Biochemical Alterations in White Yam (*Dioscorea rotundata* Poir.) under Triazole Fungicides: Impacts on Tuber Quality

Cheruth Abdul JALEEL, Ragupathi GOPI and Rajaram PANNEERSELVAM

Stress Physiology Laboratory, Department of Botany, Annamalai University, Tamil Nadu, India

Abstract

Jaleel C.A., Gopi R., Panneerselvam R. (2008): **Biochemical alterations in white yam** *(Dioscorea rotundata* Poir.**) under triazole fungicides: impacts on tuber quality**. Czech J. Food Sci., **26**: 298–307.

An investigation was conducted to find out the effects of two triazole fungicides (triadimefon (TDM) and hexaconazole (HEX) – 15 and 10 mg/l per plant, respectively) on the biochemical constituents and tuber quality of white yam (*Dioscorea rotundata* Poir.). The exposure of white yam plants to the fungicides showd increased chlorophyll, carotenoids, xanthophylls, and anthocyanin contents and altered the membrane integrity in terms of electrolytic leakage and lipid peroxidation. The triazole treatments enhanced the accumulation of proline and total phenols in tubers. The visible symptoms of fungicides appeared as thickening and darkening of leaves. Both the triazoles increased the antioxidants (ascorbic acid, reduced glutathione, and tocopherol) contents. The data suggests that, apart from their fungicidal properties, the application of triazole fungicides may be a useful tool to increase the tuber quality in yam plants.

Keywords: *Dioscorea rotundata;* triadimefon; hexaconazole; pigments; proline; total phenol; lipid peroxidation; electrolyte leakage; antioxidants

Roots and tubers are the most important food crops of very ancient origin in the tropics and subtropics, associated with the human existence, survival, and their socio-economic history. White yam (*Dioscorea rotundata* Poir.) is one of the important edible tuber crops cultivated in India and many other tropical countries including Africa. Yams are a valuable source of carbohydrates, fibres, and low level fats, which makes them a good dietary source (Panneerselvam *et al.* 2007). Yams also have medicinal properties. Several species of *Dioscorea* are amongst the principle sources of diosgenin, which can be converted to medicinally important steroids (Jaleel *et al.* 2007a).

The manipulation of the crop production with chemicals is one of the most important advancements achieved in agriculture. The tuber yields and qualities of the crop plants can be increased by the application of biofertilisers and plant growth regulators (Jaleel *et al.* 2007b, c). Triazoles are a group of compounds, which have fungicidal as well as plant growth regulatory properties (Jaleel *et al.* 2006a, b). Triazole compounds induce a variety of morphological and biochemical responses in plants including the retardation of shoot growth, stimulation of rooting, inhibition of gibberellin biosynthesis and increases in cytokinin and abscisic acid (Jaleel *et al.* 2007d). Triazole compounds have been shown to improve the yields of many root crops such as yam, carrot, tapioca, and Chinese potato (Kishorekumar *et al.* 2006, 2007; Gopi et al. 2007; Jaleel *et al.* 2007b, c, e; Manivannan *et al.* 2007). Triazoles inhibit cytochrome P-450 mediated oxidative demethylation reaction, which is necessary for the synthesis of ergosterol and the conversion of kaurene to kaurenoic acid in the gib-

berellin biosynthetic pathway and can affect the isoprenoid pathway and alter the levels of certain plant hormones by inhibiting gibberellin synthesis, reducing ethylene evolution and increasing cytokinin levels (Sankar *et al.* 2007). These qualities make them ideal for use in the edible root tuber cultivation. Some of the previous works carried out in our lab revealed the morphological and physiological changes associated with the triazole treatment in various plants, including the inhibition of the plant growth, decreased internodal elongation, increased chlorophyll levels, enlarged chloroplasts, thicker leaf tissue, increased root to shoot ratio, increased antioxidant potentials, and an enhancement in alkaloid production (Jaleel *et al.* 2006a, b, 2007a–g).

Carotenoids are a large class of isoprenoid molecules that are synthesised *de novo* by all photosynthetic and many non-photosynthetic organisms. The colours provided by these pigments are of an important agronomical value in horticultural crops (Naik *et al*. 2003). Anthocyanin pigments are widespread in the plant kingdom and provide many of the orange, red, and blue colours found in fruits, vegetables, flowers, leaves, roots, and other storage organs (Sudha & Ravishankar 2003). The role of proline in plants is related to the survival rather than to the maintenance of growth (Jaleel *et al.* 2007h). Electrolyte leakage is a common indicator of the membrane damage and this leakage is closely related to the loss of water potential (Iswari & Palta 1989). Cellular damage or oxidative injury arising from free radicals or reactive oxygen species (ROS) now appears to be the fundamental mechanism underlying a number of human diseases (Jaleel *et al.* 2007i). Free radicals can be scavenged through chemoprevention utilising natural antioxidant compounds present in foods and medicinal plants (Jaleel *et al.* 2007j). Ascorbic acid (AA) is a very important reducing substrate for H_2O_2 detoxification in photosynthetic organisms (Jaleel *et al.* 2007k). Reduced glutathione (GSH) is another most important non-enzymatic antioxidant molecule that functions as an effective ROS detoxifier (Jaleel *et al.* 2007l). α-Tocopherol (α-toc) was consumed predominantly as a radical scavenging antioxidant (Manivannan *et al.* 2008).

Many works have already been covered in different aspects of this plant (Panneerselvam *et al.* 2007; Jaleel *et al.* 2007a–c). But little attention has been drawn to the changes in tuber quality in terms of pigments, biochemicals, membrane integrity and antioxidant potentials in white yam under fungicide applications. The application of fungicides is a common practice in the cultivation of this plant. It is therefore seems important to test the changes that occur in this food crop under triazole treatment in order to identify the extent to which it tolerates the fungicide application and thereby makes it an economical food crop. Therefore, the present investigation has been carried out with an objective of evaluating the effect of triazole compounds like triadimefon (TDM) and hexaconazole (HEX) on pigments (chlorophylls, carotenoids, anthocyanin, xanthophylls), biochemical contents (proline, total phenol), membrane integrity (electrolytic leakage, lipid peroxidation), and antioxidant potential (ascorbic acid, reduced glutathione, ∞-tocopherol) of *D. rotundata* under field conditions.

