Original Article

Journal of Medicinal Herbs and Ethnomedicine 2015, 1(1): 13-23
http://scienceflora.org/journal/jmhe/
doi:10.5455/jmhe.2015-06-06

Influence of indole-3-butyric acid and triazole compounds on the growth and antioxidant constituents in Ashwagandha (*Withania somnifera* L.) Dunal

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Received: 30.06.2015
Revised: 14.07.2015
Accepted: 15.07.2015
Published: 15.07.2015

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ABSTRACT

Withania somnifera (L.) Dunal belongs to the family Solanaceae and commonly known as Ashwagandha, Indian ginseng and winter cherry is major ayurvedic medicinal plant cultivated specifically for its root portion, which has medicinal and nutritional values due the presence of clinically important active compounds such as steroidal lactones, alkaloids, flavonoids, and tannins etc. Ashwagandha root is the medicinal part of the plant and is used in the ayurvedic formulations and pharmaceutical industries. The present study was undertaken to investigate the influence of indole-3-butyric acid (IBA) and triazole compounds *viz.,* triadimefon (TDM) and propiconazole (PCZ) on the growth and non-enzymatic antioxidant content of Ashwagandha. Plants were treated with IBA 2.5 mg/L, TDM 20 mg/L and PCZ 20 mg/L separately by soil drenching on 50, 90, 130 days after sowing (DAS). Plants were analyzed on 60, 100 and 140 DAS and it's parameters such as shoot length, internodal length, number of leaves, total leaf area, fresh and dry weight of shoot, root length, root diameter, number of roots, fresh and dry weight of root, ascorbic acid (AA), α-tocopherol (α-toc) and total phenol (TP) contents were determined. It was observed that shoot length, internodal length, the number of leaves, total leaf area, fresh and dry weight of shoot decreased, but the root length, root diameter, number of roots fresh and dry weight of roots were increased higher level. Likewise, the non-enzymatic antioxidants constituents such as AA, α-toc and TP contents were also increased by TDM and PCZ treatments. But IBA treatment increased all the parameters studied. Among the treatments, triazole showed beneficial growth by increasing the biomass of root system antioxidant constituents when compared to IBA treatment. This finding suggested that the triazole compounds showed a great significant for the cultivation of medicinally important root crops, which is helpful to meet the needs of root production by enhancing antioxidant constituents in Ashwagandha.

KEY WORDS: Ashwagandha, antioxidant constituents, medicinal plants, indole-3-butyric acid, propiconazole, triadimefon

INTRODUCTION

Ashwagandha (*Withania somnifera* L.) is one of the most important medicinal plants that have been used in Indian Systems of Medicine like Ayurveda, Unani, and Siddha since ancient times. In Ayurvedic and indigenous medicine for over 3000 years, the plants are used for various physiological disorders (Asthana and Raina, 1989). It is used for the treatment of male sexual debility disorders and its rejuvenating effect of stimulates the increasing sperm motility and sperm count (Mishra *et al*., 2008; Singh *et al*., 2010; Mahdi *et al*., 2011). It's also has anticancer, antistress, anti-inflammatory, immouno-modulatory and antioxidant properties (Amritpal *et al*., 2010; Rasool and Varalakshmi, 2006; Bhattacharya *et al*., 1997). Moreover, the modern medicines contain about 25% of drugs derived from plants. India and China is the two major production centers of medicinal plants having more than 40% of global biodiversity. India is blessed with a wide variety of soils and agro-climatic situations that support the existence of large number and varieties of medicinal plants. Of these, 65 plants have large and consistent demand in world trade. India however, produces only limited quantities of these materials. Since there is a high demand for these medicinal

plants in the pharmaceutical industry, it is essential to increase the commercial cultivation so as to reduce the exploitation from the forests and meet the demand. Furthermore, to ensure proper supply of medicinal plants to the drug industries and save the biodiversity of the plants, it has become necessary that these plants be propagated properly and cultivated scientifically (Faroogi and Sreeramu, 2004).

