

## Triazole Induced Alterations in the Peroxidation of Membrane Lipids and Antioxidant Status of *Manihot esculenta* Crantz

<sup>1</sup>M. Gomathinayagam, <sup>2,3</sup>M.M. Azooz, <sup>1</sup>Cheruth Abdul Jaleel and <sup>1</sup>R. Panneerselvam

<sup>1</sup>Stress Physiology Lab, Department of Botany,  
Annamalai University, Annamalainagar 608 002, Tamilnadu, India

<sup>2</sup>Department of Botany, Faculty of Science, South Valley University, 83523 Qena, Egypt

<sup>3</sup>Department of Biology, Faculty of Science, King Faisal University,  
P.O. Box: 380, Al-Hassa 31982, Saudi Arabia

**Abstract:** The main objectives of this study were to assess the effect of Triadimefon and Hexaconazole on the non-enzymatic antioxidant potentials of *Manihot esculenta* Crantz during the growth and maturation period. One litre of 20 mg L<sup>-1</sup> triadimefon and 15mg L<sup>-1</sup> hexaconazole solution per plant was used for the treatment and control was treated with one litre of irrigation water. The treatment was given on 25,45,65 and 100 DAP by soil drenching. Plants were harvested randomly on 40, 80, 120, 160, 200 and 240 DAP and separated into stem, leaf, root and tuber was used for determining antioxidant status. From these results it is clear that triadimefon, hexaconazole can be used as enhancer for non-enzymatic antioxidant potentials of *Manihot esculenta*.

**Key words:** Lipid Peroxidation, Ascorbic acid,  $\alpha$ -Tocopherol, Triadimefon, Hexaconazole, *Manihot esculenta*

### INTRODUCTION

Number of natural and synthetic substances like Auxins, Gibberellins, Abscissic acid, Ethylene, Cytokinin, Brassinosteroids, Terpenoids, Aliphatic alcohols, Polyamines and Triazoles have growth regulating properties in various plants. These substances induce specific responses in specific plants [1]. Chemicals like Lunaric acid, Batasins, Jasmonic acid, Turgorins, Tuberonic acid and Cucurbitic acids induce specific responses in specific plants. The responses of plant growth regulators may vary with plant species, variety, age, plant, environmental conditions, physiological and nutritional status, stage of development and endogenous hormonal balance [2]. Plant growth retardants are widely used to modify canopy structure, yield and stress tolerance in many crop plants [3].

Triazole compounds are mostly used as the systemic fungicides to control fungal diseases in plants and animals. They inhibit gibberellin biosynthesis and modify the sterol metabolism in the host and parasite organism. They also have plant growth regulating properties and effectively retard the shoot growth in monocotyledons and dicotyledons. [4].

The tuber crops become the most important food crop after cereals and legumes. They form a rich source of energy for people living near sustenance level in Tropical East and West Africa, East and South Pacific islands and part of South America and India [5-7]. Tapioca (*Manihot esculenta* Crantz) also known as Cassava, Mandioca and Yucca is a bushy shrub belongs to the family Euphorbiaceae is an important food crop grown throughout the tropics for its enlarged tuberous roots [8]. The tubers are used for sago industry for starch extraction and grown in rainfed areas where a number of sago and starch mills exist. In addition the boiled tubers are consumed as staple food. The leaves of Tapioca are rich in protein serving as an excellent cattle feed. It is an important daily source of starch for 300 – 600 million of the people around the world. The tuber contains 30 to 35 % starch and appreciable amount of calcium and vitamin-C [8].

Triazole compounds increase the fresh and dry weight of tubers and alter the partitioning by increasing the translocation of photosynthates to the tuber [6,8,9]. It also influence the carbohydrate and antioxidant metabolism in various plants [10-12]. The enhancement of yield in tuber crops like tapioca will be beneficial to

the farmers if right type of triazole compound and concentration is determined by experiments. A lot of work has been done on the effect of triazole compounds on stress protection in various plants. However, work on increasing the growth, yield and modification of antioxidant potential using triazole compounds in tuber crops like tapioca is scanty. Hence, a study becomes essential to evaluate the effect of triazole compounds on the growth and metabolism of Tapioca. In this study, it is proposed to evaluate the effect of triazole compounds viz. Triadimefon and Hexaconazole on growth and metabolism of *Manihot esculenta* Crantz.

