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Triadimefon Mediated Changes in Antioxidant and Indole Alkaloid Content in Two Species of *Datura*

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ABSTRACT

The triadimefon induced changes in antioxidant potential and alkaloid production of *Datura fastuosa* and *Datura innoxia* were studied. Both varieties were subjected to 15 mg L^{-1} triadimefon treatment by soil drenching on 55, 65 and 80 Days after Planting (DAP). The plants were harvested on 60, 75 and 90 DAP and used for analyzing antioxidant contents, antioxidative enzymes and indole alkaloid activities. The antioxidants like ascorbic acid, α-tocopherol and reduced glutathione were found increased under Triadimefon (TDM) treatment when compared to control plants. The antioxidative enzymes like superoxide dismutase, ascorbate peroxidase and catalase activities and indole alkaloid content increased in both the varieties under triadimefon treatment when compared to control (con) plants. The antioxidant potentials viz., Ascorbic Acid (AA), α-tocopherol and reduced Glutathione (GSH) were found increased under triadimefon treatment. The antioxidant enzymes like Superoxide Dismutase (SOD), Ascorbate Peroxidase (APX) and Catalase (CAT) activities showed slight changes in both the species under triadimefon treatment when compared to control plants. Indole alkaloid content increased significantly under triadimefon treatment. These preliminary results suggest that, the application of triadimefon may be a useful tool to increase the antioxidant potential and alkaloid content in medicinal plants.

Key words: *Datura fastuosa*, alkaloid, *Datura innoxia,* antioxidation, triadimefon

INTRODUCTION

Datura fastuosa auct. non L. is one of the most important medicinal plant, belongs to the family Solanaceae and cultivated mainly for its alkaloids (Drake *et al*., 1996). The compounds of these plants are of commercial interest to the pharmaceutical industry (Griffin and Lin, 2000). Two distinct varieties, the black flowered *fastuosa* and the white flowered *innoxia* were taken for the present study. Black flowered cultivar gives higher yield of foliage, roots and total alkaloids. The main alkaloids present in *D. fastuosa* and *D. innoxia* are atropine, hyoscyamine and scopolamine (Bruneton, 1993). *Datura fastuosa* is commercially important due to the presence of alkaloids which have anticancer activities (CSIR, 1992). Moreover, the ethanolic extract of the leaf has significant hypoglycemic activities, rendering it as a good antidiabetic drug (Kar *et al*., 2003).

Plant growth regulating properties of triazoles are mediated by their ability to alter the balance of important plant growth substances including gibberellic acid, abscisic acid and cytokinins (Fletcher *et al*., 2000). Triazoles inhibit gibberellin and ergosterol biosynthesis in plants and induce

a variety of morphological and biochemical responses in plants. Besides triazole compounds inhibited the shoot elongation, stimulated root growth, increased cytokinin synthesis and a transient rise in abscisic acid as well as conferring protection from various environmental stress, chilling stress (Sivakumar *et al*., 2009; Ali, 2010). The non-enzymatic antioxidants including ascorbic acid were important components of plant antioxidative systems (Noctor and Foyer, 1998; Smirnoff and Wheeler, 1999). α-tocopherol was major lipid soluble antioxidant in membranes which can break the chain of lipid peroxidation and acts as cell membrane stabilizer (Davis and Swanson, 2001).

Triazole compounds not only protect the plant from stress but also induce stress like symptoms. Since, it is reported that a biotic stress has increased the alkaloid content (Jaleel *et al*., 2006). Protection of plants from apparently unrelated stress by triazole is also mediated by a reduction in free radical damage and increase in the antioxidant potential and ajmaline production (Jaleel *et al*., 2007a) in *Catharanthus*. Triazole compounds like triadimefon may also increase the alkaloids in *D. fastuosa*. Besides triazole compounds increased the root production where the alkaloids accumulate in larger quantity than in shoot.

The present investigation was made to study the effect of triadimefon in increasing biosynthesis of non enzymatic antioxidants like Ascorbic Acid (AA), α-tocopherol and reduced Glutathione (GSH) content, antioxidative enzymes like SOD, APX and CAT activities and indole alkaloid content which are of highly pharmaceutical values in *D. fastuosa* and *D. innoxia*.

MATERIALS AND METHODS

Plant materials and cultivation methods: The seeds of *D. fastuosa* and *D. innoxia* were collected from the Department of Agriculture, Annamalai University, Tamil Nadu, India. The triazole compound triadimefon (Bayleton-25 WP) was obtained from Bayer (India) Ltd., Mumbai. During the study, average temperature was 32/26°C (Maximum/minimum) and the Relative Humidity (RH) varied between 60 and 75%. The experiments were carried out in Botanical Garden and Physiology Laboratory in the Department of Botany, Annamalai University, Tamil Nadu, India.