This is a new phenomenon to increase plant productivity scientifically, as well as improve the biochemical constituents by using synthetic chemical compounds. Plant growth regulators are widely used to modify canopy structure, yield and stress tolerance in many crop plants. Manipulating the crop morphology by using plant growth regulators also increases the utilization of solar radiation and alter assimilates distribution in favor of yield increments. In addition to other agronomic tools, synthetic plant growth regulators are increasingly used to modify growth, development and stress behavior and the qualitative and quantitative yield of crop plants. Over the past few years, several triazole derivatives, collectively described as sterol biosynthesis inhibitors, have been developed and used as fungicides, and they also have plant growth regulating properties. The triazole compounds *viz*., triadimefon (TDM) (Bayleton), propiconazole (PCZ) (Banner), paclobutrazol (Bonzi) and uniconazole (Sumagic) are used as growth regulators or retardants. However, all of these products can be exhibit both fungicidal and growth regulating properties to varying degrees (Fletcher *et al*., 2000). Triazole acts as plant growth regulator also influence hormonal balance, photosynthetic rate, enzyme activities, lipid peroxidation and yield components in various crop plants. Triazole compounds have been shown to improve the yield of many root crops (Sridharan *et al*., 2006a; 2006b; Jaleel *et al*., 2007a). The plant growth regulating properties of triazoles are mediated by their ability to alter the balance of important plant hormones including gibberellic acids, abscisic acid and cytokinins (Fletcher and Hofstra, 1988; Fletcher *et al*., 2000). Indole-3-butyric acid (IBA) is a synthetic auxin used commercially for enhancing crop production and regulation of plant growth and development, rapid growth of shoot tissue and young leaves and also to promote lateral root development (Ludwig-Muller, 2000; Nagel *et al*., 2001). Hence in the present study, triazoles and IBA have been successfully used to increase the growth, root yield and antioxidant content of *W. somnifera*.

MATERIALS AND METHODS

Pot Culture Experiment and Plant Treatments

The seeds of Ashwagandha (*W. somnifera* L.) Dunal variety. "Jawahar Asgandh-20" were obtained from Horticultural College and Research Institute, Department of Medicinal and Aromatic Crops, Tamil Nadu Agricultural University, Coimbatore, India. IBA was obtained from Sigma Chemicals, Bangalore. Bayleton™ (TDM) was obtained from Bayer India Ltd., Mumbai. Banner™ (PCZ) was obtained from Rallis India Ltd., Mumbai and they were used for this research study.

The pot culture experiments were conducted at the Botanical Garden and in Plant Growth Regulation Laboratory, Annamalai University during the months of January-June, 2012. The pots were filled with the mixture of red soil + sand + farm yard manure in 1:1:1 ratio and each pot were filled with 15 kg mixture. 25 seeds were sown in each pot, and finally 3 seedlings were maintained under shade net. The experiment was conducted in completely randomized block design (CRBD) with six replications. Fertilizer was not used throughout the experiments. In preliminary experiments, 0.5, 1.5, 2.0, 2.5, 3.0, and 3.5 mg/L of IBA, 5, 10, 15, 20, 25, and 30 mg/L of TDM and PCZ were used for the treatment and to determine the it optimum concentration. 2.5 mg/L of IBA, 20 mg/L of TDM and 20 mg/L of PCZ were found to increase the dry weight significantly and in higher concentration they slightly decreased the growth and dry weight. Hence, 2.5 mg/L IBA, 20 mg/L TDM and 20 mg/L PCZ concentrations were used to determine the effect of these chemicals on the growth of *W. somnifera*. Each pot was treated with one litter of respective treatment (2.5 mg/L IBA, 20 mg/L TDM and 20 mg/L PCZ) separately and control plant was treated with 1 L of tap water. Each pot has three plants, and the treatments were given on 50th, 90th and 130th DAS by soil drenching. The electrical conductivity of the soil was 0.21/dsm and pH was 7.5 after the treatments. The average temperature was 32/26°C (maximum and minimum) and relative humidity varied between 60% and 75% during the experimental period. Plants were harvested on 60th, 100th (Plate 1) and 140th DAS, and they were used for the determination of growth and non-enzymatic antioxidant constituents of *W. somnifera*.

Growth Analysis

The shoot length was measured and recorded from the root/shoot differentiation zone to the tip and expressed in cm per plant. The length of the internodes between the fourth and fifth pair of leaves were measured and recorded in cm. The total number of leaves were counted for each plant and expressed as numbers per plant. The total leaf area of the plant was measured using Systronics® Leaf Area Meter (Model 211, Ahmadabad, India) and expressed in cm^2 per plant. The plant root length was measured from

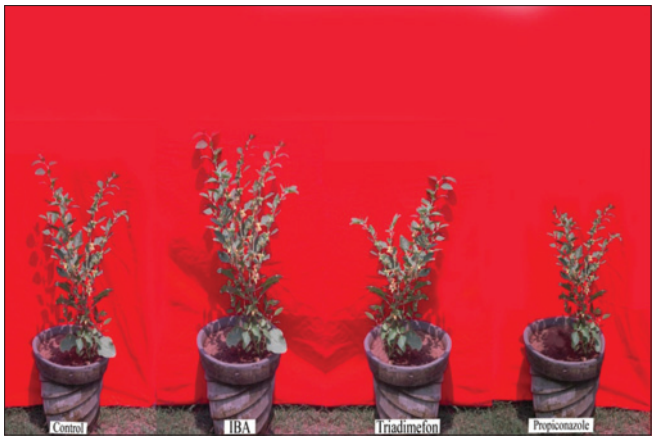

Plate 1: Influence of indole-3-butyric acid, triadimefon and propiconazole treatments on the growth of *Withania somnifera* at 100 days after sowing under pot culture experiments

nodal initiation of the shoot to the tip of the longest root and expressed in cm. The number of fully developed roots were counted and expressed as a number of roots per plant. The diameter of the roots at the thickest portion was measured using vernier calipers. The mean diameter of four randomly selected roots per plant was calculated.