MATERIALS AND METHODS

Plant material and triazole treatments. Tubers of *Dioscorea rotundata* (family *Dioscoreacae*) cv. Sree Priya were obtained from the Central Tuber Crop Research Institute (CTCRI), Kerala, India, and planted in the Botanical Garden of Annamalai University. The concentrations of 5 mg/l to 25 mg/l at 5 mg/l increments were tested initially for the growth and dry weight increase in white yam. Among these concentrations, 15 mg/l TDM and 10 mg/l HEX were found to increase the dry weight significantly while higher concentrations slightly decreased the growth and dry weight and increased leaf curling, consequently, 15 mg/l TDM and 10 mg/l HEX were used for the treatments. The treatments were applied by soil drenching on 40, 55, 70, and 85 days after planting (DAP). The plants were uprooted randomly on 45, 60, 75, and 90 DAP for analysing the pigment composition, membrane integrity, biochemical contents, and antioxidant potentials.

Estimation of chlorophyll and carotenoid contents. Chlorophyll and carotenoids were extracted from the leaves and estimated by the method of Arnon (1949). Five hundred milligrams of fresh leaf material was ground with 10 ml of 80% acetone at 4°C and centrifuged at 2500 rpm for 10 min at 4°C. This procedure was repeated until the residue became colourless. The extract was transferred to a graduated tube and made up to 10 ml with 80% acetone and assayed immediately.

Three-milliliter aliquots of the extract were transferred to a cuvette and the absorbance was read at 645, 663, and 480 nm with a spectrophotometer (U-2001-Hitachi) against 80% acetone as blank. The chlorophyll content was calculated using the formula of Arnon (given below) and expressed in milligrams per gram fresh weight.

Total chlorophyll (mg/ml) =
$$= (0.0202 \times A_{645}) + (0.00802 \times A_{663})$$

Chlorophyll *a* (mg/ml) =
$$= (0.0127 \times A_{663}) - (0.00269 \times A_{645})$$

Chlorophyll *b* (mg/ml) =
$$= (0.0229 \times A_{645}) - (0.00468 \times A_{663})$$

The carotenoid content was estimated using the formula of Kirk and Allen (1965) and expressed in milligrams per gram fresh weight.

$$\text{Carotenoid} = A_{480} + (0.114 \times A_{663} - 0.638 \times A_{645})$$

Estimation of anthocyanin content. Anthocyanin was extracted and estimated by the method of Kim *et al.* (2002). Using a pestle and mortar, five hundred mg of fresh tissue taken from the third leaf and from the periphery of the tuber tissue (0.5 cm from the epidermis and 1 cm from the head of the root tuber) was ground in liquid nitrogen and extracted with 20 ml of 50% acetic acid overnight. The homogenate was centrifuged at 19 000 g for 15 minutes. The resultant supernatant was made up to 20 ml and 80 ml of McIIvaine's buffer (pH 3.0) was added and the absorption was measured at 530 nm in a spectrophotometer (U-2001-Hitachi).

Estimation of xanthophyll content. The xanthophyll contents were estimated by the method of Neogy *et al.* (2001). Five hundred mg of fresh weight of tissues taken from the 3rd leaf and periphery of the tuber were used for the assay. The tissue was ground with 10 ml of 80% acetone at 4°C using a pestle and a mortar and centrifuged at 1000 rpm for 15 minutes. The residue was re-extracted with 80% acetone until the colour completely disappeared from the residue. The aqueous acetone extract was shaken thrice with an equal volume of hexane in a separating funnel and the combined hexane fractions were washed with equal volumes of water. To separate xanthophylls from carotenes, the hexane fraction containing carotenoid was extracted repeatedly with 90% methanol. The methanol fraction containing xanthophylls was measured for absorbance at 450 nm in a spectrophotometer.

Estimation of proline content. The PRO content was estimated by the method of Bates *et al.* (1973). The plant material was homogenised in 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 10 000 rpm. The supernatant was used for the estimation of the PRO content. The reaction mixture consisted of 2 ml of acid ninhydrin and 2 ml of glacial acetic acid, and was boiled at 100°C for 1 hour. After the termination of the reaction in an ice bath, the reaction mixture was extracted with 4 ml of toluene and the absorbance was read at 520 nm.

Estimation of total phenols content. Total phenol was estimated by the method of Malick and Singh (1980). 500 mg of fresh plant tissue was ground using a pestle and a mortar with 10 ml of 80% ethanol. The homogenate was centrifuged at 10 000 rpm for 20 minutes. The supernatant was evaporated to dryness. The residue was dissolved in 5 ml of distilled water and used as the extract. 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 in throughly. The mixture was kept in boiling water for exactly 1 min and after cooling the absorbance was read at 650 nm. The total phenol was determined using a standard curve prepared with different concentrations of gallic acid.

Measurement of electrolyte leakage. Leaf and tuber tissues electrolyte leakage was measured. One gram of tissue was cut into two-centimeter segments, rinsed in distilled water to remove the contents of cut cells, and placed in a test tube containing 15 ml of double distilled water at 24°C for 12 hours. The electrical conductivity of the solution in the tube was determined using a conductivity meter. The tube was then placed in a boiling water bath for 20 min after which it was cooled to 24°C and the electrical conductivity was measured. The percentage leakiness of the tissue was calculated as the ratio of the conductivity after 12 h to the conductivity after boiling (Pinhero & Fletcher 1994).

