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REGULAR ARTICLE

BIOCHEMICAL CONSTITUENTS OF *WITHANIA SOMNIFERA* UNDER THE INDOLE-3-BUTYRIC ACID AND TRIAZOLE SOIL DRENCHING TECHNIQUES

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ABSTRACT

The present study is aimed at understanding the effect of indole-3-butyric acid (IBA) and triazole compounds *viz.,* triadimefon (TDM) and propiconazole (PCZ) on the biochemicals of ashwagandha. Treatments were given on 50, 90 and 130 d after sowing (DAS). Biochemical constituents such as proline, glycine betaine and total alkaloids content were determined. It was observed that proline, glycine betaine and alkaloids content were enhanced by TDM and PCZ than followed by IBA treatment when compared to control for respective growth stages. Among the treatments, triazole compounds caused pronounced effect to the biochemical accumulation in higher level when compared to IBA treatment. These results suggest that, triazole active compounds act as a growth regulator also influence hormonal balance and great significance, which is helpful to satisfy the needs of enhance the biochemical contents in Ashwagandha.

Keywords: Medicinal Plants, Ashwagandha, Triazole, Proline, Glycine betaine, Alkaloids

INTRODUCTION

Ashwagandha (*Withania somnifera* L. Dunal) (Family: Solanaceae) is a medicinally important root crop and cultivated for tropical and subtropical region of India and vernacular name as Amukkra kizhangu in Tamil. The dried roots of ashwagandha are used as various form herbal medicine by Ayurveda, Siddha and Unani formulation. The root quality basically determined secondary metabolites contents such as alkaloids and steroidal lactone (Withanolides). It has household remedy for various ailments and is known by various local names, as Ashgandh in Hindi, Amukkiran Kizhangu in Tamil, Hiremaddinagida in Kannada, Askandha in Marathi. The plant species is widely distributed all over the India subcontinent [1-3]. The ashwagandha roots diverse therapeutic effect, it has been used in many groups of formulations for the treatment of an array of ailments [4,5]. The root drug is used various physiological disorders [6-7]. Many active compounds are being isolated from various parts of this plant [8-12].

Triazoles are fungicides which can be used as plant growth regulators and also influence by hormonal balance such as inhibition of gibberellic acid biosynthesis and increase abscisic acid and cytokinin contents [14, 13]. Triazole induced changes in morphological, physiological and metabolic changes are reported earlier in plants [13, 15]. Triazoles proved a better growth regulator and stress protectant in many plants [16-23]. Auxins if supplied externally has many profound effects in growth and metabolism of plants [24, 25]. Exogenous applied auxins induced the biomass production, biochemicals and secondary metabolite accumulation [26, 27]. The present study aimed to estimate the effects of triazoles and IBA on biochemical constituents of *Withania somnifera*.

MATERIALS AND METHODS

The seeds of Ashwagandha (*Withania somnifera* L.) variety "Jawahar Asgandh-20" were obtained from Tamil Nadu Agricultural University, India. Indole-3-butyric acid (Sigma Chemicals), Bayleton™ (Triadimefon) (Bayer India Ltd.) and Banner™ (Propiconazole) (Rallis India Ltd.) were used for this study.

The experiment was in Completely Randomized Block Design (CRBD) with six replications. 2.5 mgL^{-1} IBA, 20 mgL^{-1} TDM and 20 mgL^{-1} PCZ concentrations were used to determine the effect of these chemicals on the growth of *W. somnifera* and the treatments were given on 50^{th}, 90^{th} and 130^{th}DAS by soil drenching. Plants were harvested on 60^{th}, 100^{th} and 140^{th}DAS and they were used for the determination of proline, glycine betaine and alkaloids content of *W. somnifera*.

Proline content was extracted and estimated by following the method [28]. The glycine betaine content for method of

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Grieve and Grattan [29]. Total alkaloids content estimated by standard method [30].

Statistical analysis

SPSS software version 16.0 was to make statistical analysis. The data analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The values are mean±standard deviation (SD) for six samples in each group. *P* values ≤ 0.05were considered as significant.

