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Triadimefon and Hexaconazole Alters the Antioxidant Enzyme Profile of Radish

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Abstract: In the present study to estimate the effect of triazole viz. triadimefon and hexaconazole on the antioxidant metabolism of *Raphanus sativus*. Triadimefon (TDM) 10 mg^{l-1} and hexaconazole (HEX) 5 mg L^{-1} who treated to per plant in one pot, on 8, 23, 38 and 53 days after sowing (DAS). The antioxidant enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) were extracted and assayed on 30 and 60 DAS form shoot and tuber of both control and triazole treated plants. Triazole treatment increased the enzymatic antioxidants on the plant.

Key words: Triazole · Antioxidant enzymes · Membrane integrity · Superoxide dismutase · Ascorbate peroxidase · Catalase

INTRODUCTION

Triazole compounds are the chemicals belong to a class of compounds known as ergosterol biosynthesis inhibitors and are used as fungicides as wells as plant growth regulators [1-3]. Triazole compounds are characterized by a ring structure containing 3 nitrogen atoms, a chlorophenyl and a carbon side chain [2-5]. Effectiveness as fungicide or PGR is determined by the stereochemical configuration of the substituents on the carbon chain [6]. Triazole compounds such as triadimefon (TDM), Hexaconazole (HEX) widely used as fungicides and they also posses varying degrees of plant growth regulating properties [7].

They also protect plants from biotic and abiotic stresses including fungal pathogen, drought, salinity, air pollutions and low and high temperature and also it affect the isoprenoid pathway and alter the level of certain plant hormones by inhibiting gibberellin synthesis, reducing ethylene evolution and increasing cytokinin, kinetin levels [8-10]. Therefore, there is a need to investigate the efficient of this compound in the enhancement of antioxidant potentials in white radish plants in order increase their medicinal properties and making them valuable tuber crop. Hence, this study aims to evaluate the ability of triazole to enhance the antioxidant potentials and membrane integrity, with special emphasis to antioxidant potential and membrane integrity constituents [11-14].

Examples of enzymes antioxidant defenses include ascorbate peroxides and glutathion reductase, which are believed to scavenge H_2O_2 in chloroplasts and mitochondria, respectively [15-17]. The catalases and peroxidases that remove H_2O_2 efficiently and superoxide dismutases that scavenge the superoxide anion. Among these CAT and SOD are the most efficient antioxidant enzymes and their combined action reutralize the potentially dangerous superoxide radical ($O-_2$) and hydrogen peroxidase (H_2O_2) to water (H_2O) and molecular oxygen (O_2), thus averting cellular damage [18-20]. The activities of antioxidant enzymes such as ascorbic peroxidase, catalase and superoxide dismutase and up-regulated in response to several abiotic stresses such as drought, high light intensities, ozone, So2, UV-B and salinity [11-20].

Raphanus sativus (white radish) is an important vegetable crop in India and south east countries. The leaves and tubers of radish are used to prepare salad and also cooked as vegetables. It is rich in vitamin 'C' and minerals like sulphur. It is also used as a medicine in currying liver disorders and jaundice. This tuber crops are rich source of energy for people living under sustenance level since, it is available at a cheaper price for the poor people.

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MATERIALS AND METHODS

The seeds of *Raphanus sativus* L. cv. Pusa chetki were obtained from mahyco-Maharashtra hybrid seeds Co. Ltd. Maharashtra. India and planted at the botanical garden of the Annamalai University. Two seeds were sown in each plastic pot of 30cm diameter and 30cm height containing 3 kg of soil mixture containing red soil, sand and farm yard manure at 1:1:1 ratio. Then the seedling thinned to one per pot on 6th day after sowing. Triadimefon was obtained from Bayer, Germany and hexaconazole was obtained from imperial chemical industries, England.

The preliminary experiments 2, 5, 10, 15 and 20 mgL^{-1} triadimefon and hexaconazole were used, among these treatments, 10 mgL^{-1} triadimefon (TDM) and 5 mgL^{-1} hexaconazole (HEX) concentrations was found to enhance the antioxidant potentials and membrane integrity and the higher concentrations slightly decreased the growth and dry weight, hence 10 mgL^{-1} triadimefon and 5mgL^{-1} hexaconazole were used for this study. The seedlings were treated with deionized water (control), 10 mgL^{-1} triadimefon and 5 mgL^{-1} hexaconazole solution alone per plant on 8, 23, 38 and 53 days after sowing (DAS). Then the plants were harvested randomly on 30 and 60 DAS and separated into tuber and shoot and used for extraction and assay of antioxidant potentials and membrane integrity of radish plant.

