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Triazole Alters Antioxidants in Two Medicinal Herbs of Lamiaceae Family

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Abstract: A pot culture experiment was conducted to estimate the changes occurring in antioxidants like ascorbic acid, α-tocopherol and total phenols of *Plectranthus aromaticus* and *Plectranthus vettiveroids* 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 determining antioxidant potentials. The ascorbic acid, α-tocopherol and total phenols contents of the tissues increased with triazole treatments when compared with control plants. From these results it is clear that triazole treatments can be used as enhancer for antioxidant potentials in *Plectranthus aromaticus* and *Plectranthus vettiveroids*.

Key words: Propiconazole, Hexaconzole, Lamiaceae, *Plectranthus vettiveroides*

INTRODUCTION

The family Lamiaceae is one of the largest and most distinctive families of flowering plants, with about 220 genera and almost 4000 species. Lamiaceae are best known for the essential oils content to many members of the family [1]. The family is also famous for the presence of diterpenoids in its members. Species of *Mentha*, *Thymeus, Salvia*, *Coleus* and *Ocimum* are used as food flavorings, vegetables and in industry. Members of the family are used for different purposes, nut their use falls in to these categories such as medicinal, ornamental and aromatic plants which are used in perfume industry and culinary herbs and vegetables [2].

Plectranthus is a member of Lamiaceae family. It is an aromatic plant containing essential oils and diterpenes in its roots. The roots are slender and long forming a tuft like appearance. It is widely cultivated in Southern India (Tamil Nadu) for its fragrant roots, used for the decoration of temple images and for dressing hair. Its main use lies in pharmacological industry for the isolation of essential oils and diterpenoids from its fragrant roots, which are used in perfumery. This plant also has medicinal values in traditional as well as modern medicine [3].

The plant growth regulating properties of triazoles are mediated by their ability to alter the balance of important plant hormones including Gibberellic acid, ABA and cytokinins [4]. 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 [5-8]. Triazoles are known to shift assimilate partitioning from leaves to roots and alter mineral uptake and plant nutrition [9-11]. Triazoles affect the activities of several enzymes, especially those related to detoxyfication of active oxygen species and antioxidant metabolism [12-14]. To estimate the following changes occurring in *Plectranthus aromaticus* and *Plectranthus vettiveroids* on treatments with propiconazole and hexaconazole on Antioxidants like ascorbic acid, α-tocopherol and total phenols.

MATERIALS AND METHODS

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

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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 antioxidant potentials.

Antioxidants

Ascorbic Acid: Ascorbic acid content was assayed as described by Omaye *et al.* [15].

Extraction: One gram of fresh material was ground in a pestle and mortar with 5 ml of 10 per cent TCA, the extract was centrifuged at 3500 rpm for 20 minutes. The pellet was re-extracted twice with 10 percent TCA and supernatant was made to 10 ml and used for estimation.

Estimation: To 0.5 ml of extract, 1 ml of DTC reagent (2,4-Dinitrophenyl hydrazine-Thiourea-$CuSO_4$ reagent) was added and mixed thoroughly. The tubes were incubated at 37 °C for 3 hours and to this 0.75 ml of ice cold 65 per cent H_2SO_4 was added. The tubes were then allowed to stand at 30 °C for 30 minutes. The 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 milligrams per gram dry weight.

α-Tocopherol: α-Tocopherol activity was assayed as described by Backer *et al.* [16].

Extraction: 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 ofα-tocopherol.

Estimation: 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.

Total Phenols: Total phenols were estimated by the method of Malick and Singh [17].

Extraction: 500 miligrams of fresh plant tissue was ground in a pestle and mortar with 10 ml of 80 per cent ethanol. The homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was evaporated to dryness. The residue was dissolved with 5ml of distilled water and used as extract.

Estimation: To 2 ml of the extract, 0.5 ml of Folin-Ciocalteau reagent was added. After 3 min, 2 ml of 20% Na_2CO_3 solution was mixed thoroughly. The mixture was kept in boiling water for exactly one min. and after cooling the absorbance was read at 650 nm. The total phenol was determined using a standard curve prepared with different concentration of gallic acid.

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

Ascorbic Acid Content (Table 1)

Leaf: The ascorbic acid content of the leaf tissue increased with triazole treatments when compared with control plants. Treatment with triazole compounds significantly increased the ascorbic acid content of leaves when compared to control, it was 152.39 and 169.36 per cent over control with propiconazole treatment in *Plectranthus vettiveroides* and *Plectranthus aromaticus* respectively on 85 DAP. In the case of hexaconazole treatment the increase was 168.02 and 165.62 per cent over control in *Plectranthus vettiveroides* and *Plectranthus aromaticus* respectively.