RESULTS AND DISCUSSION

Proline content

IBA and Triazole compounds such as TDM and PCZ treatments significantly increased the proline content in the roots, stem and leaves of *Withania somnifera*. Among the treatments, TDM and PCZ caused pronounced effect to induced the proline accumulation to a higher level when compared IBA treated plants (Table-1). Active ingredients of triazole gradually increased the proline content in *Aesculus hippocastanum* [31]. Triazole compounds enhanced the accumulation of proline content in *Dioscorea rotundata* and *Abelmoschus esculentus* [21,32]. Paclobutrazol concentrations resulted that higher proline content in *Festuca arundinacea* and *Lolium perenne* [33] and *sativa* plant [34]. Triadimefon treatments increased the proline content in *Lycopersicom esculentum* [35] and *Cajanus cajan* [36]. Triadimefon and propiconazole treatments enhanced proline accumulation in all the stages of growth of *Raphanus sativus* [37].

Proline and glycine betaine are through to function as osmoprotectants for proteins [38]. Proline acts as a free radical scavenger and may be more important in overcoming stress than in acting as a simple osmolyte [39]. Mulberry treated with triadimefon and uniconazole showed an appreciable increase in free proline content and this increase was directly proportional to the triazole concentrations [40]. Triazole induced the abscisic acid biosynthesis in *Phaseolus vulgaris* [41]. The increased the abscisic acid content induced by TDM and PCZ might be the reason for increased proline content in *W. somnifera*

Endogenous applied IBA significant increased the proline content in weights in *Pisum sativum* [42]. IBA concentrations gave the highest values for proline content observed in onion plants [43-44]. Teng [45] reported that cultivars variation occurs in levels of free proline and gibberellins and a lower level of ABA, along with higher pollen vigour and germination rate even after prolonged higher drought stress in rice. Thus they suggested a possible correlation between IAA and ABA free proline content in maize plant [46]

Glycine betaine content

Glycine betaine content significantly increased under the PCZ, TDM and IBA treated plants. Among the treatments, triazole had higher level of glycine betaine when compared to IBA treated plants of *W. somnifera* (Table-2). Triazole with drought stress increased the glycine betaine accumulation in *Abelmoschus esculentus* [32]. Paclobutrazol treatment significantly increased the osmoregulation content like proline and glycine betaine content in *Sesamum indicum* [47] and *Arachis hypogaea* [48]. Ketoconazole treatment increased the proline and glycine betaine content in Cowpea [49]. Osmotic potential of their cells by synthesizing and accumulating compatible osmolytes such as proline and glycine betaine, which participates in the osmotic adjustments in *Helianthus annuus* under abiotic stress [38]. Glycine betaine is an osmoprotectant which can improve stress tolerance in plants [50]. Triazole treatments increased glycine betaine contents, triazole treatment modified biochemical content and antioxidant enzyme activity. Plants are highly regulated by triazole compounds and can give stress tolerance [32,51]. The increased the abscisic acid content induced by triazole might be the reason for increased glycine betaine content in *W. somnifera*

Table 1: Influence of IBA, TDM and PCZ on the proline content of Ashwagandha

Biochemical constituents	Growth stages (DAS)	Control	Indole-3-butyric acid (IBA)	Triadimefon (TDM)	Propiconazole (PCZ)
Proline content (mg g^{-1} fr. wt.)					
Leaf	60	6.346±0.484^{a}	6.725±0.510^{a}	7.720±0.589^{b}	7.891±0.600^{b}
	100	8.605±0.658^{a}	9.593±0.725^{ab}	11.015±0.841^{b}	11.396±0.859^{bc}
	140	9.965±0.757^{a}	10.711±0.815^{a}	12.121±0.922^{b}	12.406±0.945^{b}
Stem	60	6.656±0.506^{a}	7.065±0.537^{a}	8.201±0.627^{b}	8.351±0.636^{b}
	100	8.963±0.687^{a}	9.990±0.756^{ab}	11.496±0.880^{b}	11.865±0.900^{bc}
	140	10.213±0.777a	10.708±0.815a	11.868±0.900^{b}	12.205±0.929^{b}
Root	60	7.231±0.550^{a}	7.781±0.591^{a}	8.981±0.685^{b}	9.131±0.694^{b}
	100	9.566±0.732^{a}	10.705±0.819^{b}	12.426±0.951^{c}	12.546±0.960^{c}
	140	10.925±0.833^{a}	11.661±0.887^{a}	12.815±0.976^{b}	13.131±0.998^{b}