Measurement of lipid peroxidation. Peroxidation of the membrane lipids was assayed by the method of Heath & Packer (1968). The degradation of malonaldialdehyde (MDA) is a product of peroxidised lipids. The estimation of MDA can account for the lipid peroxidation. MDA reacts with thiobarbituric acid (TBA) to form TBA-MDA

chromophore proportional to the extent of peroxidation of lipids.Using a pestle and a mortar, 0.3 g of fresh tissue was homogenised in 5 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10 000 g for 5 minutes. Then 1 ml of supernatant was added to 4 ml of the reaction solution containing 0.5% TBA in 20% TCA and the mixture was incubated at 95°C for 30 minutes. The solution was allowed to cool and was then centrifuged at 1000 rpm for 2 min to remove the protein precipitated by TCA. The absorbance was read at 532 nm and adjusted for non-specific absorbance at 600 nm. MDA was estimated using the extinction coefficient of 155 ml/cm.

Estimation of ascorbic acid content. AA content was assayed as described by Omaye *et al.* (1979). The extract was prepared by grinding 1 g of fresh material with 5 ml of 10% TCA, centrifuged at 3500 rpm for 20 min, re-extracted twice, and the supernatant was made up to 10 ml and used for assay. To 0.5 ml of the extract, 1 ml of DTC reagent (2,4-dinitrophenyl hydrazine-thiourea-$CuSO_4$ reagent) was added, the mixture was incubated at 37°C for 3 h and then 0.75 ml of ice-cold 65% H_2SO_4 was added. The solution was allowed to stand at 30°C for 30 min, the resulting colour was read at 520 nm in a spectrophotometer. The AA content was determined using a standard curve prepared with AA.

Estimation of reduced glutathione content. The GSH content was assayed as described by Griffith and Meister (1979). 200 mg of fresh material was ground with 2 ml of 2% metaphosphoric acid and centrifuged at 17 000 rpm for 10 minutes. The following addition of 0.6 ml 10% sodium citrate neutralised the supernatant. 1 ml of the assay mixture was prepared by adding 100 µl extract, 100 µl distilled water, 100 µl 5,5-dithio-bis-(2-nitrobenzoic acid), and 700 µl NADPH. The mixture was stabilised at 25°C for 3–4 minutes. Then 10 µl of glutathione reductase was added, and the absorbance was read at 412 nm in a spectrophotometer.

Estimation of α-tocopherol content. The α-toc content was assayed as described by Backer *et al.* (1980). 500 mg of fresh tissue was homogenised 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 min, the supernatant was used for the estimation of α-toc. To one ml of the extract, 0.2 ml of 2% 2,2-dipyridyl in ethanol was added, the resulting solution was mixed thoroughly and kept in the dark for 5 min. The red coloured product 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-toc content was calculated using a standard graph made with known amounts of α-toc.

Statistical analysis. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The values are mean ± SD for six samples in each group. *P* values ≤ 0.05 were considered as significant.

RESULTS AND DISCUSSION

Under triazole treatments, increased chlorophyll contents were found in the leaves of yam plants at

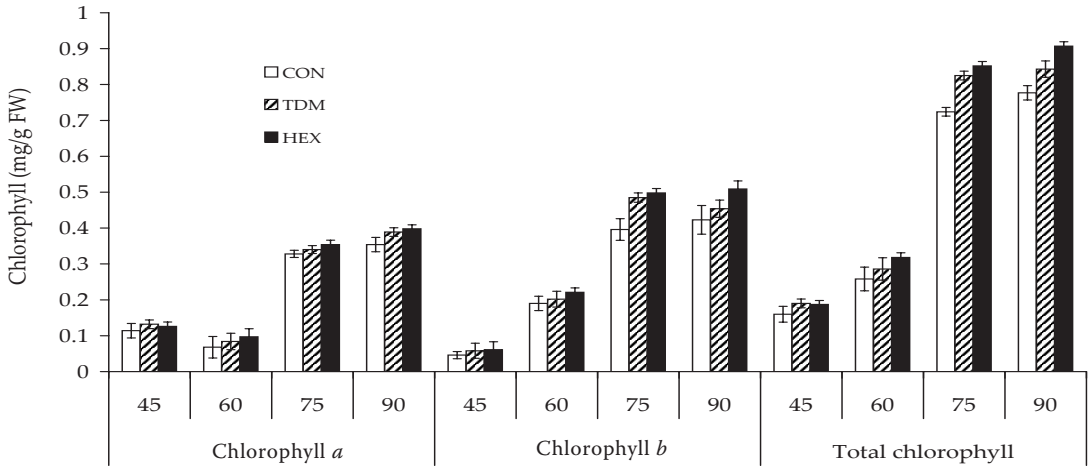

Figure 1. Differential effects of triadimefon (TDM) and hexaconazole (HEX) on the chlorophyll contents of *Dioscorea rotundata* Poir. at different growth stages. Values are given as mean ± SD of six samples in each group. Bar values not sharing a common superscript (a, b, c) differ significantly at $P \leq 0.05$ (DMRT)

different growth stages when compared to those in untreated plants (Figure 1a). There was no significant variation in the chlorophyll content within the triazole treated plants, however, the content was increased when compared to control. An increase in the chlorophyll contents was previously reported in TDM treated plants. The increase in the chlorophyll content due to the triazole treatment is attributed to the ability of triazoles to increase the cytokinin level and thereby the stimulation of the chlorophyll biosynthesis (Jaleel *et al.* 2007g, 2008b).

The carotenoids content increased in the triazole treated yam plants when compared to control plants (Figure 2a). Of the triazole treatments, the TDM treated plants showed higher carotenoid contents in tubers, whereas HEX proved to be best for anthocyanins (Figure 2c). Triazole compounds lead to alterations in many of the plant metabolites and pigments (Jaleel *et al.* 2008a, b). The treatment with ABA increased anthocyanin accumulation in strawberry fruits (Jiang & Joyce 2003). Triazole induced a transient raise in ABA content and this increased ABA content induced by triazole might be the cause for the increased anthocyanin content (Gopi *et al.* 2007). Triazole treated yam leaves showed increased xanthophyll contents at all stages of the growth (Figure 2b). Xanthophyll participates in light harvesting in photosynthetic machinery and protects the photosynthetic apparatus from excessive light energy by quenching chlorophylls and singlet oxygen (Manivannan *et al*. 2007, 2008).