Table 1: Effect of propiconazole (PCZ) and hexaconazole (HEX) on ascorbic acid content of *Plectranthus aromaticus* and *Plectranthus vettiveroides* (values are mean±S.D. of 3 samples expressed in mg/g fresh weight)

Growth Stages	*Plectranthus aromaticus*			*Plectranthus vettiveroides*		
DAP	Control	PCZ	HEX	Control	PCZ	HEX
Leaf						
45	0.66±0.026	0.68±0.027	0.67±0.026	1.33±0.053	1.99±0.071	1.85±0.068
65	1.64±0.060	2.42±0.089	1.86±0.068	1.84±0.073	1.94±0.069	1.85±0.066
85	4.57±0.147	5.17±0.109	4.87±0.156	5.63±0.181	5.95±0.113	5.83±0.147
Stem						
45	0.95±0.036	1.60±0.075	1.06±0.036	0.87±0.035	1.51±0.053	1.18±0.063
65	2.48±0.076	2.93±0.076	2.86±0.064	2.144±0.073	2.50±0.053	2.19±0.064
85	3.34±0.123	3.54±0.123	3.43±0.095	5.06±0.131	5.80±0.233	5.25±0.130
Root						
45	0.96±0.013	1.88±0.068	1.56±0.068	0.34±0.013	1.37±0.068	1.02±0.042
65	2.09±0.074	3.89±0.076	3.06±0.073	1.25±0.048	2.49±0.095	1.44±0.076
85	3.69±0.066	4.33±0.123	4.01±0.090	1.87±0.066	2.90±0.116	2.34±0.123

Table 2: Effect of propiconazole (PCZ) and hexaconazole (HEX) on ∝ - tocopherol content of *Plectranthus aromaticus* and *Plectranthus vettiveroides* (values are mean±S.D. of 3 samples expressed in mg/g fresh weight)

Growth Stages	*Plectranthus aromaticus*			*Plectranthus vettiveroides*		
DAP	Control	PCZ	HEX	Control	PCZ	HEX
Leaf						
45	2.54±0.097	6.89±0.246	3.97±0.141	4.05±0.155	5.85±0.066	5.76±0.063
65	1.44±0.053	2.39±0.015	1.90±0.074	1.79±0.071	2.57±0.021	2.41±0.015
85	0.42±0.016	0.93±0.157	0.85±0.321	0.67±0.026	1.59±0.023	1.49±0.019
Stem						
45	1.07±0.068	2.38±0.162	1.45±0.337	5.06±0.158	8.12±0.333	7.59±0.023
65	0.32±0.026	1.09±0.157	1.01±0.064	4.35±0.173	7.41±0.285	5.52±0.264
85	0.43±0.023	1.08±0.043	1.03±0.095	4.62±0.031	7.49±0.323	6.06±0.130
Root						
45	0.65±0.024	0.79±0.026	0.78±0.183	5.01±0.192	6.09±0.109	5.78±0.037
65	0.44±0.017	0.47±0.017	0.46±0.328	4.92±0.172	6.99±0.376	5.91±0.344
85	0.68±0.027	0.84±0.023	0.83±0.094	2.75±0.111	3.89±0.023	3.06±0.042

Stem: The ascorbic acid content in stem increased with triazole treatments at all stages of growth and the increase was 151.77 and 176.73 per cent over control in the propiconazole and 164.46 and 184.62 per cent over control in hexaconazole treated *Plectranthus vettiveroides* and *Plectranthus aromaticus* plants respectively on 85 DAP.

Root: The ascorbic acid content in root increased with triazole treatments at all stages of growth and the increase was 154.11 and 163.14 per cent over control in the propiconazole and 135.08 and 181.24 per cent over control in hexaconazole treated *Plectranthus vettiveroides* and *Plectranthus aromaticus* respectively on 85 DAP.

A-Tocopherol Content (Table 2)

Leaf: The α-tocopherol content in leaves increased with triazole treatment at all stages of growth and the increase was 188.05 and 135.71 per cent over control in propiconazole and 193.64 and 168.64 per cent over control in hexaconazole treated *Plectranthus vettiveroides* and *Plectranthus aromaticus* respectively on 85 DAP.