Fresh and Dry Weight of the Plant

After washing the plants in the tap water, fresh weight was determined by using an electronic balance and the values expressed in gram. After taking the fresh weight, the plants were dried at 60°C in a hot air oven for 24 h. Then the dry weight was taken, and the values expressed in gram.

Estimation of Antioxidant Constituents

Ascorbic acid (AA) 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% trichloroacetic acid, centrifuged at 3500 rpm for 20 min using table top refrigerated centrifuge Hermle, Labortechnik Z36HK, re-extracted twice the supernatant was made up to 10 ml and used for the assay. To 0.5 ml of extract, 1 ml of 2,4-dinitrophenyl hydrazine-thiourea-$CuSO_4$ reagent was added, incubated at 37°C for 3 h and 0.75 ml of ice-cold 65% H_2SO_4 was added, allowed to stand at 30°C for 30 min. The resulting color was read at 520 nm in a Systronics® 118 ultraviolet-visible spectrophotometer. The AA content was determined using a standard curve prepared with AA. The results were expressed in milligram per gram fresh weight.

α-Tocopherol (α-toc) content

α-toc content was assayed as described by Backer *et al.* (1980). 500 mg of fresh tissue was homogenized 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 and the supernatant was used for estimation of α-toc. To 1 ml of extract, 0.2 ml of 2% 2, 2-dipyridyl in ethanol was added and mixed thoroughly and kept in dark for 5 min. The resulting red color was diluted with 4 ml of distilled water and mixed well. The resulting color in the aqueous layer was measured at 520 nm. The α-toc content was calculated using a standard graph made with the known amount of α-toc and expressed in milligram per gram fresh weight.

Total phenols (TP)

TP amounts were estimated by the method of Malick and Singh (1980). 50 mg of fresh plant tissue was ground with a pestle in a mortar with 10 ml of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was evaporated to dryness. The residue was dissolved with 5 ml of distilled water and used as an 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 thoroughly. The mixture was kept in boiling water for exactly 1 min, and the absorbance was measured at 650 nm after cooling. The TP was determined using a standard curve prepared with the different concentration of gallic acid. The results were expressed in milligram per gram fresh weight.

Statistical Analysis

The pot culture experiment was conducted in CRBD SPSS software version 16.0 was to make a statistical analysis. The data analysis was performed using one-way analysis of variance followed by Duncan's multiple range test. The values are mean ± standard deviation for six samples in each group. $P \leq 0.05$were considered as significant.

RESULTS AND DISCUSSION

The Influence of IBA and Triazole Compounds on Shoot System

In the present investigation, the triazole treatments significantly reduced the shoot length and internodal length when compared to control plants. But IBA treatment increased shoot length and internodal length at a higher level when compared to a control plant. Among the triazole treatments, PCZ treated plants exhibited the more pronounced effect of reduced stem length than TDM treatment [Table 1]. Similar results were observed in PCZ and hexaconazole treated *Plectranthus aromaticus* and *Plectranthus vettiveroides* (Meena Rajalekshmi *et al.*, 2009). Paclobutrazol suppressed the shoot height in tomato plant

Table 1: Influence of IBA, TDM, and PCZ on the shoot length, internodal length, number of leaves, total leaf area, shoot fresh and dry weight of Ashwagandha

