Middle-East Journal of Scientific Research 4 (1): 52-56, 2009
ISSN 1990-9233

Membrane Integrity and Riboflavin Content of *Raphanus sativus* L. as Affected by Triazole Growth Retardants

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Abstract: In the present study to estimate the effect of triazole viz. triadimefon and hexaconazole on the membrane integrity of radish (*Raphanus sativus* L.). triadimefon (TDM) 10 mgl^{-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 membrane integrity like electrolyte leakage and lipid peroxidation (MDA) were extracted and assayed on 30 and 60 DAS form shoot and tuber of both control and triazole treated plants. The riboflavin content also estimated. Triazole treatment increased the riboflavin content while it is decrease the membrane leakage on the plant *Raphanus sativus* is important food crops and also it have some medicinal value in curing piles, liver disorders, enlarge spleen and jaundice.

Key words: Triazole · Electrolyte leakage · Lipid peroxidation · Riboflavin · *Raphanus sativus*

INTRODUCTION

Highly reactive species can react with unsaturated fatty acids to cause peroxidation of essential membrane lipids in the plasmalemma or intracellular organelles. Peroxidation damage of the plasma lemma leads to leakage of cellular contents rapid desiccation and cell death, intra cellular membrane damage can affect respiratory, in mitochondria, cause pigment breakdown and loss of carbon fixing ability in chloroplasts [1-4]. The active oxygen species are very reactive and cause a cascade of oxidative reactions resulting in chlorophyll bleaching, protein degradation and membrane destruction [3-5]. Hydrogen peroxide, unlike superoxide can easily diffuse through phospholipids biolayers of chloroplast to the cytosol and interfere with cellular metabolism [4-6]. They are also responsible for protein, lipid and nucleic acid degradation and play a major role in aging and cell death [7-9].

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 [10].

Triazole compounds such as triadimefon (TDM), Hexaconazole (HEX) widely used as fungicides and they also posses varying degrees of plant growth regulating properties [8,9,11-13]. Triazoles have been called plant multiprotectants because of their ability to induce tolerance in plants to environmental and chemical stresses [12-14] protection of plants from apparently unrelated stress by triazoles is mediated by a reduction in free-radical damages and increase in antioxidant potential [15]. Triazole treated plants have a more efficient free-radical scavenging system that enables them to detoxify active oxygen [11-15].

MATERIALS AND METHODS

Plant Materials and Triazole Treatment: 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

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from Bayer, Germany and hexaconazole was obtained from imperial chemical industries, England.

The preliminary experiments 2, 5, 10, 15 and 20 mg L^{-1} triadimefon and hexaconazole were used, among these treatments, 10 mg L^{-1} triadimefon (TDM) and 5 mg L^{-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 mg L^{-1} triadimefon and 5 mg L^{-1} hexaconazole were used for this study. The seedlings were treated with deionized water (control), 10 mg L^{-1} triadimefon and 5 mg L^{-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 membrane integrity of radish plant.

MEMBRANE INTEGRITY

Electrolyte Leakage: The electrolyte leakage was measured by [16]. The percentage leakages of the tissue were calculated as the ratio of conductivity after 12 h to the conductivity after boiling (Total inic conductivity).

Lipid Peroxidation: LPO was estimated as thio barbituric acid reactive substances (TBARS) [17]. The TBARS content was calculated according to its extinction coefficient of 155 $mM^{-1}cm^{-1}$ and expressed in units (U). One 'U' is defined as µ mole of MDA formed min^{-1} mg^{-1} protein.

Riboflavin: Riboflavin activity was assayed and described by the standard extraction methods.

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

Membrane Integrity: Changes in membrane leakage and injury can be measured the extent of electrolyte leakage [18]. Electrolyte leakage was inhibited in the leaf and tuber tissue of radish by triadimefon and hexaconazole to a larger extent when compared to control plant (Table 1, Fig. 1). Triazole altered the sterol biosynthesis and changed the composition of sterol in the plasmamembrane [19]. This change in sterol composition may induce changes in cell membrane that may be reflected in increased membrane stability, acclimatization and frost hardiness as observed in plants [1-4]. Triazoles altered the membrane properties and facilitated the removal of damaged area in the membranes [20-22]. These altered sterol composition, removal of damage area in the membrane and increased kinetin content induced by the triadimefon and hexaconazole might have facilitated the increased membrane stability in radish there by lowered the electrolyte leakage.

Lipid peroxidation is a measurement of injury to the membrane. TDM and HEX treated plant showed a lower lipid peroxidation in radish plant when compared to control plants (Table 2, Fig. 2). Lipid peroxidation is

Table 1: Effect of Triazole on electrolyte leakage of shoot and tuber tissue of radish plant

DAS	Control	TDM	HEX
Shoot			
30	0.156	0.106 (67.94)	0.110 (70.51)
60	0.214	0.151 (70.56)	0.162 (75.70)
Tuber			
30	0.289	0.219 (75.77)	0.228 (78.89)
60	0.378	0.313 (82.82)	0.317 (83.86)

Table 2: Effect of MDA activities of shoot and tubers of radish plant (values are given as mean ± SD of six replicates expressed in µmol of MDA min^{-1} $^{g-1}$ protein)

