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Antioxidant Enzyme Changes with Triazole Compounds in *Coleus*

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Abstract: In this study we tried to explore the changes occurring in antioxidant enzymes Ascorbate peroxidase (APX, EC: 1.11.1.11), Catalase (CAT, EC: 1.11.1.6) and Superoxide dismutase (SOD, EC: 1.15.1.1) of two *Coleus* speceis (*Plectranthus aromaticus* Benth and *Plectranthus vettiveroides* K.C. Jacob.) on treatments with propiconazole and hexaconazole. The treatments were given as soil drenching 30, 50 and 70 days after planting (DAP). The plants were uprooted randomly on 45, 65 and 85 DAP and separated into roots, stems and leaves and used for estimating APX, CAT and SOD. The APX, CAT and SOD acativities increased with triazole treatments when compared with control plants.

Key words: Ascorbate peroxidase, Catalase, Superoxide dismutase, Propiconazole, Hexaconzole, *Coleus*

INTRODUCTION

Plant growth retardants are applied in crop plants to reduce unwanted longitudinal shoot growth without lowering plant productivity [1]. Besides, the naturally occurring plant hormones like indole acetic acid, gibberellic acid, zeatin, cytokinins, abscisic acid and ethylene, a wide range of chemicals with well defined influence on the growth and development have been synthesized and these chemicals are widely used for many agricultural and horticultural plants [2].

The triazoles are the largest and most important group of systemic compounds developed for the control of fungal diseases in plants and animals. These compounds have both fungitoxic and plant growth-regulating properties [3-6]. The broad-spectrum PGR properties of the triazoles have been reported to mediate through the alteration of the balance of plant hormones, including GA, ABA, cytokinins and ethylene [7,8]. Inhibition of GA biosynthesis is the primary site of plant regulation by the triazoles, explaining reduced internodal elongation and decreased leaf area. The triazoles inhibit the first three steps of ent-kaurene oxidation which are catalyzed by the Cyt P-450 dependent microsomal hydroxylase, ent-kaurene oxidase thus inhibiting the formation of ent-kaurenol, ent-kaurenal and ent-kaurenoic acid [9-13].

The family Lamiaceae contains several genera, such as sage (*Salvia*), basil (*Ocimum*) and mint (*Mentha*), with a rich diversity of ethnobotanical uses. Another important genus is *Plectranthus*, a large genus containing about 300 species found in Tropical Africa, Asia and Australia. Some species of *Plectranthus* are difficult to identify because of a lack of clear-cut morphological criteria to discriminate not only among species within the genus but also among the closely related genera.

Coleus (*Plectranthus aromaticus* Benth and *Plectranthus vettiveroides* K.C. Jacob.) commonly called Indian Borage, are medicinal plants and several medicinal properties are attributed to these plants in the Indian system of medicine. *Plectranthus vettiveroides* is also aromatic plant containing essential oils and diterpenes in its roots.

Objectives of this Study: To estimate the changes occurring in Antioxidant enzymes like ascorbate peroxidase (Apx), superoxide dismutase (SOD) and catalase (CAT) of *Plectranthus aromaticus* and *Plectranthus vettiveroids* on treatments with propiconazole and hexaconazole.

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MATERIALS AND METHODS

Plant Materials and Triazole Compounds: The cuttings of *Plectranthus aromaticus* and *Plectranthus vettiveroids* were obtained from local farmers of Kollidam, Nagai District, Tamilnadu. The triazole compound propiconazole was obtained from Syngenta, India Ltd., Mumbai. Hexaconazole was obtained from Imperial Chemical Industrial, England.

During the study, average temperature was 32/26°C (maximum/minimum) and relative humidity (RH) varied between 60-75 per cent. The experimental part of this work was carried out in Botanical Garden and Stress Physiology Lab, Department of Botany, Annamalai University, Tamil Nadu. The methodologies adopted are described below.

Cultivation Methods: The plants were raised in Botanical Garden, during the months of February – May, 2006. The experiments were carried out in plastic pots. The pots were filled with 3 kg uniform soil mixture containing red soil: sand: farm yard manure (FYM) in 1:1:1 ratio. The plants were supplied with 25 gms of socked groundnut oil cake per plant. The experiment was laid out in a Completely Randomized Block Design (CRBD).

