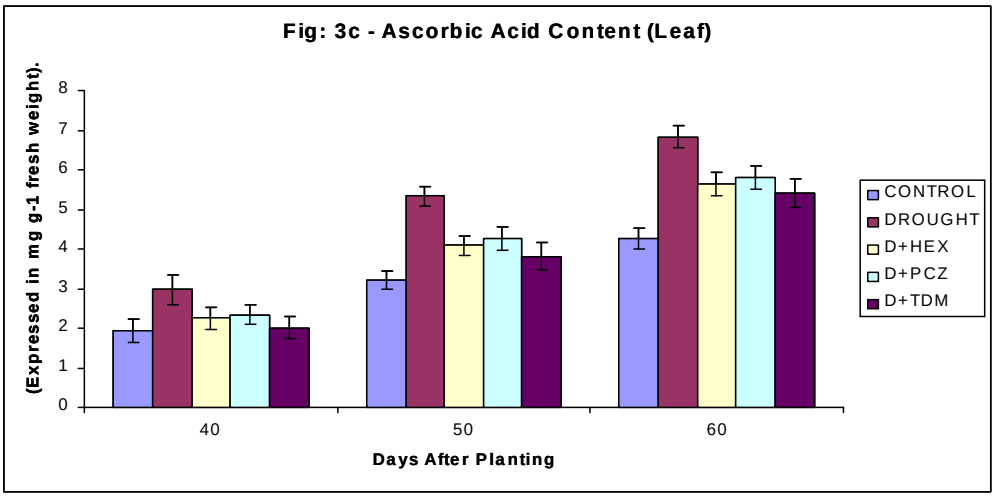
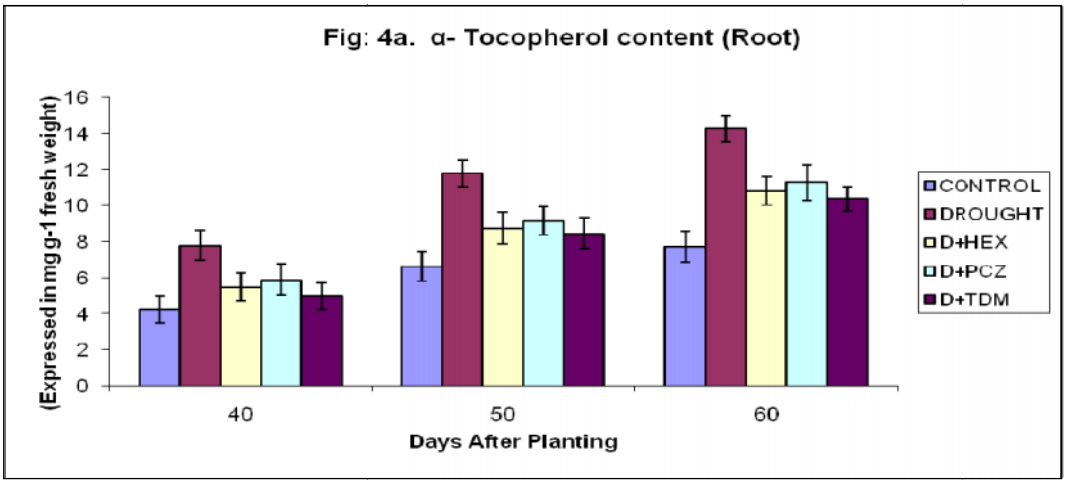
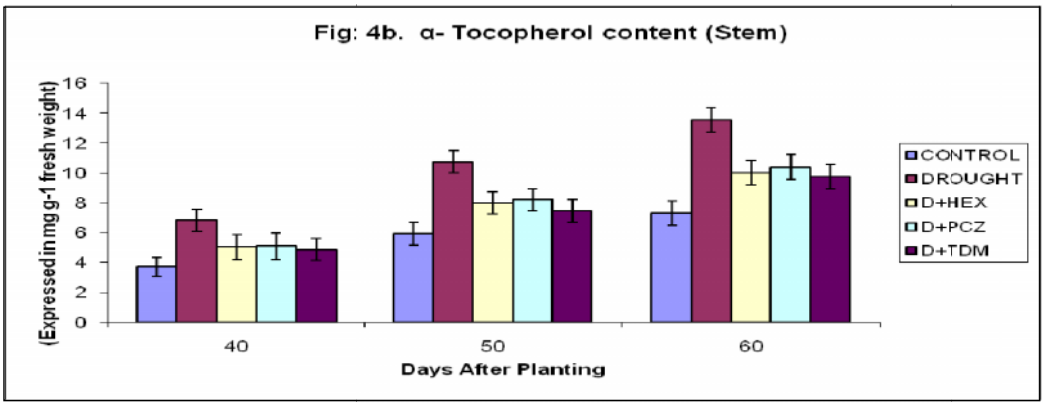