The proline content of yam shoots and tubers increased under the triazole treatments as compared to control. The HEX treatment resulted in higher amounts of proline when compared to the TDM treatment (Figure 3a). Triazole induced a transient rise in the ABA content and this increased ABA content due to the triazole treatment could

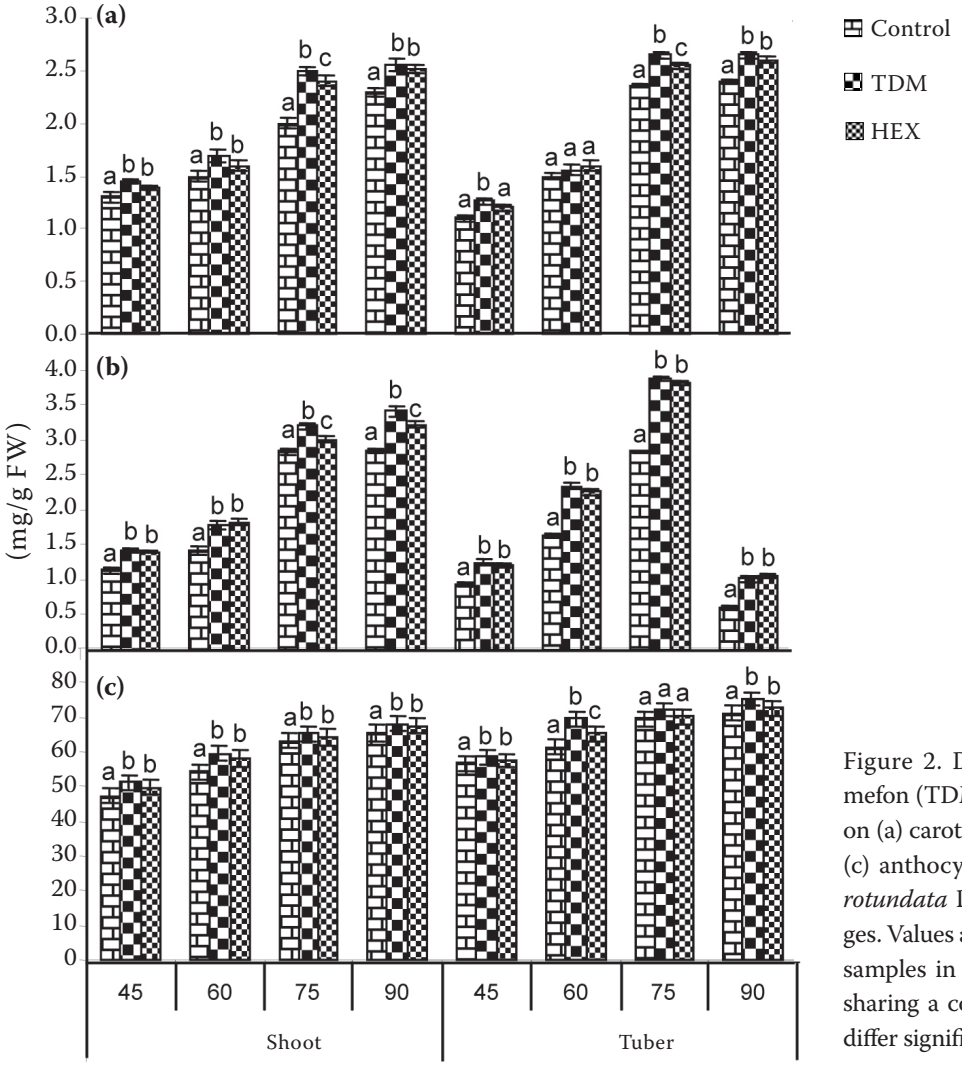

Figure 2. Differential effects of triadimefon (TDM) and hexaconazole (HEX) on (a) carotenoids, (b) xanthophylls and (c) anthocyanin contents of *Dioscorea rotundata* Poir. at different growth stages. Values are given as mean ± SD of six samples in each group. Bar values not sharing a common superscript (a, b, c) differ significantly at $P \leq 0.05$ (DMRT)

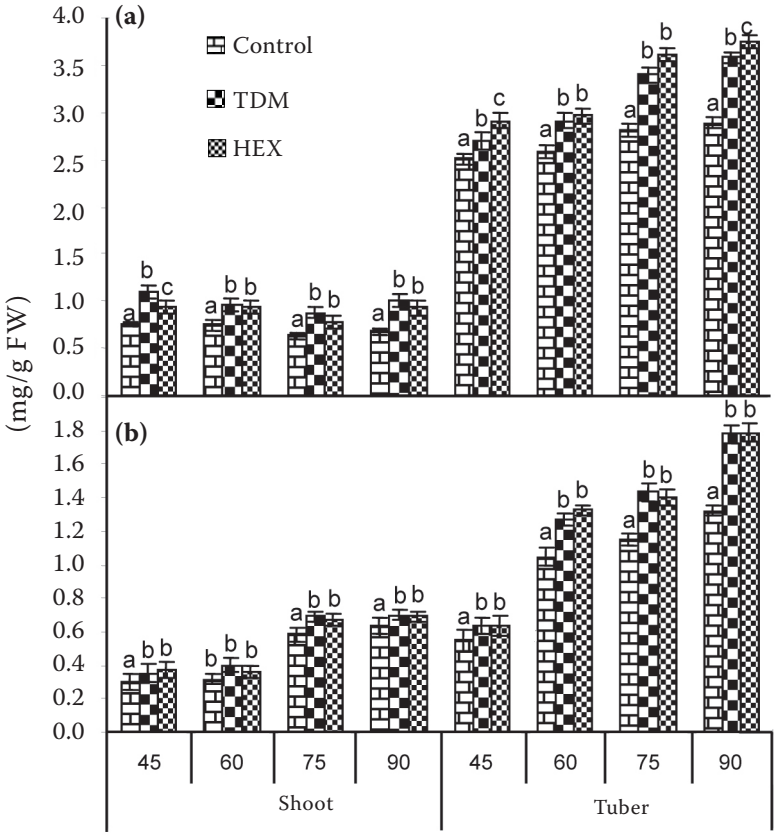

Figure 3. Differential effects of triadimefon (TDM) and hexaconazole (HEX) on (a) proline and (b) total phenol contents of *Dioscorea rotundata* Poir. at different growth stages. Values are given as mean ± SD of six samples in each group. Bar values not sharing a common superscript (a, b, c) differ significantly at $P \leq 0.05$ (DMRT)