Expressed values are the mean±SD of six replicates in each group. Values, that are not sharing a common superscript (a, b, c,) differ significantly at $P \leq 0.05$

Table 2: Influence of IBA, TDM and PCZ on the glycine betaine content of Ashwagandha

Biochemical constituents	Growth stages (DAS)	Control	Indole-3-butyric acid (IBA)	Triadimefon (TDM)	Propiconazole (PCZ)
Glycine betaine content (mg g^{-1} fr. wt.)					
Leaf	60	3.805±0.291^{a}	3.916±0.300^{a}	4.436±0.335^{b}	4.701±0.434^{c}
	100	6.251±0.476^{a}	6.997±0.521^{ab}	7.630±0.582^{b}	7.990±0.602^{b}
	140	7.223±0.548^{a}	7.618±0.580^{a}	8.551±0.649^{b}	8.981±0.685^{b}
Stem	60	2.926±0.224^{a}	3.100±0.237^{ab}	3.340±0.255^{bc}	3.460±0.264^{c}
	100	5.473±0.418^{a}	5.973±0.459^{ab}	6.418±0.490^{bc}	6.670±0.510^{c}
	140	6.363±0.486^{a}	6.723±0.589^{a}	7.606±0.578^{b}	7.733±0.589^{b}
Root	60	4.530±0.345^{a}	4.879±0.356^{a}	5.341±0.407^{b}	5.651±0.430^{b}
	100	7.00±0.535^{a}	7.795±0.595^{b}	8.797±0.671^{c}	8.986±0.689^{c}
	140	8.133±0.620^{a}	8.613±0.656^{ab}	9.791±0.743^{c}	9.998±0.685^{c}
Total alkaloids content (mg g^{-1} fr. wt.)					
Root	60	16.333±1.245^{a}	17.903±1.361^{ab}	19.003±1.447^{b}	19.403±1.478^{b}
	100	26.903±2.057^{a}	29.915±2.288^{b}	32.015±2.449^{bc}	33.126±2.536^{c}
	140	33.605±2.557^{a}	38.006±2.894^{b}	41.996±3.198^{c}	43.056±3.274^{c}

Expressed values are the mean ± SD of six replicates in each group. Values that are not sharing a common superscript (a, b, c) differ significantly at $P \leq 0.05$

Total alkaloids content

The total alkaloid content was gradually increased in all stages of growth, but high level of alkaloids production in the later root at maturity stages. Triazole treated *W. somnifera* enhanced the alkaloids content in higher level than IBA treatment when compared to control plant (Table-2). Triazoles application could well be used as an antioxidant potential tool to increase the antioxidant production and alkaloid production in *Gloriosa superba* [52]. Triadimefon treatment increased the accumulation of alkaloid "ajmalicine" content in *Catharanthus roseus* [53-54] Similar results observed in *Plectrantus forskohlii* alkaloid "forkolin" content under the triadimefon and hexaconazole treatments [23]. Triadimefon mediated increased the indole alkaloids content in Datura species [55]. Triazole treatment has good significance, as these increases the secondary metabolites of *Catharanthus roseus* [53]. These increased alkaloids content might be due to triazole effect on Geranylgeranyldiphosphate (GGPP) which is the precursor for the synthesis of terpenoids, carotenoids, ABA and cytokinin [56]. Triazole treatments increased the cytokinin content and it might also be a reason for the increased alkaloid content in triazole treated plants of *Withania somnifera* as observed in *Coleus forskohlli* culture cells treated with cytokinin [57].