Propiconazole and Hexaconazole Treatments: In the preliminary experiments 5, 10, 15 and 20 mg L^{-1} of propiconazole and hexaconazole were used for treatments to determine the optimum concentration of these compounds at which the dry weight increased significantly. Among these concentrations 15 mg L^{-1} of propiconazole and 5 mg L^{-1} hexaconazole were found to increase the dry weight significantly and the higher concentration recorded downward trend in growth and dry weight. Hence these active principle concentrations were used to determine the effect of these triazole compounds on *Plectranthus aromaticus* and *Plectranthus vettiveroids*.

The treatments were given as soil drenching 30, 50 and 70 days after planting (DAP). The plants were uprooted randomly on 45, 65 and 85 DAP and separated into roots, stems and leaves and used for determining growth and antioxidant potentials.

Antioxidant Enzymes

Superoxide Dismutase (SOD, EC: 1.15.1.1): Crude enzyme extract was prepared, for the assay of Superoxide dismutase by the method of Hwang *et al.* [14].

Extraction: One gram of fresh tissue was homogenized with 10 ml of ice-cold 50 mM sodium phosphate buffer containing 1 mM PMSF. The extract was filtered through a double-layered cheesecloth. The extract was centrifuged at 12,500 rpm for 20 minutes at 4 °C. The supernatant was saved and made up to 10 ml with extraction buffer and used for estimation of the SOD enzyme activity. The enzyme protein was determined by Bradford [15] method.

Estimation: Superoxide dismutase activity was assayed as described by Beauchamp and Fridovich [16]. The reaction medium was prepared and to 3 ml reaction medium, 1 ml of enzyme extract was added. The reaction mixture contained 1.17×10^{-6} M riboflavin, 0.1 M methionine, 2×10^{-5} potassium cyanide and 5.6×10^{-5} M nitroblue tetrasodium salt (NBT), dissolved in 0.05 M sodium phosphate buffer (pH 7.8). The mixture was illuminated in glass test tubes by two sets of Philips 40 W fluorescent tubes. Illumination started to initiate the reaction at 30 °C for one hour. Those without illumination saved as blank and kept in dark. The absorbance was read at 560 nm in the spectrophotometer against blank. Superoxide dismutase activity was expressed in units. One unit is defined as the amount of change in the absorbance by 0.1 per hour per milligram protein under the assay condition.

Ascorbate Peroxidase (APX, EC: 1.11.1.11): Ascorbate peroxidase was extracted and estimated by the method of Asada and Takahashi [17].

Extraction: Five hundred milligrams of fresh plant tissue was ground in a pestle and mortar under liquid nitrogen and 10 ml of 50mM potassium phosphate buffer (pH 7.0) containing 1 mm EDTA, 1 per cent PVP and 1 mM ascorbic acid. The homogenate was filtered through double-layered cheesecloth and centrifuged at 15,000 rpm for 20 minutes at 4 °C. The supernatant was used as source of enzymes.

Estimation: One ml of reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 200 ?l 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 μg per gram dry weight.

Catalase (CAT, EC: 1.11.1.6): Catalase activity was assayed as described by Chandlee and Scandalios [18].

Extraction: Five hundred milligrams of frozen material was homogenized in 5 ml of ice cold 50 mM sodium phosphate buffer (pH 7.5) containing in 1mM PMSF. The extract was centrifuged at 4°C for 20 minutes at 12,500 rpm. The supernatant was used for enzyme assay.

Assay: The activity of enzyme catalase was measured using the method of Chandlee and Scandalios (1984) with modification. The assay mixture contained 2.6 ml of 50 ml of 50 mM potassium phosphate buffer (pH 7.0) 0.4 ml, 15 mM H_2O_2 and 0.04 ml of enzyme extract. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm. The enzyme activity was expressed in units 1 mM of H_2O_2 reduction per minute per mg protein.

Statistical Analysis: Each treatment was analysed with at least three replicates and a standard deviation (SD) was calculated and data are expressed in mean ± SD of three replicates.

RESULTS

Superoxide Dismutase Activity (Fig. 1)

Leaf: Superoxide dismutase activity increased in leaves of triazole treated plants. The triazoles significantly increased the superoxide dismutase activity, it was higher by 168.64 and 206.64 in propiconazole *Plectranthus vettiveroides and Plectranthus aromaticus* respectively on 85 DAP. Hexaconazole increased the superoxide dismutase activity in the leaves upto 108.34 and 103.08 per cent over control on 85 DAP.