be the reason for the increased proline content in the triazole treated yam plants. The total phenol content (Figure 3b) increased with the triazole treatment both in the shoot and the tuber at all stages of growth. Phenolics are oxidised to phenoxyl radicals (Lakshmanan *et al.* 2007). This phenoxyl radical reduces the AA into monodehydroascorbate. Phenolic constituents of plants have an anti-oxidant

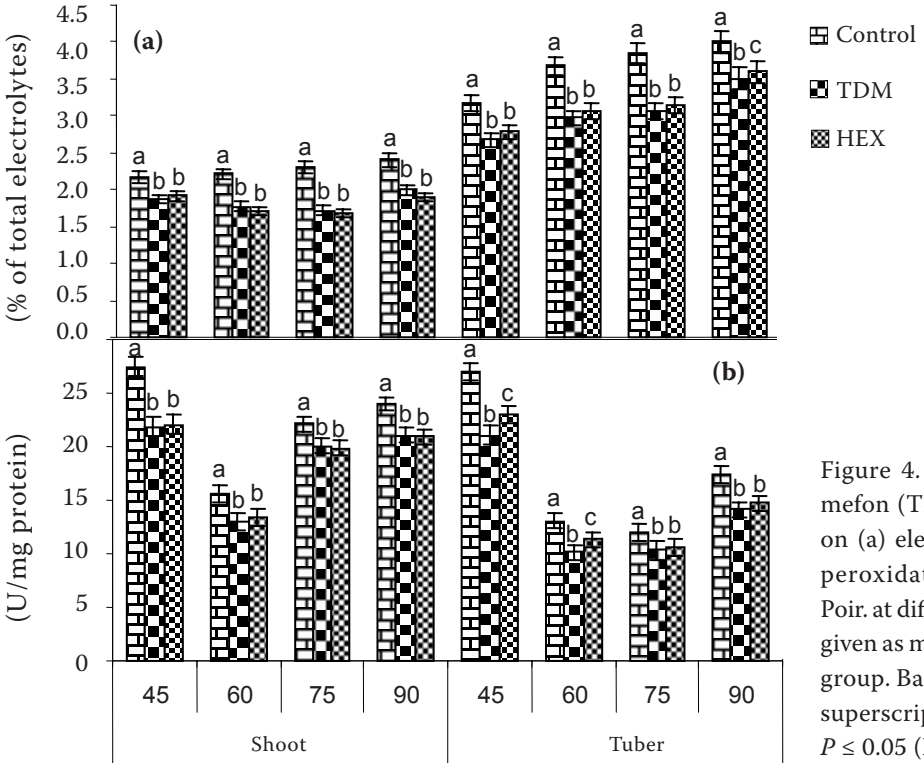

Figure 4. Differential effects of triadimefon (TDM) and hexaconazole (HEX) on (a) electrolytic leakage and (b) lipid peroxidation of *Dioscorea rotundata* Poir. at different growth stages. Values are given as mean ± SD of six samples in each group. Bar values not sharing a common superscript (a, b, c) differ significantly at $P \leq 0.05$ (DMRT)