The main objectives of this study are to assess the effect of Triadimefon and Hexaconazole on the non-enzymatic antioxidant potentials of *Manihot esculenta* Crantz during the growth and maturation period.

## MATERIALS AND METHODS

The land was prepared by ploughing thoroughly five times to a depth of 35 cm and the soil was sandy loam without any stones and pebbles. The Farm yard manure (FYM) was applied at the rate of 10 tonnes per hectare. The stem cuttings of *Manihot esculenta* Crantz. (Tapioca) CV-H-226 were obtained from Tamil Nadu Agricultural University (TNAU) India. The stem cuttings of uniform thickness having three nodes were used for planting. The stem cuttings are dipped for 10 minutes in 1% Bavestin before planting to avoid fungal infections. Each stem cuttings was planted in a plot of  $1.5 \times 1.5$  to a depth of 5 cm inside the soil and Completely Randomized Block Design (CRBD) was used for this experiment.

No inorganic fertilizer was used throughout the experiment and no systemic pesticide or fungicide was used during the experiment. Only ground water was used for irrigation. In preliminary experiments, 5, 10, 15, 20, 25 and  $30 \text{ mg L}^{-1}$  triadimefon and hexaconazole were used for treatment to determine the optimum concentration of triadimefon and hexaconazole.

Among these treatments,  $20 \text{ mg L}^{-1}$  triadimefon and  $15 \text{ mg L}^{-1}$  hexaconazole concentrations were found to increase the dry weight significantly and in higher concentrations they slightly decreased the growth and dry weight. Hence  $20 \text{ mg L}^{-1}$  triadimefon and  $15 \text{ mg L}^{-1}$  hexaconazole concentrations were used to determine the effect of these chemicals on the growth and metabolism of tapioca. One litre of  $20 \text{ mg L}^{-1}$  triadimefon and  $15 \text{ mg L}^{-1}$  hexaconazole solution per plant was used for the treatment and control was treated with one litre of irrigation water. The treatment was given on 25,45,65 and

100 DAP by soil drenching. The EC of the soil was  $0.21 \text{ dSm}^{-1}$  and pH was 6.8 after the treatment. The average temperature was  $32/26^\circ\text{C}$  (maximum and minimum) and relative humidity (RH) varied between 60-75 percent during the experimental period.

Triadimefon [1-(4-chlorophenoxy)-3,3-dimethyl-1H-1,2,4-triazole-5-yl]-2-butanone [ $\text{C}_{14}\text{H}_{16}\text{ClN}_3\text{O}_2$ ] M.W. 293.75 has been obtained from Bayer India Ltd., Mumbai and Hexaconazole (2-(2,4-dichlorophenyl)-1H-1,2,4-triazole-5-yl)hexan-2-ol [ $\text{C}_{14}\text{H}_{17}\text{Cl}_2\text{N}_3\text{O}$ ] M. W. 314.2 has been obtained from Rallis India Ltd., Mumbai used for this study. Plants were harvested randomly on 40, 80, 120, 160, 200 and 240 DAP and separated into stem, leaf, root and tuber was used for determining antioxidant status.

**Statistical Analysis:** The data was analysed using the analysis of variance (ANOVA) as described by the method outlined by Ridgman [13]. Means were compared between treatments from the error mean square by LSD (Least Significant Difference) at the  $P = 0.05$  and  $P = 0.01$  confidence level using Tuckey's [14] test.

**Lipid Peroxidation:** Peroxidation of membrane lipids was assayed by the method of Heath and Packer [15].

Degradation of malonaldehyde (MDA) is a product of peroxidised lipids. Estimation of MDA can account for the lipid peroxidation. MDA reacts with TBA (thiobarbituric acid) to form TBA-MDA chromophore proportional to the extent of peroxidation of lipids.

Using a pestle and mortar fresh tissue 0.3 g of was homogenized in 5 ml of 0.1 % trichloroacetic acid. The homogenate was centrifuged at 10,000 g for 5 minutes. Then 1ml of supernatant was added to 4 ml of reaction solution containing 0.5% thiobarbituric acid (TBA) in 20% TCA and incubated at  $95^\circ\text{C}$  for 30 minutes. The solution was allowed to cool and centrifuged at 1000g for 2 minutes to remove the protein precipitated by TCA. Absorbance was read at 532 nm and adjusted for non specific absorbance at 600nm. MDA was estimated using extinction coefficient of  $155 \text{ mL}^{-1} \text{ cm}^{-1}$ .