The plants were raised in the Botanical Garden, during the months of January-April, 2007. The seeds of two varieties were sown separately in raised seed beds by broadcasting method and covered with fine soil to ensure proper germination. The nursery beds were watered twice a day and weeded regularly in order to ensure healthy growth of the seedlings. The field was repeatedly ploughed and brought to fine tilt and divided into four plots prior to transplantation. Two plots for each variety were prepared thirty plants per plot were planted for both the varieties at a distance of 35×50 cm. Only ground water was used for irrigation and subsequent irrigation was done two times in a week to keep the optimum moisture level in the soil.

Triadimefon treatment: One plot for each variety was subjected to triadimefon treatment and another one was kept as control. Triadimefon 15 mg L^{-1} was given to each plant by soil drenching. The treatment was given on 50, 65 and 80 Days After Planting (DAP). The plants were uprooted randomly on 60, 75 and 90 DAP and separated into root, stem and leaves and used for estimating the non-enzymatic antioxidant content, antioxidant enzymes and indole alkaloid content.

Antioxidants: Ascorbic acid, α-tocopherol and reduced glutathione content were assayed from root, stem and leaves by following the methods of Omaye *et al*. (1979), Baker *et al*. (1980) and Griffith and Meister (1979), respectively.

Antioxidant enzymes: Superoxide Dismutase (SOD, EC: 1.15.1.1), Ascorbate Peroxidase (APX, EC: 1.11.1.11) and Catalase (CAT, EC: 1.11.1.16) activity was estimated from leaves, stem and roots by the following methods. Crude enzyme extract was prepared, for the assay of SOD (Hwang *et al*., 1999). The enzyme protein was determined (Bradford, 1976). SOD activity is expressed in unit mg^{-1} protein. One unit (U) is defined as change in 0.1 absorbance h^{-1} mg^{-1} protein under the assay condition. APX was extracted, estimated (Asada and Takasaki, 1987) and expressed in units mg^{-1} protein (U = change in 0.1 absorbance min^{-1} mg^{-1} protein). CAT was extracted, assayed (Chandlee and Scandalios, 1984) and expressed in units mg^{-1} protein (U = 1 mM of H_2O_2 reduction min^{-1} mg^{-1} protein).

Indole alkaloid extraction and quantification: Indole alkaloid extraction from the roots was carried out by following the standard extraction method (Lounasmaa and Tamminen, 1993). Identification and quantification of indole alkaloid was done by preparative thin layer chromatography using silica gel G (Merk) in chloroform: Methanol (98:2). Alkaloid was spotted with Dragendorff's reagent (Stahl, 1969).

Statistical analysis: The mean values for the biochemical studied were calculated for each treatment of triadimefon and expressed are percentage over the control. The values are Mean±SD for three samples in each group. The p-values≤0.05 were considered as significant.

RESULTS

Effect of triadimefon on ascorbic acid: Triadimefon induced changes in the ascorbic acid contents of the two varieties of *D. fastuosa* (Fig. 1). The AA content increased with the age of the plant in both the varieties in control and triadimefon treated plants in the leaf, stem and root samples. Among the plant parts, the root recorded the highest AA content. The higher AA content (14.158 mg g^{-1} fresh weight) was recorded in the triadimefon treated roots of *innoxia* variety on 90 DAP sampling and the lowest content (2. 021 mg g^{-1} FW) was recorded in the control plant of *Datura* variety on 60 DAP.

Effect of triadimefon on α-tocopherol: The α-tocopherol content in the TDM treated plants was higher than that of control on 60, 75 and 90 DAP (Fig. 2). The highest α-tocopherol content

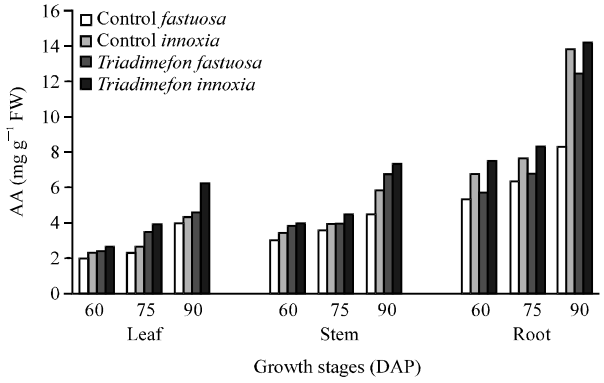

Fig. 1: Efficacy of triadimefon treatment on AA content of leaf, stem and root in two varieties of *D. fastuosa* auct. non L. Values are Mean±SD of three samples