Growth parameters	Growth stages (DAS)	Control	IBA	TDM	PCZ
Shoot length (cm/plant)	60	36.00±2.74[a]	41.33±3.149[b]	33.66±2.562[a]	32.96±2.513[a]
	100	53.18±4.068[b]	62.73±4.800[c]	48.02±3.676[a]	47.13±3.607[a]
	140	66.04±5.026[b]	79.01±6.016[c]	57.36±4.367[a]	56.61±4.309[a]
Internodal length (cm/plant)	60	3.60±0.273[ab]	3.90±0.295[b]	3.40±0.260[a]	3.30±0.25[a]
	100	4.10±0.315[b]	4.55±0.347[c]	3.80±0.288[ab]	3.70±0.282[a]
	140	4.40±0.336[b]	4.90±0.37[c]	4.00±0.304[a]	3.93±0.295[a]
Number of leaves (N/plant)	60	86.01±5.549[a]	95.00±6.235[b]	82.00±6.245[a]	81.00±5.168[a]
	100	168.08±11.86[a]	193.00±12.78[b]	156.00±11.94[a]	154.08±10.79[a]
	140	230.04±15.5[b]	266.04±17.48[c]	210.04±13.99[ab]	206.04±12.68[a]
Total leaf area (cm^2/plant)	60	1323.5±87.780[a]	1397.0±96.431[b]	1283.2±83.711[a]	1270.4±80.739[a]
	100	2768.0±162.02[ab]	2985.5±178.67[b]	2609.6±149.82[a]	2592.2±148.41[a]
	140	3849.5±223.20[a]	4233.2±256.4[b]	3583.6±207.87[a]	3548.6±203.2[a]
Shoot fresh weight (g/plant)	60	98.77±6.52[a]	108.61±7.269[b]	93.01±6.082[a]	92.34±6.033[a]
	100	163.33±10.501[a]	185.54±12.203[b]	150.28±9.505[a]	148.86±9.393[a]
	140	216.94±13.517[b]	251.31±16.133[c]	194.20±12.788[a]	192.53±12.660[a]
Shoot dry weight (g/plant)	60	16.15±1.227[a]	18.00±1.370[b]	15.37±1.169[a]	15.003±1.142[a]
	100	28.91±2.212[a]	33.12±2.536[b]	26.84±2.053[a]	26.13±1.999[a]
	140	39.73±3.028[a]	46.46±3.539[c]	36.03±2.741[a]	35.42±2.697[a]

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 $P \leq 0.05$.
IBA: Indole-3-butyric acid, TDM: Triadimefon, PCZ: Propiconazole, DAS: Days after sowing, SD: Standard deviation

(Still and Pill, 2003) and in barley (Sarkar *et al*., 2004) and it also reduced the internodal length and stem diameter in *Arbutus unedo* (Navarro *et al*., 2009). TDM treatment reduced stem elongation and plant height in *Catharanthus roseus* (Jaleel *et al*., 2008b), *Plectranthus forskholli* (Alagu Lakshmanan *et al*., 2007), *Cassava* (Gomathinayagam *et al*., 2007), radish (Sridharan *et al*., 2006a) and also decreased the plant height and internodes length in *Oryza sativa* (Sakamoto *et al*., 2004). IBA treatment enhanced plant growth as indicated by plant height in *Alnus glutinosa* (San Jose *et al*., 2012), *Pisum sativum* (Amal *et al*., 2009) and in *Rosa* species (Hussain and Khan, 2004). The highest shoot length was recorded in response to IBA treatment in *Avicennia marina* (Abdulaziz *et al*., 2004).

Triazole treated plants showed reduced number of leaves and total leaf area when compared to control while it was increased under IBA treatment. Among the triazole treatments, PCZ inhibited the number of leaves and leaf area to a larger extent when compared to TDM [Table 1]. Similar results were observed in PCZ and TDM treated radish (Sridharan *et al*., 2006a) and PCZ treatment reduced the leaf area in *C. roseus* (Jaleel *et al*., 2008a) and TDM treated *Solenostemon rotundifolius* (Kishorekumar *et al*., 2007a). Paclobutrazol treatment also reduced the leaf area in *C. roseus* (Jaleel *et al*., 2006), barley (Sunitha *et al*., 2004) and in *Beta vulgaris* (Velayutham *et al*., 2003). IBA treatment caused significant increased leaf area in all stages of growth in *Cucurbita pepo* (Sure *et al*., 2012), *Berberis thungbergii* (Pacholczak, 2006) and *Tylophora indica* (Faisal and Anis, 2006).

Triazole treatments significantly lowered the shoot fresh and dry weight. However, IBA treatment increased the shoot fresh and dry weight of *W. somnifera* when compared to a control plant. Among the triazole treatments, PCZ treatment decreased it to a larger extent when compared to TDM [Table 1]. Difenoconazole treatment reduced shoot fresh and dry weight in *Mentha piperita* (Kavina *et al*., 2011a). TDM and hexaconazole treatments suppressed plant height in radish (Sankari *et al*., 2006) and in barley (Sarkar *et al*., 2004). Triazole treatments reduced the stem elongation, plant height and decreased the fresh and dry weight in *Citrus* (Mehouachi *et al*., 1996). IBA treatment increased the root and shoot fresh weight in *Allium sativum* (Bideshki *et al*., 2013). IBA showed significantly increased the leaves number and plant fresh and dry weight in *P. sativum* (Amal *et al*., 2009), African violet plant (Martin-Max *et al*., 2005) and in three *Vitex* species (Priya, 2008). The total fresh and dry weight of shoot per cutting increased in IBA treatment in grapevine cuttings (Kaur *et al*., 2002) and also increased the leaf fresh weight in *T. indica* (Faisal and Anis, 2006).