### Antioxidants

**Ascorbic Acid:** Ascorbic acid was assayed as described by Omaye *et al.* [16].

**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 g for 20 minutes. The pellet was re-extracted twice with 10 per cent TCA and supernatant was made to 10 ml and used as extract.

**Estimation:** To 0.5 ml of extract, 1 ml of DTC 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<sub>2</sub>SO<sub>4</sub> was added. The tubes were then allowed to stand at 30°C for 30 minutes. The resulting colour was read at 520 nm in a Spectrophotometer. The ascorbic acid content was determined using a standard curve prepared with ascorbic acid and the results were expressed in mg per gram dry weight.

**a-Tocopherol:**  $\alpha$ -tocopherol activity was assayed as described by Baker *et al.* [17].

**Extraction:** 0.5 grams of fresh tissue was homogenised with 10 ml of a mixture of petroleum ether: ethanol (2:1.6 v/v) and extract was centrifuged at 10,000 g for 20 minutes and supernatant was used for the estimation of  $\alpha$ -tocopherol.

**Estimation:** To 1 ml of extract 0.2 ml of 2 per cent 2, 2 dipyridial in ethanol was added and mixed thoroughly and kept in dark for 5 minutes. The resulting red colour developed was diluted with 4 ml of distilled water and mixed well. The resulting colour in the aqueous layer was measured at 520 nm. The  $\alpha$ -tocopherol content was calculated using a standard graph made with known amount of  $\alpha$ -tocopherol.

## RESULTS AND DISCUSSION

### Lipid Peroxidation

**Leaf (Fig. 1):** The Lipid peroxidation in the leaf cells was inhibited by the triazole treatment. The inhibition of lipid peroxidation was higher in triadimefon and hexaconazole treated plants and it was 67.88 and 64.8 on 240 DAP.

**Tuber (Fig. 2):** Triazole treatment significantly inhibited the lipid peroxidation in the tuber and it was 82.40 and 79.61 percent as compared to control in triadimefon and hexaconazole treated plants on 240 DAP.

Triadimefon and hexaconazole treated tapioca plants showed a lower level of lipid peroxidation when compared to control. Malonodialdehyde (MDA) a product of lipid peroxidation damages enzymes and plant membranes as observed in *Daucus carota* tubers [9]. The lipid peroxidation is a consequence of higher oxidative stress [18]. Uniconazole reduced the electrolyte leakage and MDA accumulation and consequently decreased the heat induced lipid peroxidation in rape plants [19].

### Antioxidants

#### Ascorbic Acid

**Leaf (Table. 1):** The ascorbic acid content increased with the age in the leaves of the control and treated plants.

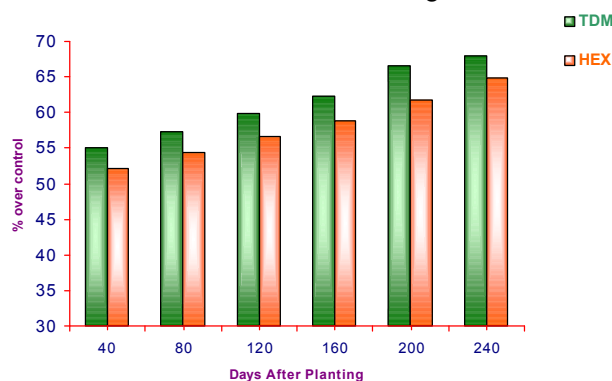


Fig. 1: Triazoles induced changes in the lipid peroxidation in the leaves of Tapioca

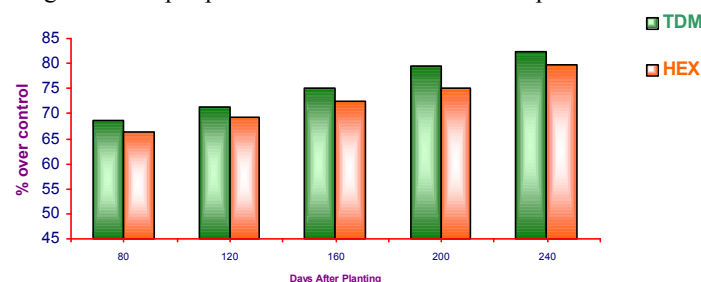


Fig. 2: Triazoles induced changes in the lipid peroxidation in the tubers of Tapioca