Foliar application of IAA, IBA and NAA enhancement in the total alkaloids of leaves and roots, contents of *vincristine* and *vinblastine* production in *Catharanthus roseus* [26]. According to Ataei-Azimi [58] 2,4-D, KIN, and IAA enhanced the production of *vincristine* and *vinblastine* alkaloids during *in vitro* culture. IBA supplementation is useful tool for growth and secondary metabolite production in adventitious roots of *Morinda citrifolia* [59]. An increased alkaloid content was also reported in *Catharanthus roseus* by the application of 2,4-D and IAA [60]. Auxin appears to be the primary factor controlling growth and morphology of roots, while the effects of cytokinins vary depending on secondary metabolite formation as well as the relevant plant species [61].

CONCLUSION

The present investigation, it can be concluded that the exogenous applied indole-3-butyric acid, triadimefon and propiconazole at low concentrations (IBA 2.5 mgL^{-1}, TDM 20 mgL^{-1}and PCZ 20 mgL^{-1}) significantly enhanced the proline, glycine betaine and total alkaloids content for respective growth stages of ashwagandha. Among the treatments, triazole caused pronounced effect to enhance the biochemicals accumulation in higher level when compared to IBA. Triazole application could well be used as a potential agronomical tool to enhanced primary and secondary metabolites production in medicinally important root crops.

REFERENCES

1. Sangwan RS, Chaurasiya ND, Misra LN, Lal P, Uniyal GC. 2004. Phytochemical variability in commercial herbal products and preparations of Withania somnifera (Ashwagandha). Curr Sci 2004;86:461–465.
2. Gupta GL, Rana AC. PHCOG MAG: Plant review. *Withania somnifera* (Ashwagandha): A review Pharmacognosy 2007;1:129–136.
3. Murthy HN, Dijkstra C, Anthony P, White DA, Davey MR, Power JB, Hahn EJ, Paek KY. Establishment of *Withania somnifera* hairy root cultures for the production of withanolide A. J Int Plant Biol 2008; 50:975-981.
4. Singh G, Sharma PK, Singh, D. Biological activities of Withania Somnifera. Annals of Biol Res 2010;1: 56-63.
5. Kumar A, Kaul MK, Bhan MK, Khanna PK, Suri KA. *Withania somnifera* (L.) Dunal (Solanaceae). Genet Resour Crop Evol 2007;54:655-660.
6. Kaur P, Mathur S, Sharma M, Tiwari M, Srivastava KK, Chandra R. A biologically active constituent of *Withania somnifera* (Aswagandha) with anti-stress activity. Ind J Clin Biochem 2001;16:195–198.
7. Mishra L, Mishra P, Pandey A, Sangwan RS, Sangwan NS, Tuli R. Withanolides from *Withania somnifera* roots. Phytochemistry 2008;69;1000–1004
8. Matsuda H, Murakami T, Kishi A, Yoshikawa M. Structures of withanosides I, II, III, IV, V, VI, and VII, new withanolide glycosides, from the roots of Indian *Withania somnifera* Dunel and inhibitory activity for tachyphylaxis to clonidine in isolated guinea-pig ileum. Bioorg Med Chem 2001;9:1499–1507.
9. Mishra L, Lal P, Sangwan RS, Sangwan NS, Uniyal GC, Tuli R. Unusually sulphated oxygenated steroids from Withania somnifera. Phytochemistry 2005;66:2702–2707.