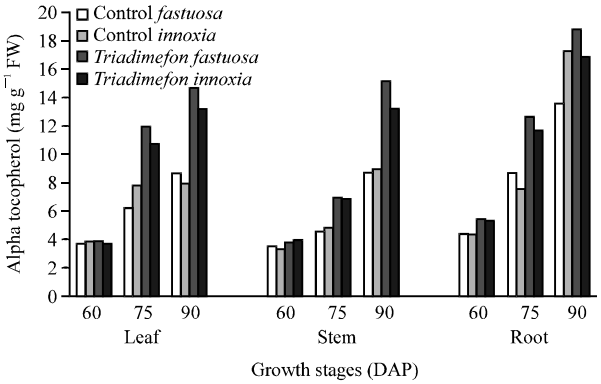

Fig. 2: Efficacy of triadimefon treatment on α-tocopherol content of leaf, stem and root in two varieties of *D. fastuosa* auct. non L. Values are Mean±SD of three samples

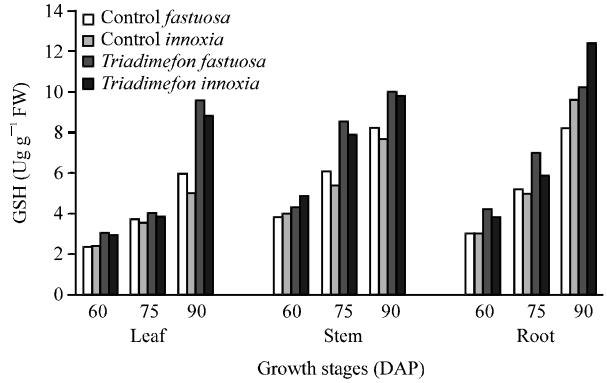

Fig. 3: Efficacy of triadimefon treatment on GSH content of leaf, stem and root in two varieties of *D. fastuosa* auct. non L. Values are Mean±SD of three samples

(18. 853 mg g^{-1} FW) was recorded on 90 DAP root sampling of triadimefon treated *D. fastuosa* variety. The lowest α-tocopherol content (3.332 mg g^{-1} FW) was recorded in 60 DAP stem samples of untreated *innoxia* variety.

Effect of triadimefon on reduced glutathione: The variations found in the reduced glutathione content under triadimefon treatment in *fastuosa* and *innoxia* varieties of *Datura fastuosa* (Fig. 3). It increased with the age of the plant in both the varieties. On 90 DAP, root sample from triadimefon treated *innoxia* variety showed a highest content of GSH (12. 336 μg g^{-1} FW) and the lowest content (2.321 μg g^{-1} FW) was recorded in control *D. fastuosa* variety on 60 DAP.

Effect of triadimefon on superoxide dismutase: The activity of SOD was increased under triadimefon treatment (Fig. 4). The highest activity (9.185 U mg^{-1} protein) was recorded in 90 DAP triadimefon treated root sampling of *D. fastuosa* variety and lowest activity (0.986 U mg^{-1} protein) was recorded in the 60 DAP root sampling of control of *D. innoxia* variety.

Effect of triadimefon on ascorbate peroxidase: APX activity was higher in *Datura fastuosa* when compared to SOD and CAT activities (Fig. 5). It increased under triadimefon treatment in

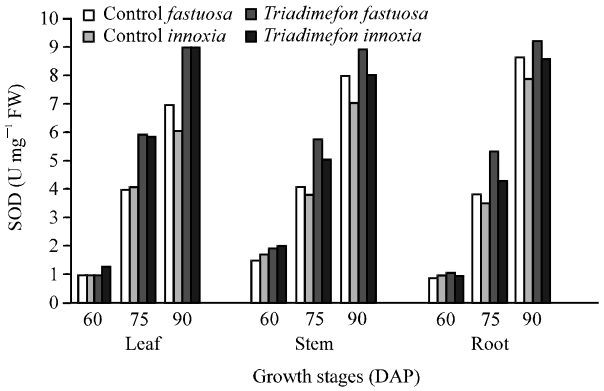

Fig. 4: Efficacy of triadimefon treatment on SOD activity of leaf, stem and root in two varieties of *D. fastuosa* auct. non L. Values are Mean±SD of three samples