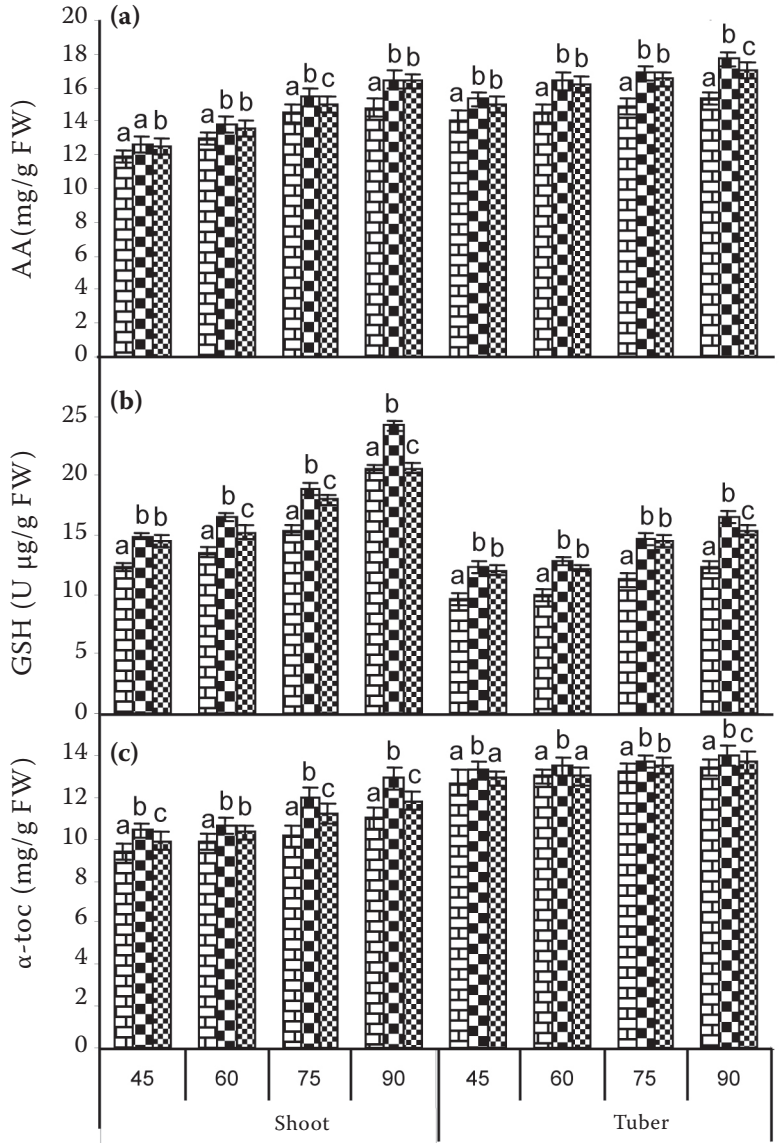

Figure 5. Differential effects of triadimefon (TDM) and hexaconazole (HEX) on (a) ascorbic acid, (b) reduced glutathione and (c) α-tocopherol contents of *Dioscorea rotundata* Poir. at different growth stages. Values are given as mean ± SD of six samples in each group. Bar values not sharing a common superscript (a, b, c) differ significantly at $P \leq 0.05$ (DMRT)

activity and offer protection against oxidative damage (Rice-Evans *et al.* 1997). The increased oxidation of phenolics due to the fungicide application may therefore contribute to the acceleration of the oxidative damage.

Electrolyte leakage (Figure 4a) and lipid peroxidation (Figure 4b) were inhibited by the triazoles treatment in yam plants when compared to control. In the tuber tissues, the electrolyte leakage decrease due to the TDM and HEX treatments when compared to that in the untreated plants. Out of the triazoles used for the plant treatment, HEX performed best in decreasing the electrolyte leakage and lipid peroxidation when compared to TDM treated plants. The inhibition of electrolyte leakage and lipid peroxidation is in agreement with the proved ability of triazoles in maintaining the plant membrane integrity. Triazoles altered the sterol biosynthesis and changed the composition of sterol in the plasma membrane. The changes in the sterol composition may induce changes in the cell membrane that may be reflected in an increased membrane stability (Burden *et al.* 1987; Gomathinayagam *et al.* 2007). TDM and HEX might have facilitated the increased membrane stability in yam due to the altered sterol composition, removal of damage area in the membrane, and increased kinetin contents.

The TDM and HEX treatments increased the levels of AA (Figure 5a), GSH (Figure 5b), and α-toc (Figure 5c) in the leaves and tubers of yam. AA is an important component of the plant antioxidant system (Jaleel *et al.* 2007j). AA is one of the major soluble antioxidants in chloroplasts

(Jaleel *et al.* 2007m). AA also has photo-protective function because of its antioxidant capacity. Triazoles increased the levels of antioxidants like AA and protected the membrane by preventing or reducing oxidative damage (Jaleel *et al.* 2008b). Glutathione has been shown to regulate the expression of genes whose products are involved in redox regulation and stress tolerance enhancement (Jaleel *et al.* 2007l). α-Toc is the most abundant form of antioxidants in nature (Jaleel *et al.* 2007n). α-Toc has antioxidant, cell signaling, and vitamin E functions. It acts as a chain breaking antioxidant, preventing the propagation of free radical reactions (Jaleel *et al.* 2007o). The increase in α-toc level in the triazole treated yam can increase the antioxidant potential in the leaves and tuber tissues. Enhanced GSH levels along with the interaction of AA and tocopherol may increase the oxidative stress tolerance resulting from triazole treatments in yam. Therefore, based on the present results, it can be concluded that the triazole compounds like HEX and TDM could be used for increasing the yield and tuber quality of yam plants under cultivation.

References

Arnon D.I. (1949): Copper enzymes in isolated chloroplasts, polyphenol oxidase in *Beta vulgaris* L. Plant Physiology, **24**: 1–15.

Backer H., Frank O., De Angells B., Feingold S. (1980): Plasma tocopherol in man at various times after ingesting free or ocetylaned tocopherol. Nutrition Reports International, **21**: 531–536.

Bates L.S., Waldern R.P., Teare I.D. (1973): Rapid determination of free proline for water stress studies. Plant and Soil, **39**: 205–207.

Burden R.S., James C.S., Cooke D.T., Anderson N.H. (1987): C-14 Demethylation in phytosterol biosynthesis – a new target site for herbicidal activity. In: Proceedings Brazilian Crop Protection Conference for Weeds, 3B-4: 171–178.

Gomathinayagam M., Jaleel C.A., Lakshmanan G.M.A., Panneerselvam R. (2007): Changes in carbohydrate metabolism by triazole growth regulators in cassava (*Manihot esculenta* Crantz); effects on tuber production and quality. Comptes Rendus Biologies, **330**: 644–655.

Gopi R., Jaleel C.A., Sairam R., Lakshmanan G.M. A., Gomathinayagam M., Panneerselvam R. (2007): Differential effects of hexaconazole and paclobutrazol on biomass, electrolyte leakage, lipid peroxidation and antioxidant potential of *Daucus carota* L. Colloids and Surfaces B: Biointerfaces, **60**: 180–186.

Griffith O.W., Meister A. (1979): Potent and specific inhibition of glutathione synthesis by buthionine sulfoximine (s-n-butylhomocysteine sulfoximine). Journal of Biological Chemistry, **254**: 7558–7560.

Heath R.L., Packer L. (1968): Photoperoxidation in isolated chloroplast I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics, **125**: 189–198.

Iswari S., Palta J.P. (1989): Plasma membrane ATPase activity following reversible and irreversible freezing injury. Plant Physiology, **90**: 1088–1095.

Jaleel C.A., Gopi R., Lakshmanan G.M.A., Panneerselvam R. (2006a): Triadimefon induced changes in the antioxidant metabolism and ajmalicine production in *Catharanthus roseus* (L.) G. Don. Plant Science, **171**: 271–276.