Fig. 3. Effects of drought stress and drought with triazole combination on the Ascorbic acid content of *Capsicum annuum* L. (expressed in µg g^{-1} fresh weight)

plants decreased the ascorbic acid content but, it was higher than that of control. Increase in ascorbic acid content was reported in the paclobutrazol treated grape fruits (Fucik and Swietlik, 1990) and citrus limon (Jain *et al.*, 2002). Uniconazole increased the level of antioxidants ascorbic acid and α-tocopherol like in tomato seedlings and protected the membrane by preventing or reducing oxidative damage (Singh, 1996). α- tocopherol content of the drought stressed plants significantly increased when compared to control plants (Fig.4).

Water stress resulted in significant increases in antioxidant α- tocopherol concentration in turf grass (Boggess *et al.*, 1976; Zhang and Schmidt, 2000). Similar results were observed in maize (Pastori and Trippi, 1993); *sorghum* (Zhang and Kirkham, 1996); *Triticum aestivum* (Carlos *et al.*, 1999) and (Srivalli *et al.*, 2003). grasses (Fu and Huang, 2001); Wheat (Baisak *et al.*,1994; Loggini *et al.*,1999); apple tree (Sircelj *et al.*, 2005); *Poncirus trifoliate* (Wu *et al.*, 2006).Triazole treatment to the drought stressed *Capsicum annuum* L. plants





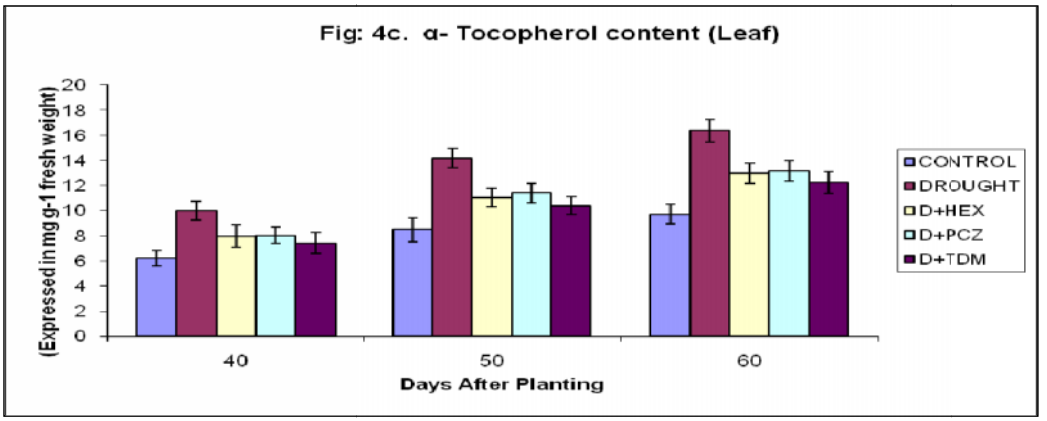
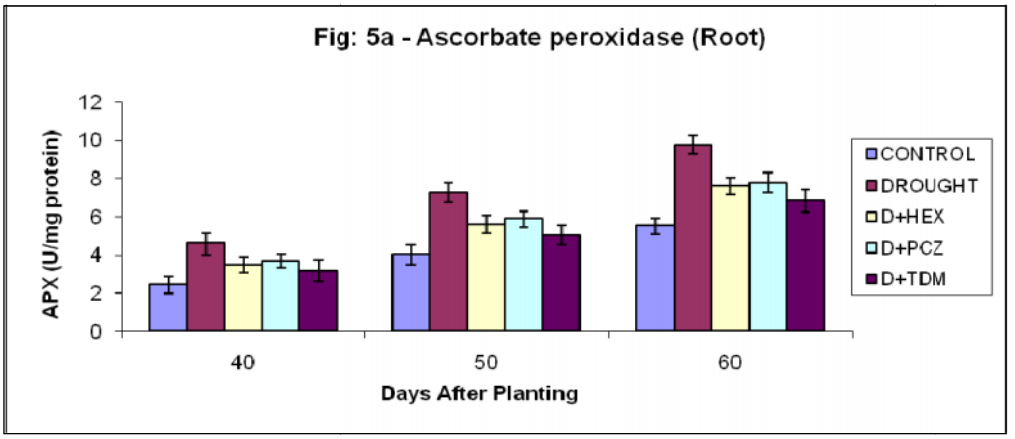
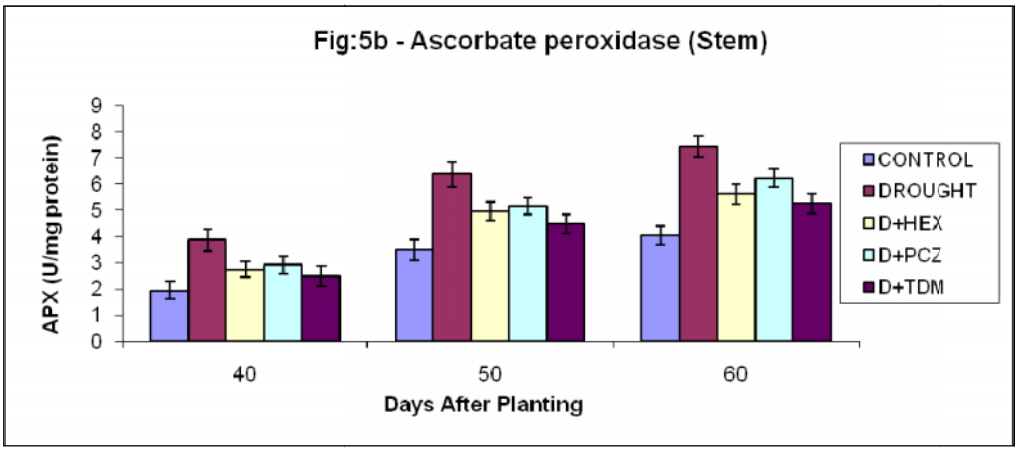