Antioxidant Enzyme Extractions and Assays

Superoxide Dismutase (SOD EC; 1.15.1.1): SOD (EC 1.15.1.1) activity was assayed according to Beauchamp and Fridovich [21]. SOD activity was expressed in units (U mg^{-1} protein). One U is defined as the amount of change in the absorbance by 0.1 hr^{-1} mg^{-1} protein.

Ascorbate Peroxidase: Ascorbate peroxidase (APX) (EC 1.11.1.1) activity was determined according to Asada and Takahashi [22]. The enzyme activity was expressed in U mg^{-1} protein (U = change in 0.1 absorbance min^{-1} mg^{-1} protein).

Catalase (CAT, 1.11.1.6): Catalase (CAT) (EC 1.11.1.6) was measured according to Chandlee and Scandalios [23]. The enzyme activity was expressed in U mg^{-1} protein (U = 1 mM of H_2O_2 reduction min^{-1} mg^{-1} protein).

Statistical Analysis: Statistical analysis was performed using the one-way analysis of variance (ANOVA) followed by the Duncan's multiple range test (DMRT). The values mean ±SD for six samples in each group p values < 0.05 were considered as significant.

RESULT AND DISCUSSION

Triazole treatment increased the enzymatic antioxidant SOD activities in shoot and tuber at all stages of the growth when compared to control plant (Table 1, Fig. 1). Like wise APX (Table 2, Fig. 2) and CAT activities (Table 3, Fig. 3) also increased by triazole viz-10mg L-1 triadimefon and 5mg L-1 hexaconazole treatments when compared to control plant. SOD is a major scavenger of reactive oxygen species and it catalyses the dismutation

Table 1: Effect of Triazole on SOD activities of shoot and tubers of radish plant (values are given as mean ± SD of six replicates expressed in units per hour mg-1protein)

Das	Control	TDM	HEX
Shoot			
30	1.268	1.427 (112.53)	1.427 (112.53)
60	2.331	2.876 (123.38)	2.947 (126.42)
Tuber			
30	0.526	0.559 (106.27)	0.569 (108.17)
60	0.924	1.053 (113.96)	1.093 (118.29)

Table 2: Effect of Triazole on APX activities of shoot and tubers of radish plant (values are given as mean ± SD of six replicates expressed in μg^{g-1} f.w)

Das	Control	TDM	HEX
Shoot			
30	0.225	0.280	0.248
60	0.298	0.341	0.340
Tuber			
30	0.137	0.151	0.148
60	0.212	0.260	0.258

3: Effect of Triazole on CAT activities of shoot and tubers of radish plant (values are given as mean ± SD of six replicates expressed in µmol of H_2O_2 decomposed per min mg $^{g-1}$ f.w)

Das	Control	TDM	HEX
Shoot			
30	0.508	0.552 (108.66)	0.551 (107.85)
60	0.542	0.643 (118.63)	0.617 (113.83)
Tuber			
30	0.376	0.401 (106.64)	0.405 (107.71)
60	0.413	0.490 (118.64)	0.486 (117.67)

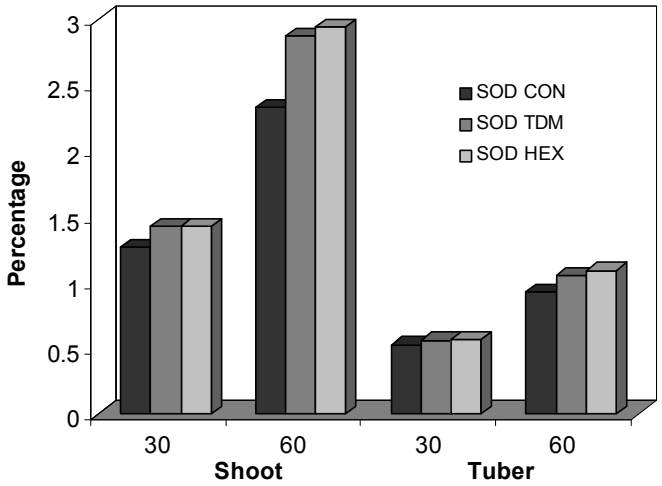

Fig. 1: Effect of Triazole on SOD activities of shoot and tubers of radish plant (values are given as mean ± SD of six replicates expressed in units per hour mg-1protein)