Jaleel C.A., Gopi R., Manivannan P., Kishorekumar A., Sankar B., Panneerselvam R. (2006b): Paclobutrazol influences on vegetative growth and floral characteristics of *Catharanthus roseus* (L.) G. Don. Indian Journal of Applied and Pure Biology, **21**: 369–372.

Jaleel C.A., Gopi R., Manivannan P., Kishorekumar A., Gomathinayagam M., Panneerselvam R. (2007a): Changes in biochemical constituents and induction of early sprouting by triadimefon treatment in white yam (*Dioscorea rotundata* Poir.) tubers during storage. Journal of Zhejiang University Science: B, **8**: 283–288.

Jaleel C.A., Manivannan P., Gomathinayagam M., Sridharan R., Panneerselvam R. (2007b): Responses of antioxidant potentials in *Dioscorea rotundata* Poir. following paclobutrazol drenching. Comptes Rendus Biologies, **330**: 798–805.

Jaleel C.A., Kishorekumar A., Manivannan P., Sankar B., Gomathinayagam M., Gopi R., Somasundaram R., Panneerselvam R. (2007c): Alterations in carbohydrate metabolism and enhancement in tuber production in white yam (*Dioscorea rotundata* Poir.) under triadimefon and hexaconazole applications. Plant Growth Regulation, **53**: 7–16.

Jaleel C.A., Gopi R., Manivannan P., Panneerselvam R. (2007d): Responses of antioxidant defense system of *Catharanthus roseus* (L.) G. Don. to paclobutrazol treatment under salinity. Acta Physiologiae Plantarum, **29**: 205–209.

Jaleel C.A., Manivannan P., Sankar B., Kishorekumar A., Gopi R., Somasundaram R., Panneerselvam R. (2007e): Induction of drought stress tolerance by ketoconazole in *Catharanthus roseus* is mediated by enhanced antioxidant potentials and secondary

metabolite accumulation. Colloids and Surfaces B: Biointerfaces, **60**: 201–206.

Jaleel C.A., Manivannan P., Sankar B., Kishorekumar A., Sankari S., Panneerselvam R. (2007f): Paclobutrazol enhances photosynthesis and ajmalicine production in *Catharanthus roseus*. Process Biochemistry, **42**: 1566–1570.

Jaleel C.A., Gopi R., Panneerselvam R. (2007g): Alterations in lipid peroxidation, electrolyte leakage, and proline metabolism in *Catharanthus roseus* under treatment with triadimefon, a systemic fungicide. Comptes Rendus Biologies, **330**: 905–912.

Jaleel C.A., Gopi R., Sankar B., Manivannan P., Kishorekumar A., Sridharan R., Panneerselvam R. (2007h): Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress. South African Journal of Botany, **73**: 190–195.

Jaleel C.A., Manivannan P., Lakshmanan G.M.A., Sridharan R., Panneerselvam R. (2007i): NaCl as a physiological modulator of proline metabolism and antioxidant potential in *Phyllanthus amarus*. Comptes Rendus Biologies, **330**: 806–813.

Jaleel C.A., Gopi R., Manivannan P., Panneerselvam R. (2007j): Antioxidative potentials as a protective mechanism in *Catharanthus roseus* (L.) G. Don. plants under salinity stress. Turkish Journal of Botany, **31**: 245–251.

Jaleel C.A., Manivannan P., Sankar B., Kishorekumar A., Gopi R., Somasundaram R., Panneerselvam R. (2007k): Water deficit stress mitigation by calcium chloride in *Catharanthus roseus*; effects on oxidative stress, proline metabolism and indole alkaloid accumulation. Colloids and Surfaces B: Biointerfaces, **60**: 110–116.

Jaleel C.A., Gopi R., Manivannan P., Sankar B., Kishorekumar A., Panneerselvam R. (2007l): Antioxidant potentials and ajmalicine accumulation in *Catharanthus roseus* after treatment with giberellic acid. Colloids and Surfaces B: Biointerfaces, **60**: 195–200.

Jaleel C.A., Manivannan P., Kishorekumar A., Sankar B., Panneerselvam R. (2007m): Calcium chloride effects on salinity induced oxidative stress, proline metabolism and indole alkaloid accumulation in *Catharanthus roseus*. Comptes Rendus Biologies, **330**: 674–683.

Jaleel C.A., Manivannan P., Kishorekumar A., Sankar B., Gopi R., Somasundaram R., Panneerselvam R. (2007n): Alterations in osmoregulation, antioxidant enzymes and indole alkaloid levels in *Catharanthus roseus* exposed to water deficit. Colloids and Surfaces B: Biointerfaces, **59**: 150–157.

Jaleel C.A., Panneerselvam R. (2007o): Variations in the antioxidative and indole alkaloid status in different parts of two varieties of *Catharanthus roseus*, an important folk herb. Chinese Journal of Pharmacology and Toxicology; **21:** 487–494.

Jaleel C.A., Gopi R., Manivannan P., Gomathinayagam M., Murali P.V., Panneerselvam R. (2008a): Soil applied propiconazole alleviates the impact of salinity on *Catharanthus roseus* by improving antioxidant status. Pesticide Biochemistry and Physiology, **90**: 135–139.

Jaleel C.A., Lakshmanan G.M.A., Gomathinayagam M., Panneerselvam R. (2008b): Triadimefon induced salt stress tolerance in *Withania somnifera* and its relationship to antioxidant defense system. South African Journal of Botany, **74**: 126–132.

Jiang Y., Joyce D.C. (2003): ABA effects on ethylene production, PAL activity, anthocyanin and phenolic contents of strawberry fruit. Plant Growth Regulation, **39**: 171–174.

Kim H.S., Mizuno K., Sawada S., Fujimura T. (2002): Regulation of tuber formation and ADP-glucose pyrophosphorylase (AGPase) in sweet potato (*Ipomoea batatas* (L.) Lam.) by nitrate. Plant Growth Regulation, **37**: 207–213.