Triazoles reduced the cell number, length and width of the xylem cells (Fletcher *et al*., 2000). The reduced plant height and increased the thickness of the young plant stem, as well as the accelerated root formation are the significant of the triazole treatment in *Lycopersicon esculentum* (Berova *et al*., 2000). The inhibition of GA biosynthesis, as well as increased ABA content induced by triazole treatment, could be the reason for the inhibition of stem and leaf expansion in the triazole treated *W. somnifera*. Hence, by

the above reasons the triazole decreased shoot length, internodal length, the number of leaves, total leaf area, the fresh and dry weight of shoot in Ashwagandha.

Auxins have been shown to regulate different aspects of plant growth and development by affecting numerous processes including cell division, cell elongation, and differentiation. (Woodward and Bartel, 2005). Auxin also plays a significant role in stimulates the cell division in the cambium, enhances epical dominance by suppression of lateral bud growth. An osmotic equilibrium exists in a cell where the turgor pressure developed is counterbalanced by the wall pressure acting in the opposite direction. Regarding the mechanism of cell elongation, it is thought that auxins stimulate cell elongation by modifying certain conditions responsible for this equilibrium (Devlin, 1969) and this is the reason for the increase in shoot growth by IBA treatment.

The Influence of IBA and Triazole Compounds on Root System

The root length was increased by PCZ, TDM and IBA treatments in *W. somnifera*. Among the treatments, IBA increased root length at a higher level than PCZ and followed by TDM when compared to control plant [Table 2]. PCZ and hexaconazole treatments increased the root length in *P. aromaticus* and *Plectranthus vetiveroids* (Meena Rajalekshmi *et al*., 2009). TDM treatment increased the root length in *C. roseus* (Jaleel *et al*., 2008b). Paclobutrazol increased the root length and enhanced the lateral roots in tomato plants (Berova *et al*., 2000). Triazole compounds increased the root growth in cucumber, which was associated with increased cytokinin levels (Feng *et al*., 2003). IBA hormone levels significantly increased the root length and root number in micro cuttings of *Camellia sinensis* (Bidarigh *et al*., 2012). IBA also increases the root length and root number in *Poinsettia pulcherrima* (Ramtin *et al*., 2011) and *Khaya anthotheca* and *Khaya ivorensis* (Frimpong *et al*., 2008), Similar results were observed in IBA treated *Dalbergia sissoo* (Husen, 2008).

Triazole treated plants showed an increased the root diameter when compared to IBA treated plants [Table 2]. PCZ and TDM increased the root length while the tuber girth in radish (Sridharan *et al*., 2006a). Increased root diameter has been correlated with larger cortical parenchyma cell in soybean and maize (Barnes *et al*., 1989). The increased root diameter in *Chrysanthemum* was due to an increase of rows and diameter of cortical cells (Burrows *et al*., 1992). Paclobutrazol also increased the root diameter by increasing the width of the cortex and by favoring formation of more secondary xylem vessels. Depending on the plant species and the concentration, PBZ caused thickening of maize root and increased their starch content (Baluska *et al*., 1993). IBA treatment increased average adventitious root thickness, rooting capacity and quality rooting due to an increase in root carbohydrates content in apple cuttings (Karakurt *et al*., 2009).

The triazole compounds like TDM and PCZ increased the number of roots and IBA also increased the number of roots in Ashwagandha. Among the treatments, IBA increased higher level than PCZ and followed by TDM when compared to control [Table 2]. TDM and paclobutrazol effectively stimulated rooting in bean hypocotyls (Davis *et al*., 1985; Fletcher and Hofstra, 1988). Paclobutrazol treated roots of tomato plants increased the root length and enhanced the lateral roots (Berova *et al*., 2000). Triazole promoted adventitious root formation in mung bean by interfering with gibberellin biosynthesis (Porlings

Table 2: Influence of IBA, TDM and PCZ on the root length, root diameter, number of roots, root fresh and dry weight of Ashwagandha