Stem: Triazole treatment increased the superoxide dismutase activity in stems when compared with control plants. The highest activity was recorded with propiconazole treatment followed by hexaconazole treatment. Triazole treatment significantly increased the superoxide dismutase activity and it was higher by 153.82 and 211.08 per cent in propiconazole treated *Plectranthus vettiveroides and Plectranthus aromaticus* respectively on 85 DAP. In the case of hexaconazole treatment the stem superoxide dismutase activity increased up to 143.60 in *Plectranthus vettiveroides* and 198.60 in *Plectranthus aromaticus* on 85 DAP.

Root: The superoxide dismutase activity of triazole treated *Plectranthus vettiveroides* and *Plectranthus aromaticus* roots increased to a significant extent when compared to untreated control plants. The propiconazole treated plants had a higher effect in the enhancement of superoxide dismutase activity when compared to hexaconazole treated plants. It was 196.43 and 196.06 per cent over control in *Plectranthus vettiveroides* and *Plectranthus aromaticus* respectively on 85 DAP.

Ascorbate Peroxidase Activity (Fig. 2)

Leaf: Ascorbate peroxidase activity increased in the leaves of triazole treated and control plants. Significant increase in ascorbate peroxidase activity was recorded in treated plants as compared to control. Among the triazole treatments, propiconazole increased the enzyme activity to a higher level than that of hexaconazole and it was 155.46 and 110.86 per cent over the control respectively on 85 DAP.

Stem: The ascorbate peroxidase activity of triazole treated *Plectranthus vettiveroides* and *Plectranthus aromaticus* stem increased to a significant extent when compared to untreated control plants. The propiconazole treated plants had a higher effect in the enhancement of ascorbate peroxidase activity when compared to hexaconazole treated plants. It was 146.17 and 126.40 per cent over control (propiconazole treated) and 126.40 and 118.48 per cent over control (hexaconazole treated) in *Plectranthus vettiveroides* and *Plectranthus aromaticus* respectively on 85 DAP.

Root: Triazole treatment increased the ascorbate peroxidase activity in roots when compared with control plants. The highest activity was recorded with propiconazole treatment followed by hexaconazole treatment. Triazole treatments significantly increased the ascorbate peroxidase activity and it was higher by 162.60 and 118.02 per cent in propiconazole treated *Plectranthus vettiveroides and Plectranthus aromaticus* respectively on 85 DAP. In the case of hexaconazole treatment the stem ascorbate peroxidase activity increased up to 151.55 in *Plectranthus vettiveroides* and 115.64 in *Plectranthus aromaticus* on 85 DAP.

Catalase Activity (Fig. 3)

Leaf: Catalase activity increased with triazole treatments when compared to control plants. Significant increase in ascorbate peroxidase activity was recorded in both propiconazole and hexaconazole treatments when compared to control. Among the triazole treatments, propiconazole increased the catalase activity to a higher level than that of hexaconazole and it was 180.68 and 188.08 per cent over the control *Plectranthus vettiveroides and Plectranthus aromaticus* respectively on 85 DAP.