10. Rahman AU, Shahawar DE, Naz A, Jamal A, Choudhary MI. Withanolides from *Withania coagulans*. Phytochemistry 2003;63:387–390.
11. Chen WY, Chang FR, Huang ZY, Chen JH, Wu YC. Tubocapsenolide A, a novel Withanolide, inhibits proliferation and induces apoptosis in MDAMB-231 cells by thiol oxidation of heat shock proteins. J Biol Chem 2008;283:17184–17193.
12. Pan MR, Chang HC, Wu YC, Huang CC, Hung WC. Tubocapsanolide A Inhibits transforming growth factor-b-activating kinase 1 to suppress NF-kbinduced CCR7. J Biol Chem 2009;284:2746–2754.
13. Fletcher A, Gilley N, Sankhla N, Davies T. Triazoles as plant growth regulators and stress protectants. Hort Rev 2000;24:55-138.
14. Fletcher RA, Arnold, V. Stimulation of cytokinins and chlorophyll synthesis in cucumber cotyledons by triadimefon. Physiol Plantarum 1986;197-201.
15. Fletcher RA, Hofstra G. Triazole as potential plant protectants, in: D. Berg, M. Plempel, (Eds.), Sterol biosynthesis inhibitors, Ellis Horwood Ltd., Cambridge England 1988;321-331.
16. Lin KH, Tsou CC, Hwang SY, Chen LFO, Lo. HF. Paclobutrazol pre-treatment enhanced flooding tolerance of sweet potato. J of Plant Physiol 2006 163:750-760.
17. Bahram, B. Amelioration of chilling stress by paclobutrazol in watermelon seedlings. Sci Hortic-Amsterdam 2009;121:144–148.
18. Gopi R, Jaleel CA R. Sairam R Alagu Lakshmanan GM, Gomathinayagam M, R. Pannerselvam. Differential effects of hexaconazole and paclobutrazol on biomass, electrolyte leakage, lipid peroxidation and antioxidant potential of *Daucus carota* L. Colloids Surf. B: Biointerfaces 2007;60:180-186.
19. Sridharan R, Kishorekumar A, Somasundaram R, Manivannan P. Alagulakshmanan GM, Gomathinayagam M, Panneerselvam, R. Effect of triazole on growth and chlorophyll pigments in radish. Ind J Environ Ecoplan 2006;12:57-62.
20. Kishorekumar A, Jaleel CA, Manivannan P, Sankar B, Sridharan R, Somasundaram R, Panneerselvam R. Changes in biochemical constituents under salinity in *Solenostemon rotundifolius* (Poir.) Morton. Indian J App Pure Biol 2007;22:223-225.
21. Jaleel CA, Kishorekumar A, Manivannan P, Sankar B, Gomathinayagam M, Gopi R, Somasundaram R, Panneerselvam R. Alterations in carbohydrate metabolism and enhancement in tuber production in white yam (*Dioscorea rotundata* Poir.) under triadimefon and hexaconazole applications. Plant Growth Regul 2007;53:7-16.
22. Jaleel CA, Zhao C, Mohamed S, Al-Juburi HJ, Moussa HR, Gomathinayagam M, Panneerselvam R. Alterations in sucrose metabolizing enzyme activities and total phenol content of *Curcuma longa* L. as affected by different triazole compounds. Front Biol China 2009;4:419-423.
23. Alagu Lakshmanan GM. Triazole induced variation in growth, biochemical changes and forskolin content in *Plectranthus forskohlii* (Willd.) Brig. Ph. D. Thesis 2008 Cat. No. P-I, 125-A, Annamalai University, Annamalainagar, India.
24. Krikorian D. In: Davies PJ. (ed.). Plant Hormones; Physiology. Biochemistry and Molecular biology. Kluwer Academi publishers, London. 1995;774-798.
25. Nagel. Annals of Botany. 2001;88:27-31.