Growth parameters	Growth stages (DAS)	Control	IBA	TDM	PCZ
Root length (cm/plant)	60	18.26±1.388^{a}	23.66±1.801^{b}	22.55±1.715^{b}	22.90±1.742^{b}
	100	26.89±2.057^{a}	35.52±2.718^{b}	34.01±2.604^{b}	34.91±2.671^{b}
	140	34.90±2.656^{a}	48.00±3.655^{bc}	45.51±3.465^{b}	46.00±2.656^{b}
Root diameter (cm/plant)	60	1.63±0.125^{a}	1.95±0.161^{ab}	2.15±0.168^{b}	2.22±0.170^{b}
	100	2.45±0.188^{a}	3.10±0.246^{b}	3.40±0.262^{b}	3.44±0.264^{b}
	140	3.60±0.273^{a}	4.76±0.362^{b}	5.10±0.389^{b}	5.18±0.394^{b}
Number of roots (N/plant)	60	13.00±0.990^{a}	18.00±1.370^{c}	16.00±1.218^{b}	17.00±1.294^{bc}
	100	18.01±1.379^{a}	26.01±1.990^{c}	23.01±1.762^{b}	24.01±1.838^{bc}
	140	22.00±1.675^{a}	32.00±2.437^{c}	29.00±2.208^{b}	30.00±2.284^{bc}
Root fresh weight (g/plant)	60	16.55±1.258^{a}	19.00±1.447^{b}	19.90±1.514^{b}	20.44±1.558^{b}
	100	28.87±2.212^{a}	34.54±2.644^{b}	35.62±2.725^{b}	36.25±2.774^{b}
	140	39.95±3.046^{a}	49.87±3.79^{b}	51.55±3.924^{b}	51.00±3.884^{b}
Root dry weight (g/plant)	60	3.64±0.277^{a}	4.09±0.313^{b}	4.24±0.322^{b}	4.35±0.331^{b}
	100	5.91±0.450^{a}	6.87±0.526^{b}	7.16±0.546^{b}	7.30±0.559^{b}
	140	10.50±0.799^{a}	12.79±0.972^{b}	13.00±0.990^{b}	13.19±1.007^{b}

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 $P \leq 0.05$.
IBA: Indole-3-butyric acid, TDM: Triadimefon, PCZ: Propiconazole, DAS: Days after sowing, SD: Standard deviation

and Petridou, 1996). IBA has a better capacity to stimulate the root growth than indole-3-acetic acid treatment in *Arabidopsis thaliana* (Essemine *et al*., 2011). The numbers of root per cutting were increased by IBA in *K. anthotheca* and *K. ivorensis* (Frimpong *et al*., 2008). IBA treatment is more favorable for adventitious root formation in single node cuttings of *Ricinodendron heudelotii* (Tchinda *et al*., 2013) and IBA can promote root growth, including root number and length, and collar diameter, which helped the enhancement of the root system (Nagel *et al*., 2001).

The fresh and dry weight of the root increased significantly in all the treated plants. Among the treatments, PCZ and TDM increased higher level than IBA treated plants of *W. somnifera* [Table 2]. Triazole compounds increased the fresh and dry weight of tuber in radish (Sridharan *et al*., 2006a; 2006b). TDM and hexaconazole treatments increased tuber fresh and dry weights in *Cassava* (Gomathinayagam *et al*., 2007). IBA was found to be more efficient in increasing the root fresh weight and dry weight in *Morinda citrifolia* (Baque *et al*., 2010) and *A. thaliana* (Essemine *et al*., 2011) IBA significantly increased adventitious rooting percentage, the numbers of roots and root biomass for *Azadirachta indica* and *Pongamia pinnata* (Palanisamy *et al*., 1998). Similar result was observed in *Khaya anthohera,* and *Khaya irorensis* (Frimpong *et al*., 2008). IBA normally initiate formation of roots. This is due to having the meristematic activity so that the meristematic tissues give rise to new lateral roots and thus resulted in increased root fresh and dry weight in Ashwagandha plant by IBA treatment.

Increase in root growth and root length was high in triazole treatments than IBA treated plant, Because, the triazole compounds inhibited gibberellin biosynthesis and increased cytokinin and abscisic acid content and this might be the cause for reduced shoot growth, but improve the root growth by source and sink relationship so the photosynthates sinked in the storage material in the root part of the tissues higher in triazole treatment than that of IBA treated plant roots, So that the shoot length was reduced and roots length, root diameter and number of roots were increased by the triazole treated plants when compared to IBA treated plants. For that reason, triazole treated plants root length, the number of roots, root diameter and fresh and dry weight was increased, likewise the medicinal parts of Ashwagandha roots also showed increasing trend in all the root parameters when compared to IBA treatment.

The Influence of IBA and Triazole Compounds on Non-Enzymatic Antioxidants Constituents

All the treatments significantly increased the non-enzymatic antioxidant constituents like, AA, α-toc and TP contents in all parts of *W. somnifera* when compared to control plants. Among the treatments, PCZ and TDM caused a profound effect of increased antioxidant contents when compared to IBA. Among the plant parts, roots had higher AA, α-toc and TP contents when compared to stem and leaf [Table 3 and Figures 1 and 2]. TDM and hexaconazole treatments increased the non-enzymatic antioxidants like AA and α-toc contents in *Raphanus sativus* (Sridharan *et al*., 2009b; 2009c), similar results were observed in sweet potato (Sivakumar *et al*., 2010). PCZ treatment increased AA and α-toc content in *Vigna unguiculata* (Manivannan *et al*., 2007) and *Gloriosa superba* (Kavina *et al*., 2011). The increase in AA content was reported in the paclobutrazol treated grape fruits (Fucik and Swietlik, 1990) and *Citrus* lemon (Jain *et al*., 2002). TP content was increased in triazole treated radish (Sankari *et al*., 2006) and in paclobutrazol treated mango seedlings (Murti and Upreti, 2003). Phenol content was increased in coleus under hexaconazole treatments (Alagu Lakshmanan *et al*., 2007).