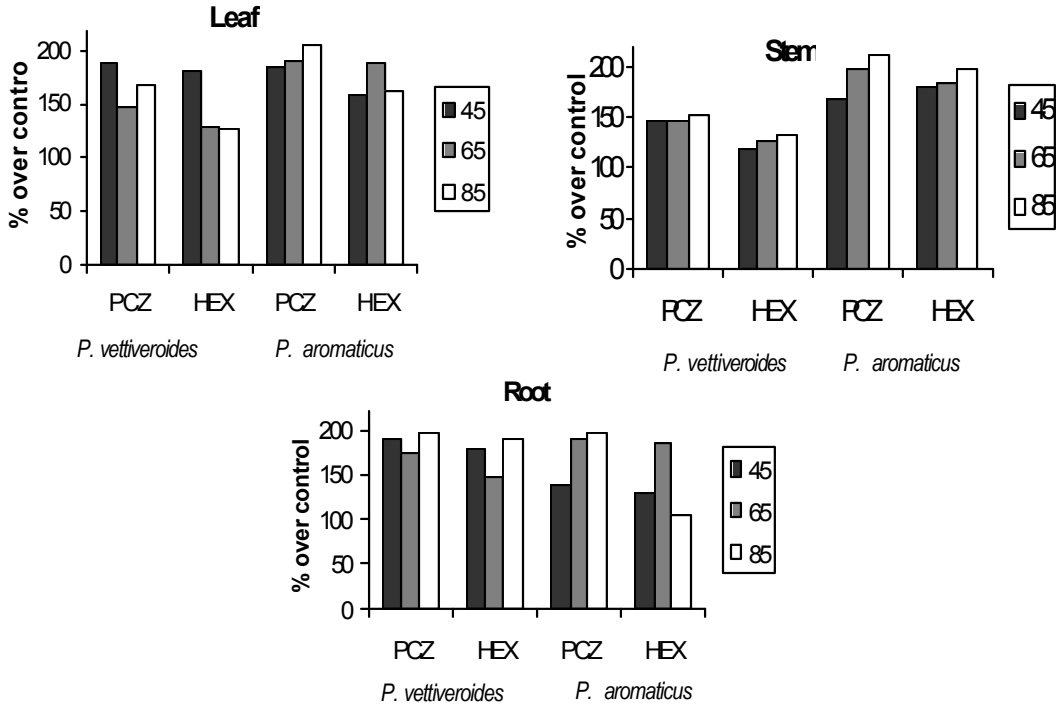

Fig. 1: Effect of propiconazole (PCZ) and hexaconazole (HEX) on superoxide dismutase activity of *Plectranthus vettiveroides* and *Plectranthus aromaticus*

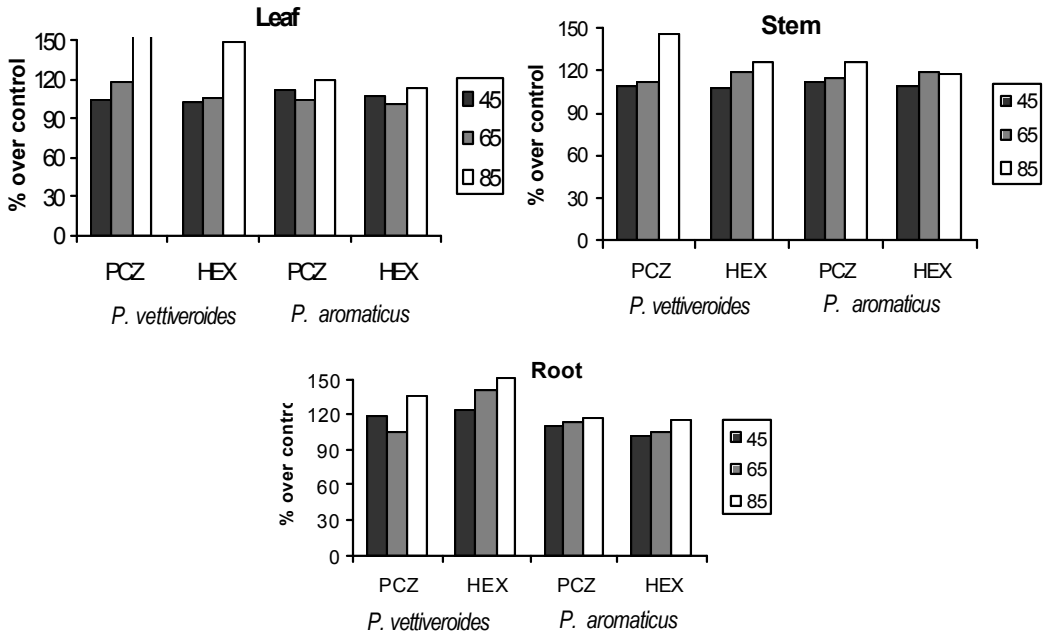

Fig. 2: Effect of propiconazole (PCZ) and hexaconazole (HEX) on ascorbate peroxidase activity of *Plectranthus vettiveroides* and *Plectranthus aromaticus*