Stem: The α-tocopherol content in stems of *Plectranthus vettiveroides* and *Plectranthus aromaticus* increased under triazole treatments in all stages of growth when compared to control. The increase was 116.06 and 103.16 per cent over control in propiconazole treated

Table 3: Effect of propiconazole (PCZ) and hexaconazole (HEX) on total phenol content of *Plectranthus aromaticus* and *Plectranthus vettiveroides* (values are mean±S.D. of 3 samples expressed in mg/g fresh weight)

Growth Stages	*Plectranthus aromaticus*			*Plectranthus vettiveroides*		
DAP	Control	PCZ	HEX	Control	PCZ	HEX
Leaf						
45	11.80±0.333	15.57±0.184	15.37±0.185	4.83±0.161	6.29±0.330	5.68±0.292
65	12.55±0.529	16.63±0.255	16.01±0.225	4.90±0.168	6.34±0.323	5.74±0.331
85	13.16±0.521	16.88±0.275	16.65±0.237	5.30±0.189	6.40±0.233	5.80±0.247
Stem						
45	3.40±0.168	4.99±0.068	3.92±0.135	2.93±0.058	3.96±0.071	3.89±0.037
65	3.57±0.176	4.11±0.076	4.19±0.124	2.94±0.073	3.10±0.077	3.05±0.064
85	3.61±0.123	4.28±0.123	4.33±0.133	3.28±0.131	4.35±0.094	3.14±0.130
Root						
45	2.05±0.082	3.51±0.068	3.51±0.089	1.30±0.052	3.85±0.038	2.46±0.017
65	2.10±0.084	3.52±0.076	3.52±0.093	1.34±0.053	3.87±0.036	2.54±0.019
85	2.15±0.086	3.56±0.123	3.64±0.097	1.43±0.055	3.91±0.043	2.77±0.030

Plectranthus vettiveroides and *Plectranthus aromaticus* respectively on 85 DAP. In the case of hexaconazole the increase was 108.64 and 148.21 per cent over control *Plectranthus vettiveroides* and *Plectranthus aromaticus* respectively on 85 DAP.

Root: The α-tocopherol content was higher in roots of triazole treated plants when compared to control. The increase in α-tocopherol content was 120.60 and 119.11 per cent over control in propiconazole treated *Plectranthus vettiveroides and Plectranthus aromaticus* respectively on 85 DAP. Hexaconazole increased the α-tocopherol content in roots of treated plants upto 126.10 and 149.60 per cent over control on 85 DAP.

Phenol Content (Table 3)

Leaf : The phenol content was higher in triazole treated plants when compared to control. The increase in phenol content was 120.75 and 152.27 per cent over control in propiconazole treated *Plectranthus vettiveroides and Plectranthus aromaticus* on 85 DAP. In hexaconazole treatment the increase was nearly 190.56 and 150.53 per cent over control respectively on 85 DAP.

Stem: The phenol content was higher in stems of triazole treated *Plectranthus* plants as compared to control. The increase in phenol content was 171.64 and 190.85 per cent over control in propiconazole treated *Plectranthus vettiveroides and Plectranthus aromaticus* respectively on 58 DAP. Hexaconazole increased the total phenol content in the stem upto 165.24 and 192. 24 per cent over control on 85 DAP.

Root: In the roots, the phenol content was higher in both propiconazole and hexaconazole treated plants when compared to control. The increase in phenol content was 163.63 and 149 per cent over control in propiconazole *Plectranthus vettiveroides and Plectranthus aromaticus* respectively on 85 DAP. Hexaconazole increased the total phenol content in the roots upto 163.84 and 122.79 per cent over control on 85 DAP.

DISCUSSION

Propiconazole and hexaconazole treatments increased the ascorbic acid content in the leaf, stem and roots of both *Plectranthus aromaticus* and *Plectranthus vettiveroides* plants. Ascorbic acid is an important component of the plant antioxidant system [18-20]. Uniconazole increased the level of the antioxidants like, ascorbic acid in tomato seedlings and protect membrane by preventing or reducing oxidative damage [21]. Similar results were observed in many plants [4-7,22].

Triazole treatments increased the α-tocopherol content in all parts of both *Plectranthus aromaticus* and *Plectranthus vettiveroides* plants when compared to control. Triazole compounds protected seedlings from stress and this protection was mediated by an increase in α-tocopherol and ascorbate and enhanced activities of glutathione reductase [23]. α-Tocopherol was consumed predominantly as radical scavenging antioxidant against the lipid peroxidation as observed [24]. Protection of plants from apparently unrelated stress by triazole is also mediated by a reduction of free radicals damage and increase in the antioxidant potential [25].

Both *Plectranthus aromaticus* and *Plectranthus vettiveroides* plants showed increased phenol content under triazole treatments when compared to control plants. Increased total phenol content was previously reported in triazole treated plants [7-8]. It has been suggested that peroxidase could act as efficient H_2O_2 scavenging system in plant vacuoles in the presence of phenolics and reduced ascorbate [12-14]. Phenolics are oxidized to phenoxyl radicals. This phenoxyl radical reduces the ascorbic acid into monodehydro ascorbate. Thus phenol acts as an intermediatory ROS acceptor in the vacuoles. This increase of phenol by triazoles may be further enhances the antioxidant capacity of radish along with other antioxidants [26-27].

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