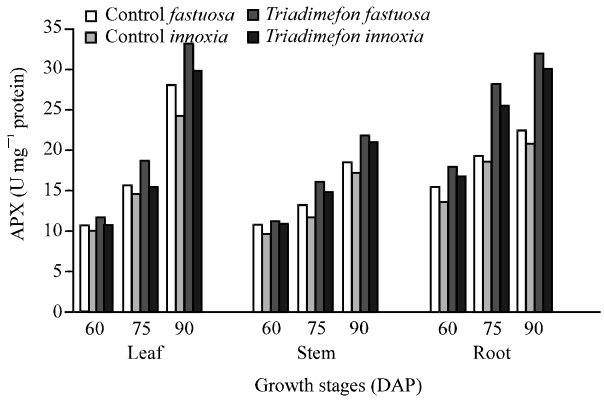

Fig. 5: Efficacy of triadimefon treatment on APX activity of leaf, stem and root in two varieties of *D. fastuosa* auct. non L. Values are Mean±SD of three samples

both *D. fastuosa* and *D. innoxia* varieties when compared to control on all the sampling days. Among the samples, the leaf samples showed higher APX activity. The highest APX activity (33.097 U mg^{-1} proteins) was recorded in triadimefon treated *D. fastuosa* leaves on 90 DAP, while the lowest activity (9.712 U mg^{-1} proteins) was recorded in 60 DAP samplings from stem of control *D. innoxia* variety.

Effect of triadimefon on catalase: The CAT activity was very low in both the varieties of *D. fastuosa* (Fig. 6). The amount of CAT varies in different parts of the plant. The highest activity (0.251 U mg^{-1} protein) was recorded in 90 DAP sampling from leaf of triadimefon treated *D. fastuosa* variety, while the lowest activity (0.117 U mg^{-1} protein) was recorded in 60 DAP sampling from stem of control *D. fastuosa* variety.

Effect of triadimefon on indole alkaloid content: During the early stages of plant growth, the indole alkaloid content was less in both the varieties (Fig. 7). The lowest content (0.076 mg g^{-1} dry weight, DW) was recorded in the roots of control *D. innoxia* variety on 60 DAP. But the content increased with the age of plant in both control and treated plants. The highest content (0.720 mg g^{-1} D.W) was recorded in the roots of triadimefon treated *D. fastuosa* variety on 90 DAP.

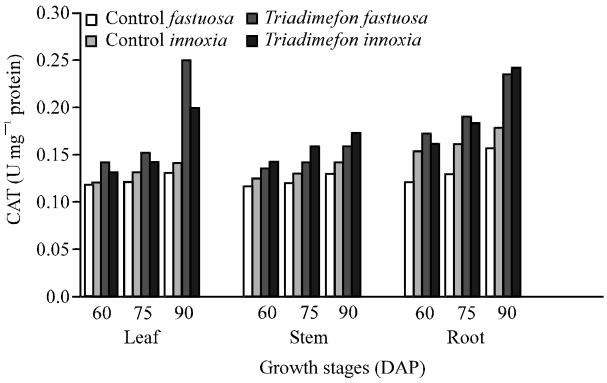

Fig. 6: Efficacy of triadimefon treatment on CAT activity of leaf, stem and root in two varieties of *D. fastuosa* auct. non L. Values are Mean±SD of three samples

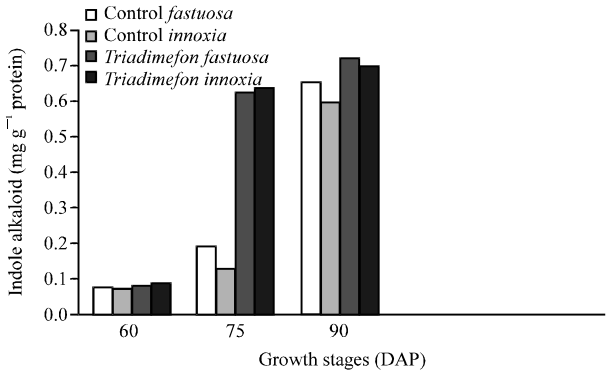

Fig. 7: Efficacy of triadimefon treatment on indole alkaloid content of leaf, stem and root in two varieties of *D. fastuosa* auct. non L. Values are Mean±SD of three samples