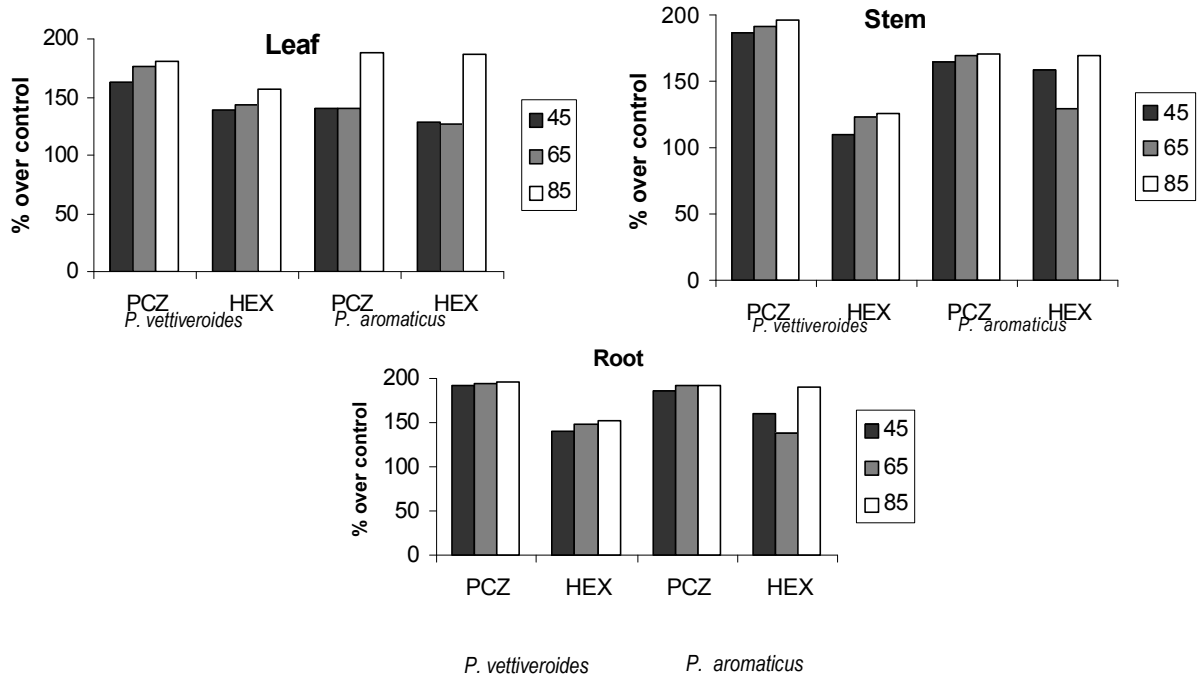

Fig. 3: Effect of propiconazole (PCZ) and hexaconazole (HEX) on catalase activity of *Plectranthus vettiverides* and *Plectranthus aromaticus*

Stem: Triazole treatment increased the catalase activity in tubers when compared with control. Triazole treatment significantly increased the catalase activity and it was higher by 196.43 and 171.61 per cent in propiconazole treated *Plectranthus vettiveroides and Plectranthus aromaticus* respectively on 85 DAP. In the case of hexaconazole treatment the stem superoxide dismutase activity increased up to 126.34 in *Plectranthus vettiveroides* and 169.36 in *Plectranthus aromaticus* on 85 DAP.

Root: In the roots, the catalase activity was higher in both propiconazole and hexaconazole treated plants when compared to control. The increase in catalase activity was 197.42 and 200.16 per cent over control in propiconazole *Plectranthus vettiveroides and Plectranthus aromaticus* respectively on 85 DAP. Hexaconazole increased the catalase activity in the roots upto 152.30 and 190.48 per cent over control on 85 DAP.

DISCUSSION

Superoxide dismutase (SOD) activity increased with triazole treatments in both *Plectranthus aromaticus* and *Plectranthus vettiveroides* to a level of higher than that of control plants. SOD plays a major role in combating oxygen radical mediated toxicity. High SOD enzyme activity in the tissue of *Mangifera indica* L. retarded the membrane permeability [19].

SOD catalyses the dismutation of $O_2^{\bullet ?}$ to H_2O_2 and O_2 while CAT scavenge H_2O_2 [20]. PBZ treatment increased the activity of SOD in *Catharanthus* [4]. Propiconazole treatment protected plants from water stress damage and the stress protection is mediated by an increased activity of antioxidant enzymes [21].

The ascorbate peroxidase (APX) activity increased in the triazole treated *Plectranthus aromaticus* and *Plectranthus vettiveroides* when compared to the control. Triadimefon increased the level of APX activity [22]. Other authors observed similar effects [23-25]. APX utilizes ascorbic acid as an electron donor in the neutralization of H_2O_2 both in the cytosol and molecular compartments [20]. PBZ stimulated increase in the activities of SOD, APX and catalase [6-8].

CAT activity has been increased with treatment with triazole in both *Plectranthus aromaticus* and *Plectranthus vettiveroides*. An increase in CAT activity was noted in *Catharanthus* under salinity [7] and further enhanced by paclobutrazol treatment to provide effective

scavenging of H_2O_2 [4]. The H_2O_2 scavenging system represented by APX and CAT are more important in imparting tolerance than SOD as reported in oxidative stressed plants [26-30].

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