DAS	Control	TDM	HEX
Shoot			
30	0.118	0.101 (85.59)	0.110 (93.22)
60	0.136	0.127 (93.38)	0.130 (95.58)
Tuber			
30	0.101	0.085 (84.15)	0.091 (90.09)
60	0.126	0.108 (85.7.1)	0.117 (92.85)

Table 3: Triazole on Rbf content 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	0.040	0.044	0.092
60	0.061	0.069	0.065
Tuber			
30	0.081	0.084	0.082
60	0.088	0.099	0.094

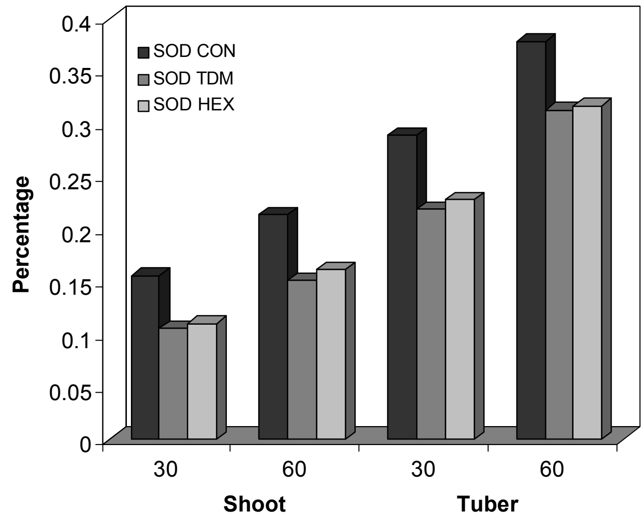

Fig. 1: Effect of Triazole on electrolyte leakage of shoot and tuber tissue of radish plant

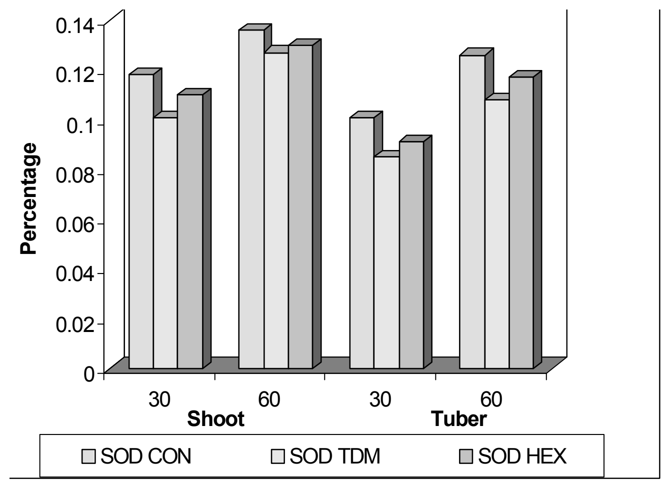

Fig. 2: Effect of MDA activaties of shoots and tubers of radish plant (values are given as mean±Sd of six replicates expressed in mmol of MDA min^{-1} protein)

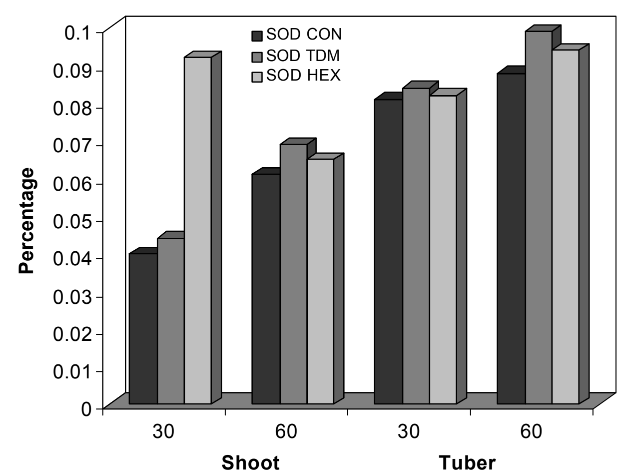

Fig. 3: Triazole on Rbf content of shoot and tubers of radish plant (values are given as mean ± SD of six replicates expressed in mg g-1 F.W)

measured by the malonoldialdehyde (MDA) released [18-21]. Uniconazole reduced the electrolyte leakage and MDA, accumulation and consequently decreased heat induced lipid peroxidation in plants [20-23].

One of the triazole of ketoconazole treated plant can increase the riboflavin content (Table 3, Fig. 3) can increase the membrane stability and prevent membrane degradation due to oxidation of the lipid component of the membrane by the reactive oxygen species. It is involved in lipid peroxidation and oxidized to act as an electron acceptor [11-14].

Triazole compounds such as triadimefon (TDM) and hexaconazole (HEX) widely used as fungicides and they also posses Varying degrees of plant growth regulating properties [6,7]. 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 non-enzymatic antioxidant of AA, RBF, α-toc, GSH and enzymatic antioxidant like SOD, APX, CAT activity under TDM, HEX treatment may be an indicator of plants protective mechanisms under abiotic stress. The inhibited electrolyte leakage and lowered lipid peroxidation in the tissue of radish can be were correlated and it might be the results of increased membrane integrity induced by triazole treatments.

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