DISCUSSION

The increase in non-enzymatic antioxidants, antioxidant enzymes and root alkaloid content suggests that, triadimefon caused a profound influence upon the regulatory mechanism of the plant as a whole (Jaleel *et al.*, 2006). Triadimefon is a triazole compound having fungicidal as well as plant growth regulation properties (Fletcher *et al.*, 2000; Sivakumar *et al.*, 2009, 2010). The application of this triazole compound, being a fungicide that can alter the metabolic equilibrium, can exhibit stress like symptoms in plants (Fletcher *et al.*, 2000). It also simultaneously can protect plants from apparently unrelated abiotic stress like NaCl stress, seawater and salinity stress (Panneerselvam *et al.*, 1997; Azooz and Al-Fredan, 2009; Amirjani, 2010). Triadimefon treatment increased the non-enzymatic antioxidant responses like AA, α-tocopherol and GSH content in all parts of plant and their content was varying in different parts of the plants (Jaleel *et al.*, 2006).

The increase in antioxidative metabolism can be correlated with plants native protective mechanism against oxidative stress raised from the fungicide application. Being the major antioxidant species in plants, the AA, α-tocopherol and GSH contents vary in different sub-cellular compartments, according to the intensity of stress, nickel stress, chilling stress (Gaspar *et al.*, 2002; Bhardwaj *et al.*, 2007; Ali, 2010). An increase in AA content was reported in PBZ treated *Dioscorea rotundata poir, C. roseus* (Jaleel *et al.*, 2007a, b). Ascorbic acid is important antioxidant

which functions as the terminal antioxidant because the redox potential of ascorbate/monodehydroascorbate pair is lower than that of most of the bioradicals (Scandalios *et al*., 1997). The triadimefon treated plants showed an increased α-tocopherol content in leaves, stem as well as in roots, thus showing very good antioxidant potentials in different parts of the plant (Jaleel *et al*., 2006; Sivakumar *et al*., 2010). GSH content was found increased under pesticides and herbicides application, drought stress (Davis and Swanson, 2001; Omidi, 2010) and in Paclobutrazol (PBZ) applied *Dioscorea rotundata poir* and *C. roseus* (Jaleel *et al*., 2007a, b). The increase in GSH can be correlated with its ability to scavenge singlet oxygen, peroxides and hydroxyl radicals and is involved in recycling of AA in the ascorbate-glutathione pathway in chloroplasts (Pastori *et al*., 2000).

In the present study, increase in antioxidant enzymes activities (SOD APX CAT) was recorded in two varieties of *Datura* plants under triadimefon treatment. According to Pastori *et al*. (2000) many stress situation caused an increase in the foliar SOD activity maize and TDM treated radish, *C. roseus*, sweet potato (Jaleel *et al*., 2006; Sivakumar *et al*., 2010), uniconazole treated cassia (Sheela and Pandy, 2003), showed an increases SOD activity.

Increased APX activity by triadimefon treatment would increase the demand for ascorbate regeneration. Similar findings were reported in paclobutrazol treated maize, *C. roseus* (Pastori *et al*., 2000; Jaleel *et al*., 2007c). The increased ascorbic acids in the triazole-treated plants were well correlated with the increased APX contents (Jaleel *et al*., 2006). Ascorbate peroxidase is the main antioxidant enzyme in the chloroplast which contains superoxide dismutase in *C. roseus* (Jaleel *et al*., 2006). In this context, it is believed that a simultaneous increase in several components of the antioxidative defense system would be necessary in order to obtain an increase in plant protective mechanism (Foyer *et al*., 1994).

The increase in CAT found under triadimefon treatment was of great importance in plants protective mechanisms under abiotic stress. Therefore, the present results were consistent with previous hypothesis that triazole induced stress tolerance in plants may be caused, at least in part, by increased antioxidant activities (Jaleel *et al*., 2006; Sivakumar *et al*., 2010). The H_2O_2 scavenging system represented by CAT is more important in importing tolerance to oxidative stress as observed in sweet potato and wheat varieties (Sivakumar *et al*., 2010; Willekens *et al*., 1997). The changes in CAT may vary according to the intensity of stress, time of assay after stress and induction of new isozyme(s) (Shim *et al*., 2003).

The alkaloid content increased in treated plants when compared to control. The plant growth regulation properties of triadimefon may be the reason for increase alkaloid content under treatment. Similar results were obtained in PGR application in *Datura* plants. Increased Indole alkaloid content was also reported in *Datura* (Zayed, 2003; Berkov and Zayed, 2004).

CONCLUSION

In conclusion, our results indicated that the triadimefon application at low concentration could be used as a potential tool to increase in antioxidant defense mechanisms and indole alkaloid production in *Datura*.

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