26. Masidur Alam M, Naeem M, Idrees M, Masroor M, Khan Moinuddin A. Augmentation of photosynthesis, crop productivity, enzyme activities and alkaloids production in sadabahar (*Catharanthus roseus* L.) through application of diverse plant growth regulators. J Crop Sci Biotech. 2012;15:117-129.
27. Kavikishor PB, Sangam S, Amrutha RN, Laxmi PS, Naidu KR, Rao KRSS, Reddy KJ, Theriappan P, Sreenivasulu N. Regulation of proline biosyn-thesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. Curr Sci 2005;88:424–43
28. Bates LS, Waldern RP, Teare ID. Rapid determination of free proline for water stress studies. Plant Soil 1973;39:205-207.
29. Grieve CM, Grattan SR. Rapid assay for determination of water soluble quaternary ammonium compounds. Plant and Soil 1983;70:303-307.
30. Anonymous. Indian Pharmacopoeia, Vol. II, Government of India, Ministry of Health and Family Welfare, New Delhi. 1986;95:81-83.
31. Percival CG, Noviss K. Triazole induced drought tolerance in horse chestnut (*Aesculus hippocastanum*). Tree Physiol 2008;28:1685–1692.
32. Amalan Rabert G, Rajasekar M, Manivannan P, Somasundaram R, R. Panneerselvam R. Effect of triazole fungicide on biochemical and antioxidant enzymes activity in okra (*Abelmoschus esculentus* L.) Plant under drought stress. Int J Agri Food Sci 2013;3:100-107.
33. Shahrokhi M, Tehranifar A, Hadizadeh H, Selahvarzi Y. Effect of drought stress and paclobutrazol-treated seeds on physiological response of *Festuca arundinacea* L. Master and *Lolium perenne* L. Barrage. J of Environ Sci 2013;14:77–85.
34. Mathur R, Bohra SP. Effect of paclobutrazol on aminotransferases; Protein and proline content in Eruca sativa var. T-23 seedlings. J Phytol Res 1992;5:93-95.
35. Mohamadi N, Rajaei P. Effect of Triamidefon fungicide on some growth parameters and antioxidant enzymes activity in tomato (*Lycopersicom esculentum* Mill.) plant under drought stress. Int J Adv Biol Biom Res 2013;1:341-350.
36. Karikalan L, Rajan SN, Gopi R, Sujatha BM, Pannersevalam R. Induction of salt tolerance by triadimefon in pigeon pea (Cajanus *cajan* L.) Mill sp. Ind J Exp Biol 1999;37:825-829.
37. Sridharan R, Manivannan P, Murali PV, Somasundaram R, Panneerselvam R. Responses of non-enzymatic antioxidant potentials in radish by triazole compounds. Global J Mol Sci 2009;4:63-67.
38. Manivannan P, Jaleel CA, Sankar B, Kishorekumar A, Somasundaram R, Alagu Lakshmanan GM, Panneerselvam R. Growth, biochemical modifications and proline metabolism in *Helianthus annuus* L. as induced by drought stress. Colloids Surf B: Biointerfaces 2007;59:141-149.
39. Reddy KK, Venkaiah K, Bramaramba B. Rooting of stem cutting of *Avicennia officinalis* Linn. and *Avicennia alba* Bl: A tool for afforestation of blanks in mangrove forests. India Forester 1994;156-161.
40. Sreedhar VM. Proline accumulation and reduced transpiration in leaves of triazole treated mulberry plant. Indian Bot Repro 1991;10:1-5.
41. Mackay CE, Hall JC, Hofstra G, Fletcher RA. Uniconazole induced changes in abscisic acid, total amino acids and proline in *Phaseolus vulgaris*. Pest Biochem Physiol 1990;37:74-82.
42. Amal MES, Amira MH. Effect of acetylsalicylic acid, indole-3-bytric acid and gibberellic acid on plant growth and yield of pea (*Pisum sativum* L.). Aust J Basic and Appl Sci 2009;3:3514-3523.