Auxin treatments increased the AA in tomato plant (Tyburski *et al*., 2006). IBA treatment increased the AA and α-toc in three species of *Vitex* (Priya, 2008). A TP content was higher in IBA treated cuttings particularly at the initiation and expression stages in *Saraca asoka* (Dash

Table 3: Influence of IBA, TDM and PCZ on the AA content in Ashwagandha

Plant parts	Growth stages (DAS)	Control	IBA	TDM	PCZ
AA (mg/g fr. wt.)					
Leaf	60	5.101±0.389^{a}	5.421±0.412^{a}	5.956±0.452^{b}	6.011±0.456^{b}
	100	7.355±0.564^{a}	7.995±0.613^{a}	8.913±0.682^{b}	9.003±0.687^{b}
	140	9.268±0.707^{a}	9.798±0.748^{ab}	10.563±0.804^{c}	10.630±0.808^{c}
Stem	60	6.90±0.524^{a}	7.341±0.559^{ab}	8.001±0.609^{b}	8.051±0.609^{b}
	100	8.125±0.622^{a}	8.728±0.667^{a}	9.705±0.743^{b}	9.905±0.756^{b}
	140	9.928±0.757^{a}	10.421±0.793^{ab}	11.212±0.855^{b}	11.403±0.868^{b}
Root	60	7.206±0.546^{a}	7.773±0.593^{a}	8.556±0.649^{b}	8.621±0.658^{b}
	100	9.905±0.756^{a}	10.995±0.84^{b}	12.081±0.924^{c}	12.200±0.935^{c}
	140	11.380±0.864^{a}	12.121±0.922^{ab}	12.971±0.990^{b}	13.102±0.998^{b}

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 $P \leq 0.05$.
IBA: Indole-3-butyric acid, TDM: Triadimefon, PCZ: Propiconazole, DAS: Days after sowing, SD: Standard deviation, AA: Ascorbic acid

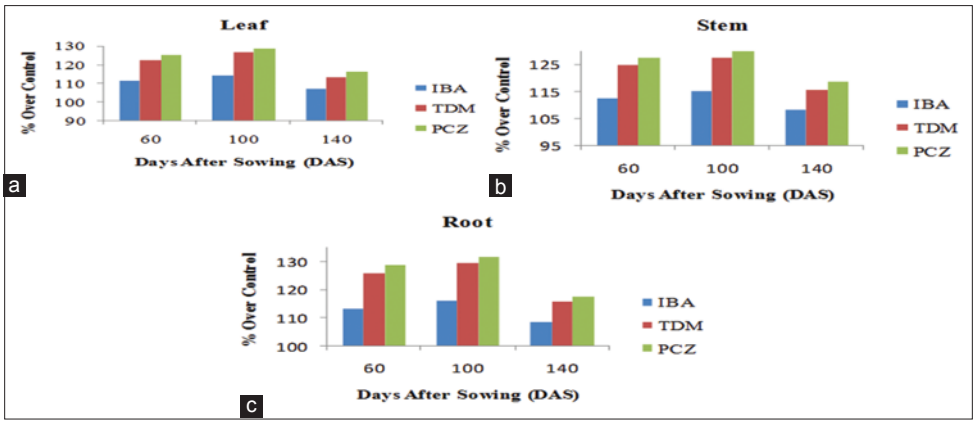

Figure 1: (a-c) Influence of indole-3-butyric acid, triadimefon and propiconazole on the α-tocopherol content in Ashwagandha