Fig. 4. Effects of drought stress and drought with triazole combination on the α- Tocopherol content of *Capsicum annuum* L. (expressed in μg g^{-1} fresh weight)





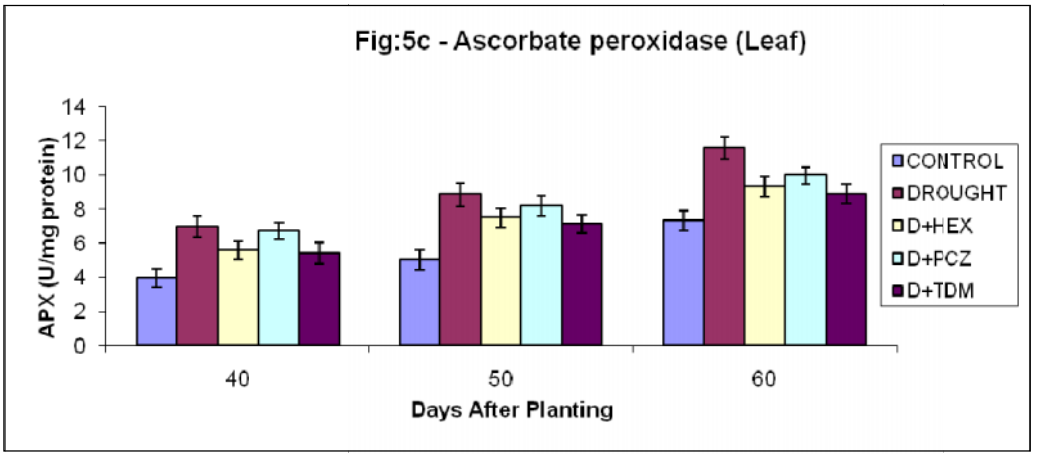
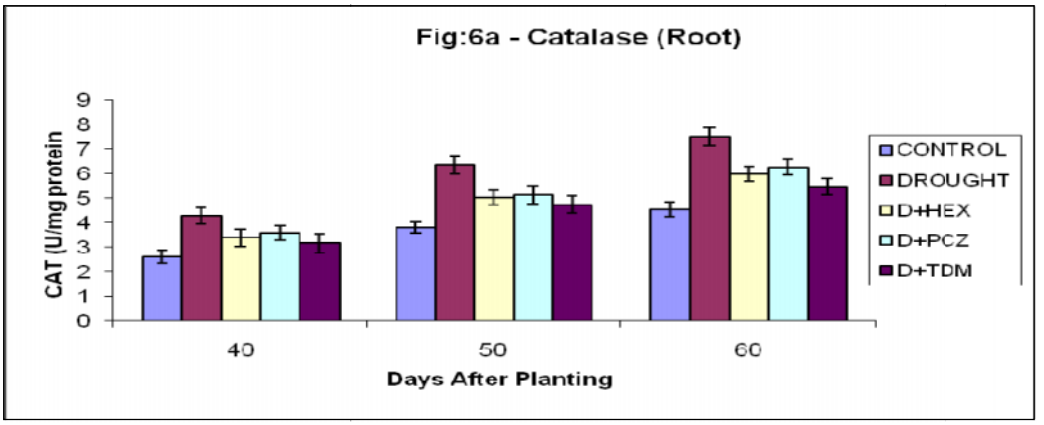
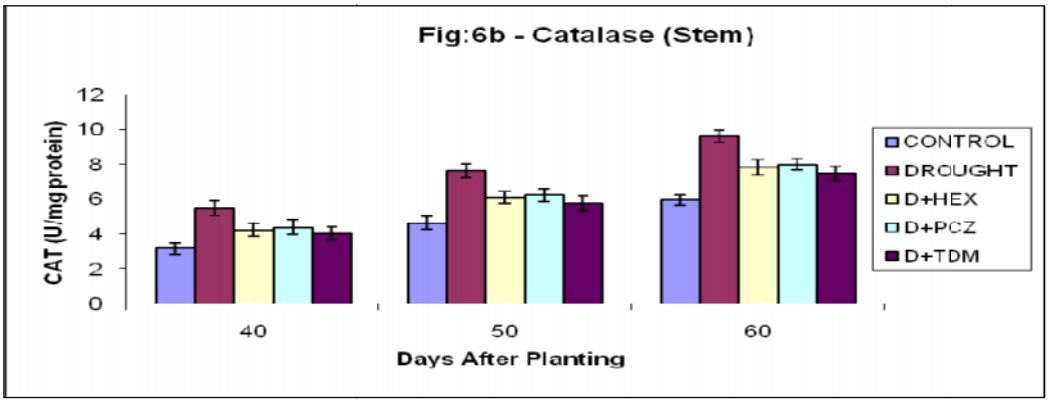