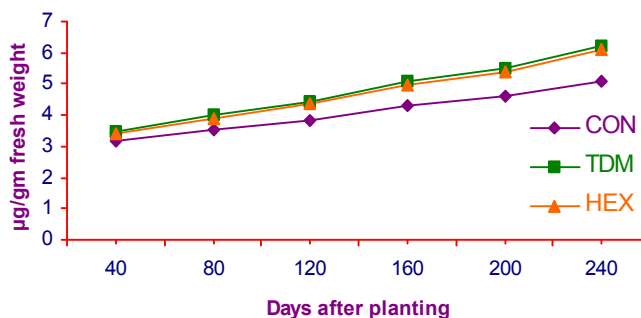


Fig. 3: Triazoles induced changes in the  $\alpha$ -tocopherol content in the leaves of Tapioca

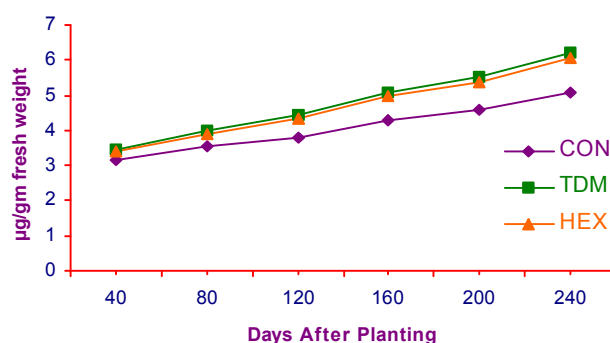


Fig. 4. Triazoles induced changes in the  $\alpha$ -tocopherol content in the tuber of Tapioca

Table 1: Triazole induced changes in the ascorbic acid content in the leaves of Tapioca (Values are the mean of three replicates expressed in µg/ g fresh weight)

DAP	CON	TDM	HEX	F- ratio	LSD
40	102.45	122.44	119.88	*	4.62
80	106.03	128.83	126.30	*	4.34
120	118.37	146.90	144.71	**	4.86
160	122.18	156.51	154.33	**	5.17
200	128.41	169.77	167.52	**	5.46
240	120.84	156.25	154.51	*	4.84

NS-Non significant; \*-Significant at 0.05 level; \*\*-Significant at 0.01 level

Table 2: Triazole induced changes in the ascorbic acid content in the tuber of Tapioca (Values are the mean of three replicates expressed in µg/ g fresh weight)

DAP	CON	TDM	HEX	F- ratio	LSD
80	12.03	15.11	14.71	*	1.38
120	17.05	22.02	21.73	*	2.42
160	21.18	28.02	27.57	*	3.67
200	38.12	51.28	50.91	**	4.12
240	42.15	58.22	57.88	**	3.86

NS-Non significant; \*-Significant at 0.05 level; \*\*-Significant at 0.01 level

The ascorbic acid content increased to a larger extent in triazole treated plants when compared to control and it was 129.31 and 127.87 percent over control in the triadimefon and hexaconazole treated plant on 240 DAP.

**Tuber (Table. 2):** In tapioca tubers triazole treatments increased the ascorbic acid content to a larger extent when compared to control and it was 58.225 and 57.88 microgram in the triadimefon and hexaconazole treated plants as compared to 42.156 microgram in the control plants.

Triadimefon and hexaconazole treatments increased the ascorbic acid content in the leaves and tubers of tapioca. Ascorbic acid is an important component of the plant antioxidant system [20-21]. Ascorbate is one of the two major soluble antioxidants in chloroplast [22]. Ascorbate also has photoprotective function because of its antioxidant capacity [23].

Uniconazole increased the level of antioxidants like ascorbic acid and  $\alpha$ -tocopherol and protected membrane by preventing or reducing oxidative damage [4,20]. Increase in ascorbic acid content was reported in the Triadimefon treated *Catharanthus roseus* plants [24].

### $\alpha$ – Tocopherol

**Leaf (Fig. 3):** The triadimefon and hexaconazole treatment increased the  $\alpha$  – tocopherol content in the leaves of tapioca. The tocopherol content increased with the age of the plant. The increased content of the  $\alpha$  – tocopherol in

the leaves of tapioca was 122.20 and 119.83 percent over control in the triadimefon and hexaconazole treated plant on 240 DAP.