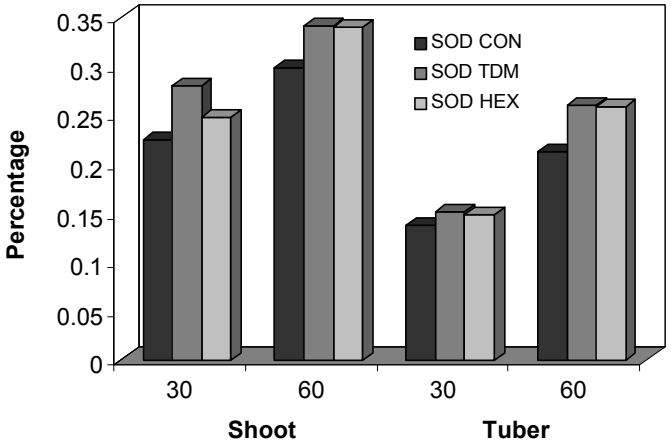

Fig. 2: Effect of Triazole on APX activities of shoot and tubers of radish plant (values are given as mean ± SD of six replicates expressed in mg g-1 f.w)

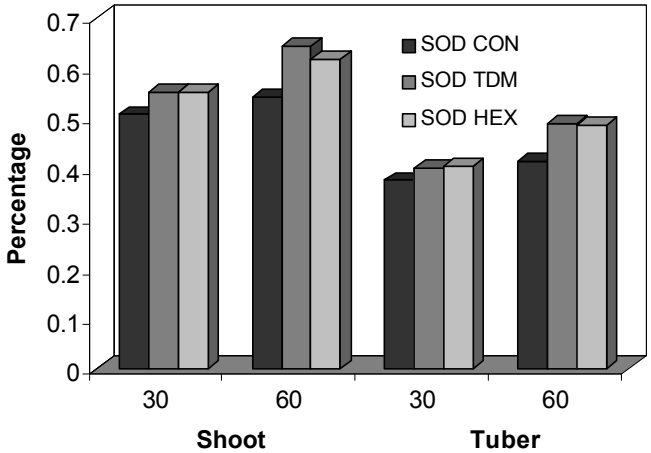

Fig. 3: Effect of Triazole on CAT activities of shoot and tubers of radish plant (values are given as mean ± SD of six replicates expressed in mmol of H2O2 decomposed per min mg g-1 f.w)

of superoxide anion radical (O^{-2}) with great efficiency, resulting in the production of H_2O_2 and O^2 [24]. Triazole treatments increased the activity of SOD, GHS and APX in the leaves and roots of plants [25-27]. APX found in organelles is belived to scavenge H_2O_2 produced from the organelles, where as the function of cytosalic Apx is probably to eliminate H_2O_2 that is produced in the cytosol or apoplats and that which has diffused from organelles. In the chloroplast, H_2O_2 can be detoxified by the ASA-GSH-NADPH system that has been catalyzed by APX [8-14]. PBZ treated plant increased APX activity [4-5]. CAT is tetrametric sheme containing enzymes that catalyze the dismutation of H_2O_2 into water and oxygen. They are localized mainly in the peroxisomes. There is proliferation of peroxisomes during stress, which might help in scavenging of H_2O_2 and diffuse from the cytosol [22-27].

Triazole compounds such as triadimefon (TDM) and hexaconazole (HEX) widely used as fungicides and they also posses Varying degrees of plant growth regulating properties. An abiotic factor like a fungicide TDM, HEX there should be an enhancement in the in the production of toxic free-radicals of H_2O_2, O_2-, O_2 or OH, which should be detoxified in terms of increased antioxidant activities. The enhancement of enzymatic antioxidant like SOD, APX, CAT activity under TDM, HEX treatment may be an indicator of plants protective mechanisms under abiotic stress.

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