Fig. 5. Effects of drought stress and drought with triazole combination on the Ascorbate peroxidase activity of *Capsicum annuum* L. (u/mg protein).





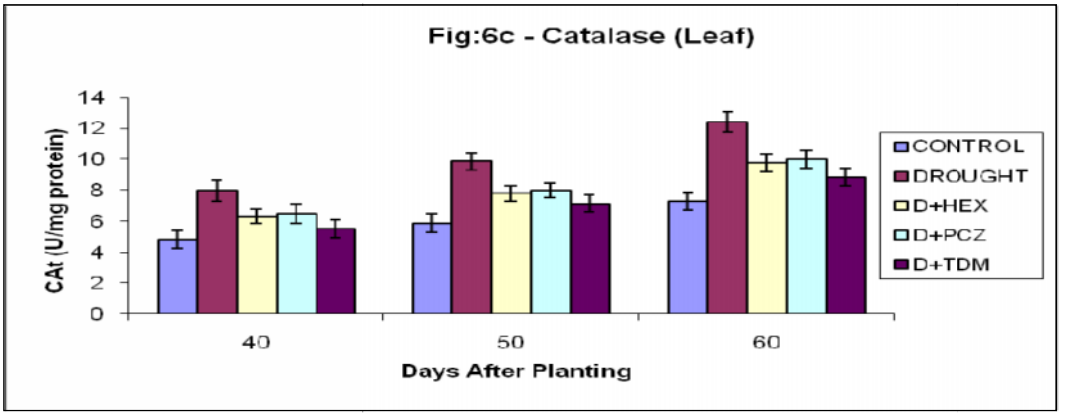


Fig. 6. Effects of drought stress and drought with triazole combination on the Catalase activity of *Capsicum annuum* L. (u/mg protein)