**Tuber (Fig. 4):** In the tubers of tapioca the  $\alpha$  – tocopherol content showed a significant increase in the triazole treated plants when compared to control. Triadimefon and hexaconazole increased the  $\alpha$  – tocopherol to 5.686 and 5.536  $\mu\text{g}$  as compared to 4.588  $\mu\text{g}$  in the control on 240 DAP.

Triadimefon and hexaconazole treatment increased  $\alpha$ -tocopherol content in the leaves and tubers of tapioca.  $\alpha$ -tocopherol is the most abundant form of antioxidant in nature [25,26].  $\alpha$ -Tocopherol has antioxidant, cell signaling and vitamin-E functions. It acts as a chain breaking antioxidant, preventing the propagation of free radical reactions [27,28]. The active oxygen species formed at the membrane of leaves under water stress was efficiently removed upon rehydration with increase in the  $\alpha$ -tocopherol and  $\beta$ -carotene [29,30]. The increase in  $\alpha$ -tocopherol level in the triazole treated tapioca can increase the antioxidant potential in the leaves and tuber tissue.

## REFERENCES

1. Abdul Jaleel, C., R. Gopi, P. Manivannan, B. Sankar, A. Kishorekumar and R. Panneerselvam, 2007. Antioxidant potentials and ajmalicine accumulation in *Catharanthus roseus* after treatment with gibberellic acid. *Colloids and Surfaces B: Biointerfaces*, 60(2): 195-200.
2. Abdul Jaleel, C., P. Manivannan, B. Sankar, A. Kishorekumar, Ragupathi Gopi, Rajaram Somasundaram and R. Panneerselvam, 2007. 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(2): 201-206.
3. Abdul Jaleel, C., P. Manivannan, B. Sankar, A. Kishorekumar, R. Gopi, R. Somasundaram and R. Panneerselvam, 2007. *Pseudomonas fluorescens* enhances biomass yield and ajmalicine production in *Catharanthus roseus* under water deficit stress. *Colloids and Surfaces B: Biointerfaces*, 60: 7-11.
4. Abdul Jaleel, C., R. Gopi, P. Manivannan and R. Panneerselvam, 2007. Responses of antioxidant defense system of *Catharanthus roseus* (L.) G. Don. to paclobutrazol treatment under salinity. *Acta Physiologiae Plantarum*, 29: 205-209.
5. Abdul Jaleel, C., R. Gopi, M. Gomathinayagam and R. Panneerselvam, 2008. Effects of Calcium chloride on metabolism of salt stressed *Dioscorea rotundata*. *Acta Biologica Cracoviensia Series Botanica*, 50(1): 63-67.
6. Abdul Jaleel, C., P. Manivannan, M. Gomathinayagam, R. Sridharan and R. Panneerselvam, 2007. Responses of antioxidant potentials in *Dioscorea rotundata* Poir. following paclobutrazol drenching. *Comptes Rendus Biologies*, 330: 798-805.
7. Panneerselvam, R., C. Abdul Jaleel, R. Somasundaram, R. Sridharan and Muthiah Gomathinayagam, 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.
8. Gomathinayagam, M., C. Abdul Jaleel, G.M.A. Lakshmanan and R. Panneerselvam, 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.
9. Gopi, R., C. Abdul Jaleel, R. Sairam, G.M.A. Lakshmanan, M. Gomathinayagam and R. Panneerselvam, 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.
10. Abdul Jaleel, C., Ragupathi Gopi and Rajaram Panneerselvam, 2007. Alterations in lipid peroxidation, electrolyte leakage and proline metabolism in *Catharanthus roseus* under treatment with triadimefon, a systemic fungicide, *Comptes Rendus Biologies*, 330(12): 905-912.
11. Alagu Lakshmanan, G.M., C. Abdul Jaleel, Muthiah Gomathinayagam and R. Panneerselvam, 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.
12. Abdul Jaleel, C., R. Gopi, P. Manivannan, M. Gomathinayagam, P.V. Murali and Rajaram Panneerselvam, 2008. Soil applied propiconazole alleviates the impact of salinity on *Catharanthus roseus* by improving antioxidant status. *Pesticide Biochemistry and Physiology*, 90(2): 135-139.