Kirk J.T., Allen R.L. (1965): Dependence of pigment synthesis on protein synthesis. Biochemical and Biophysical Research Communications, **21**: 523–530.

Kishorekumar A., Jaleel C.A., Manivannan P., Sankar B., Sridharan R., Somasundaram R., Panneerselvam R. (2006): Differential effects of hexaconazole and paclobutrazol on the foliage characteristics of Chinese potato (*Solenostemon rotundifolius* Poir., J.K. Morton). Acta Biologica Szegediensis, **50**: 127–129.

Kishorekumar A., Jaleel C.A., Manivannan P., Sankar B., Sridharan R., Panneerselvam R. (2007): Comparative effects of different triazole compounds on growth, photosynthetic pigments and carbohydrate metabolism of *Solenostemon rotundifolius*. Colloids and Surfaces B: Biointerfaces, **60**: 207–212.

Lakshmanan G.M.A., Jaleel C.A., Gomathinayagam M., Panneerselvam R. (2007): Changes in antioxidant potential and sink organ dry matter with pigment accumulation induced by hexaconazole in *Plectranthus forskholii* Briq. Comptes Rendus Biologies, **330**: 814–820.

Malick C.P., Singh M.B. (1980): Plant Enzymology and Histoenzymology. Kalyani Publishers, New Delhi.

Manivannan P., Jaleel C.A., Kishorekumar A., Sankar B., Somasundaram R., Sridharan R., Panneerselvam R. (2007): Changes in antioxidant metabolism of *Vigna unguiculata* (L.) Walp. by propiconazole under water deficit stress. Colloids and Surfaces B: Biointerfaces, **57**: 69–74.

Manivannan P., Jaleel C.A., Kishorekumar A., Sankar B., Somasundaram R., Panneerselvam R. (2008): Protection of *Vigna unguiculata* (L.) Walp. plants from salt stress by paclobutrazol. Colloids and Surfaces B: Biointerfaces, **61**: 315–318.

Naik P.S., Chanemougasoundharam A., Khurana S.M.P., Kalloo G. (2003): Genetic manipulation of caroteniod pathway in higher plants. Current Science, **85**: 1423–1430.

Neogy M., Datta J.K., Mukherji S., Roy A.K. (2001): Effect of aluminium on pigment content, hill activity and seed yield in mungbeen. Indian Journal of Plant Physiology, **6**: 381–385.

Omaye S.T., Turnbull J.D., Sauberilich H.E. (1979): Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Methods in Enzymology, **1**: 3–11.

Panneerselvam R., Jaleel C.A., Somasundaram R., Sridharan R., Gomathinayagam M. (2007): Carbohydrate metabolism in *Dioscorea esculenta* (Lour.) Burk. tubers and *Curcuma longa* L. rhizomes during two phases of dormancy. Colloids and Surfaces B: Biointerfaces, **59**: 59–66.

Pinhero R.G., Fletcher R.A. (1994): Paclobutrazol and ancymidol protect corn seedlings from high and low temperature stresses. Plant Growth Regulation, **15**: 47–53.

Rice-Evans C., Miller N.J., Papanga G. (1997): Antioxidant properties of phenolic compounds. Trends in Plant Science, **2**: 152–159.

Sankar B., Jaleel C.A., Manivannan P., Kishorekumar A., Somasundaram R., Panneerselvam R. (2007): Effect of paclobutrazol on water stress amelioration through antioxidants and free radical scavenging enzymes in *Arachis hypogaea* L. Colloids and Surfaces B: Biointerfaces, **60**: 229–235.

Sudha G., Ravishankar G.A. (2003): Elicitation of anthocyanin production in callus cultures of *Daucus carota* and involvement of calcium channel modulators. Current Science, **58**: 775–779.

Received for publication September 9, 2007
Accepted after corrections January 19, 2008

Corresponding author:

Dr. Ragupathi Gopi, Annamalai University, Department of Botany, Annamalainagar 608 002, Tamil Nadus, India
tel.: + 91 41 44 238 248/354, fax: + 91 41 44 222 265, e-mail: abdul79jaleel@rediffmail.com

The European Food Safety Authority (EFSA)
invites leading scientists to sign up its new expert database

EFSA, which is the European Union's scientific risk assessment body on food and feed safety, nutrition, animal welfare and plant protection and health, is launching a database for Europeans leading scientific experts to assist it in its work. EFSA is inviting scientific experts to sign up its new expert database, which will be used to assist its Scientific Committee and Panels in their risk assessment activities. This open invitation to scientific experts is being made within the context of EFSA's strengthened policy for the selection of scientific experts to assist EFSA with its scientific work. EFSA, in cooperation with EU Member States, has decided to set up a database of external scientific experts in order to develop a more effective and flexible response to its growing workload. In addition, one of the main objectives of setting up this database is to enhance the transparency of the process through which experts are selected and invited to participate in EFSA's scientific activities.

Experts included in the database may be invited for one of two types of assignments: (1) EFSA assignments, where a nature of a tasks may be a provision of scientific advice to EFSA's Scientific Committee, Scientific Panels, EFSA's networks (Advisory Forum, Focal Points, Zoonoses Task Force, amongst others) and related working groups; and (2) assignments on EU Member States' own scientific projects (when experts give their consent for their profile information to be shared with Member State National Food Safety Agencies). In accordance with existing EFSA rules, those experts selected from the database will be invited to a certain scientific activity. Scientific experts participated in this activity, will receive traveling and subsistence expenses and an indemnity for their contribution to EFSA's work.

External scientific experts with the required expertise could be invited (a) to an *ad hoc* basis, to attend a single meeting or for the duration of the work on a specific mandate or project; or (b) to a longer term, when the required expertise for more than one mandate or project is needed.

Interested experts from the Czech Republic will fill out an individual on line application form available at EFSA's website: *efsa.europa.eu*.