decreased the α- tocopherol content but, it was higher than that of control. Triazole caused an enhancement in α- tocopherol content under drought as well as well-watered plants. A similar result was observed in bean (Simontacchi *et al.,* 1993) under chilling stress. Singh (1996) reported an increase in α -tocopherol and β-carotene content in the fruit juice of paclobutrazol treated mango (*Mangifera indica* L). Similar results were observed in grape fruits (Fucik and Swietlik, 1990) and *Citrus limon* (Jain *et al.,* 2002). Ascorbate Peroxidase (APX) activity increased in *Capsicum annuum* L (Fig.5) under drought condition and in all the treatments. Increased APX activity was reported in *Phaseolus acutifolius* under drought stress (Turkan *et al*., 2005) and in soybean (Heerden and Kruger, 2002). Similar results were obtained by many workers under drought stress in many higher plants *Pinus halepensis* (Alonso *et al.,* 2001); maize (Jiang and Zhang, 2002); *Phaseolus acutifolius* (Turkan *et al.,* 2005), soybean (Heerden and Kruger, 2002); wheat (Baisak *et al.,*1994; Lin and Wang, 2002; Gong *et al.,* 2005) and *Kentucky bluegrass* (Liu *et al.,* 2008). Drought stress induced generation of active oxygen species is well recognized at the cellular level and is tightly controlled at both the production and consumption levels through increased antioxidant systems (Reddy *et al*., 2004). Triazole treatment in combination with drought decreased the APX activity but, it was higher than that of control plants. Increased APX activity was reported in *Phaseolus acutifolius* under drought stress (Turkan *et al*., 2005) and in soybean (Heerden and Kruger, 2002). Triazoles increased the level of APX activity in *Solenostemon rotundifolius* (Kishorekumar *et al.,* 2008). Paclobutrazol increased the APX activity in peanut plants under drought stress (Sankar *et al.,* 2007) and *C. roseus* plants under salt stress (Jaleel *et al.,* 2007a). Similar increase was also reported in *Vigna* plants under propiconazole treatments in combination with drought (Manivannan *et al.,* 2007a) and ketoconazole in *C. roseus* (Jaleel *et al.,* 2007).

Drought stress has increased the catalase (CAT) activity in all the parts of the plants to a larger extent under all the treatments in *C. annuum* L. (Fig.6). The CAT activity increased under drought in *Nicotiana plumbaginifolia* (Nicolas smirnoff, 1998) and *Pinus halepensis* (Alonso *et al.,* 2001) Similar results were observed in wheat (Sgherri *et al.,* 2000; Lin and Wang, 2002; Gong *et al.,* 2005; Shao *et al.,* 2005), *Phaseolus acutifolius* (Turkan *et al.,* 2005); *Zea mays* (Jiang and Zhang, 2002). Under salt stress the CAT activity increased in spinach (Ozturk and Demir, 2003). Triazole treatment to the stressed plants caused a decrease in CAT activity but, it was higher than that of control plants. Increased CAT activity was reported in *Phaseolus acutifolius* under drought stress (Turkan *et al*., 2005) and in soybean (Heerden and Kruger, 2002). Triazoles increased the level of CAT activity in *Solenostemon rotundifolius* (Kishorekumar *et al.,* 2008). Paclobutrazol increased the CAT activity in peanut plants under drought stress (Sankar *et al.,* 2007). Similar increase was also reported in *Vigna* plants under propiconazole treatments in combination with drought (Manivannan *et al.,* 2007a) and ketoconazole in *C. roseus* (Jaleel *et al.,* 2007). Thus, from these results, it is clear that plants are highly regulated by triazole compounds Drought stressed plants under triazole treatment leading to partial improvement of their response to drought-induced stress. It can be concluded that triazole such as Triadimefon (TDM), Hexaconazole (HEX) and Propiconazole (PCZ) may be useful to trigger drought avoidance mechanisms in plants like *Capsicum annuum* L. Further work is needed to understand the genetic mechanism behind triazole induced water stress tolerance in *Capsicum annuum* L.

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