13. Ridgman, W.J., 1975. Experimentation in biology: An introduction to design and analysis. Thomson Litho Ltd., East Kilbride, Scotland. pp; 81-100.
14. Tuckey, J.W., 1953. The problem of multiple comparisons. Princeton University Press, N.J.
15. Heath, R.L. and L. Packer, 1968. Photoperoxidation in isolated chloroplasts 1: Kinetic and Stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys., 125: 189-198.
16. Omaye, S.T., J.D. Turnbull and H.E. Sauberlich, 1979. Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Methods Enzymol. Academic Press, New York. 62: 3-11.
17. Baker, H., O. Frank, B. De Angells and S. Feingold. 1980. Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol. Nutr. Rep. Int., 21: 531-536.
18. Abdul Jaleel, C., G.M.A. Lakshmanan, M. Gomathinayagam and R. Panneerselvam, 2008. Triadimefon induced salt stress tolerance in *Withania somnifera* and its relationship to antioxidant defense system. South African Journal of Botany, 74(1): 126-132.
19. Zhou, W. and M. Leul, 1999. Uniconazole-induced tolerance of rape plants to heat stress in relation to changes in hormonal levels, enzyme activities and lipid peroxidation. Plant Growth Regul., 27: 99-104.
20. Abdul Jaleel, C., R. Gopi, A. Kishorekumar, P. Manivannan, B. Sankar and R. Panneerselvam, 2008. Interactive effects of triadimefon and salt stress on antioxidative status and ajmalicine accumulation in *Catharanthus roseus*. Acta Physiologiae Plantarum, 30: 287-292.
21. Abdul Jaleel, C., R. Gopi, P. Manivannan and R. Panneerselvam, 2008. Exogenous application of triadimefon affects the antioxidant defense system of *Withania somnifera* Dunal. Pesticide Biochemistry and Physiology, 91(3): 170-174.
22. Hong-Bo Shao, Li-Ye Chu, C. Abdul Jaleel, P. Manivannan, R. Panneerselvam and M.A. Shao, 2009. Understanding water deficit stress-induced changes in the basic metabolism of higher plants-biotechnologically and sustainably improving agriculture and the environment in arid regions of the globe. Critical Reviews in Biotechnology, 29(2): 131-151.
23. Abdul Jaleel, C., R. Gopi, P. Manivannan, M. Gomathinayagam, Ksouri Riadh, Jallali Inès, Zhao Chang-Xing, Shao Hong-Bo and R. Panneerselvam, 2009. Antioxidant defense responses: Physiological plasticity in higher plants under abiotic constraints. Acta Physiologiae Plantarum, 31(3): 427-436.
24. Abdul Jaleel, C., R. Gopi, G.M. Alagulakshmanan and R. Panneerselvam, 2006. Triadimefon induced changes in the antioxidant metabolism and ajmalicine production in *Catharanthus roseus* (L.) G. Don. Plant Science, 171: 271-276.
25. Abdul Jaleel, C., R. Gopi 2008. R. Panneerselvam, Biochemical alterations in white yam (*Dioscorea rotundata* Poir.) under triazole fungicides; impacts on tuber quality. Czech Journal of Food Sciences, 26(4): 298-307.
26. Abdul Jaleel, C., R. Gopi and R. Panneerselvam, 2008. Growth and photosynthetic pigments responses of two varieties of *Catharanthus roseus* to triadimefon treatment. Comptes Rendus Biologies, 331: 272-277.
27. Remya Vijayakumar, Zhao Chang-Xing, Rengasamy Gopal, C. Abdul Jaleel. 2009. Non-enzymatic and enzymatic antioxidant variations in tender and mature leaves of *Strychnos nux-vomica* L. (Family: Loganiaceae). Comptes Rendus Biologies, 332: 52-57.
28. Abdul Jaleel, C., P. Manivannan, B. Sankar, A. Kishorekumar, S. Sankari and R. Panneerselvam, 2007. Paclobutrazol enhances photosynthesis and ajmalicine production in *Catharanthus roseus*. Process Biochemistry, 42: 1566-1570.
29. Abdul Jaleel, C., A. Kishorekumar, P. Manivannan, B. Sankar, M. Gomathinayagam, R. Gopi, R. Somasundaram and R. Panneerselvam, 2007. 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.