43. Amin AA, Rashad ME, Sh EL, Gharib EAE. Physiological response of maize plants (*Zea mays* L.) to foliar application of morphactin CF125 and indole-3-butyric acid. J of Biol Sci 2006;6:547-554.
44. Amin AA, Rashad M, Sh EL, El-Abagy HMH. Physiological effect of indole-3-butyric acid and salicylic acid on growth, yield and chemical constituents of Onion plants. J of Appl Sci Res 2007;3:1554-1563.
45. Teng S, Rognoni, S, Bentsink L, Smeekens S. The Arabidopsis GSQ5/DOG1 Cvi allele is induced by the ABA-mediated sugar signalling pathway, and enhances sugar sensitivity by stimulating ABI4 expression. Plant J 2008;55:372-381.
46. Kaya C, Levent Tuna A, Alfredo A, C. Alves C. Gibberellic acid improves water deficit tolerance in maize plants. Acta Physiol Plant 2006;28:331-337.
47. Sindhu S, Abraham, Jaleel, CA, Chang-Xing Z, Somasundaram R, Azooz MM, Manivannan P, Panneerselvam R. Regulation of Growth and Metabolism by Paclobutrazol and ABA in *Sesamum indicum* L. under drought condition. Global J Mol Sci 2008;3:57-66.
48. Girija C, Smith BN, Swamy PM. Interactive effects of sodium chloride and calcium chloride on the accumulation of proline and glycine betaine in peanut (*Arachis hypogaea* L.). Environ Exp Bot 2002;43:1-10.
49. Somasundaram R, Manivannan P, Senthilkumar R, Gopi R, Sridharan R, Gomathinayagam M, Panneerselvam R. Stress amelioration by ketoconazole in NaCl stressed cowpea. J Curr Sci 2006;1:179-183.
50. Mahmood T, Ashraf M, Shahbaz M. Does exogenous application of glycinebetaine as a pre-sowing seed treatment improve growth and regulate some key physiological attributes in wheat plants grown under water deficit conditions. Pak J Bot 2009;41: 1291-1302.
51. Amalan Rabert G, Rajasekar M, Manivannan P, Somasundaram R, Panneerselvam R. Triazole induced modification of biochemical and antioxidant metabolism in *Capsicum annuum* L. Under drought stress. Int J of Cur Res 2013;10:3258-3267.
52. Kavina J, Gopi R, Panneerselvam R. Difenoconozole and Propiconazole's Effects on Antioxidant Potentials of *Gloriosa superba* Linn. World J of Agricul Sci 2012;8: 247-252.
53. Jaleel CA, Gopi R, Alagu Lakshmanan GM, Panneerselvam R. Triadimefon induced changes in the antioxidant metabolism and ajmalicine production in *Catharanthus roseus* (L.) G. Don. Plant Sci 2006;171:271-276.
54. Jaleel CA, Manivannan P, Kishorekumar A, Sankar B, Gopi R, Somasundaram R, Panneerselvam R. Alterations in osmoregulation, antioxidant enzymes and indole alkaloid levels in *Catharanthus roseus* exposed to water deficit. Colloids and Surfaces B: Biointerfaces 2007;59:150-157.
55. Sivakumar T, Panneerselvam R. Triadimefon mediated changes in antioxidant and indole alkaloid content in two species of Datura. American. J Plant Physiol 2011;6:252-260.
56. Rademacher W. Growth retardants: Effects on gibberellin biosynthesis and other metabolic pathways. Ann. Rev. Plant Physio. Plant Mol Biol 2000;51:501-531.
57. Mukherjee S, Ghosh B, Jha S. Higher production of forskolin in genetically transformed cultures of *Coleus forskohlii* Brig. Induced by growth regulators. J. Plant Biochem. Biotechnol 2003;12:81-85.
58. Ataei-Azimi A, Hashemloian BD, Ebrahimzadeh H, Majd A. High *in vitro* production of ant-canceric indole alkaloids from Periwinkle (*Catharanthus roseus*) tissue culture. African J Biotechnol 2008;7:2834-2839.
59. Baque MP, Hahn EJ, Paek KY. Growth, secondary metabolite production and antioxidant enzyme response of *Morinda citrifolia* adventitious root as affected by auxin and cytokinin. Plant Biotechnol 2010;4:109–116.
60. Amit KJ, Dubey PK, Rana RC. *In vitro* callus induction and biomass production of *Catharanthus roseus*. Plant Arch. 2005;5:55–60.
61. Rao SR, Ravishankar GA. Plant cell cultures: Chemical factories of secondary metabolites. Biotechnol Adv 2002;20:101-153.