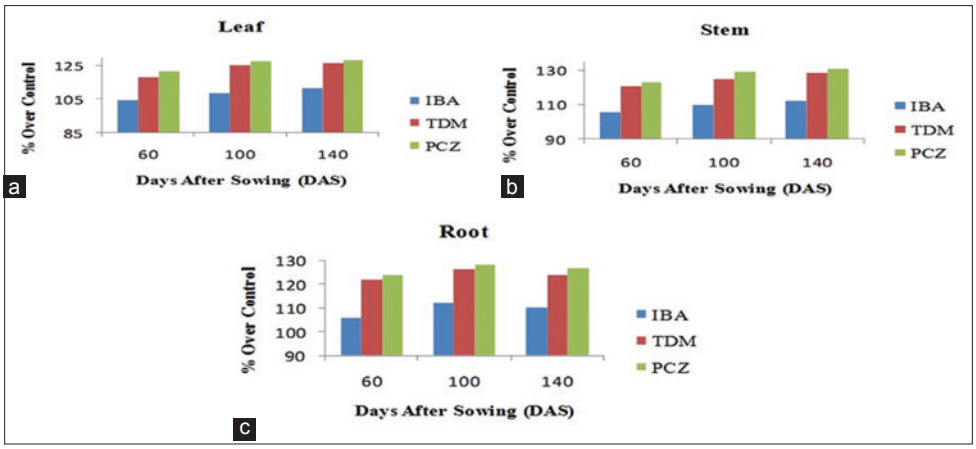

Figure 2: (a-c) Influence of indole-3-butyric acid, triadimefon and propiconazole on the total phenol content in Ashwagandha

et al., 2011) and in *R. heudelotii* (Tchinda *et al*., 2013). Enhanced production of phenolic compounds by IBA was recorded in *Thymus vulgaris* (Karalija and Paric, 2011) and *P. sativum* (Amal *et al*., 2009). Foliar application of IBA concentrations gave the highest values for TP and proline content observed in maize and onion plant (Amin *et al*., 2006; 2007). IBA treatment caused an increase in total soluble protein and phenol contents in grapevine cuttings (Sukhwant *et al*., 2002).

Triazole increased the level of the antioxidants like AA and α-toc content in *W. somnifera*. The non-enzymatic antioxidant contents play major roles in maintaining the balance between free-radical production and elimination (Jaleel *et al*., 2007a; 2007b; Lin *et al*., 2006) triazole caused a profound influence upon the regulatory mechanisms of the plant as a whole including the increase of antioxidants (Fletcher *et al*., 2000a). The enhancement of antioxidant defense mechanism under triazole treatment can be correlated with the ability of triazoles in protecting plants from abiotic stresses (Manivannan *et al*., 2007; Jaleel *et al*., 2007a; 2007b; Sridharan and Panneerselvam 2009a; Sridharan *et al*., 2009b). Protection of plants from apparently unrelated stress by triazole and growth hormones is also mediated by a reduction of free radical damage and increase in the antioxidant potential from chilling damage and the stress protection was mediated by an increase in α-toc, AA and enhanced antioxidant activities (Fletcher and Hofstra, 1988). Hypothesized a cycle where H_2O_2 is scavenged by phenolic compounds. Phenolics are oxidized to phenoxyl radicals. This phenoxy radical reduces the AA into monodehydroascorbate (Sgherri *et al*., 2003) antioxidant metabolites such as ascorcorbate, phenols, α-toc, glutathione, and carotenoids are involved in scavenging reactive oxygen species (ROS) (Vranova *et al*., 2002). From the present results, it can be concluded that the PCZ and TDM application can enhance the root growth and non-enzymatic antioxidants to a great extent and is of great importance in imparting economic and medicinal values of *W. somnifera*.

In auxin treatments increased the AA content in tomato (Tyburski *et al*., 2006). The increase in peroxidase and hydroxyl radicals is involved in recycling of AA in the ascorbate glutathione pathway in chloroplasts (Foyer, 1993). Increasing auxin concentration causes the

accumulation of H_2O_2, which in turn may function in increasing the activity of catalase to prevent the high accumulation of H_2O_2 and other ROS in plant cells (Sharifi and Ebrahimzadeh, 2010). Non-enzymatic antioxidants in potentiating antioxidative defenses system and ameliorating oxidative damage have significant implications in the mulberry in relation to stress tolerance (Guha *et al*., 2012). The metabolism of the exogenously provided auxin (IBA), especially its combination with phenolic compounds has been considered in relation to the promotion of adventitious rooting (Weisman *et al*., 1988). Phytohormones can regulate the synthesis of basic antioxidant enzymes, and some of the isoforms of antioxidant enzymes are also implicated in phytohormone catabolism (Szechynska-Hebda *et al*., 2012).

CONCLUSION

Ashwagandha is mainly cultivated for its root, which has medicinal and nutritional value due to the presence of clinically important active compounds such as steroidal lactones, alkaloids, flavonoids, tannins, etc. The root of Ashwagandha is the medicinal part of the plant and is used as herbal tonic and health food. In the present investigation, the influence of triazole and IBA on the induced alteration in terms of growth modulation, root yield and antioxidant constituents in Ashwagandha were analyzed, and the following conclusions emerged. Triazole compounds promoted the underground root growth by suppressing the shoot growth. Triazole compounds, *viz.,* PCZ and TDM increased the root growth in terms of root length, the number of roots, root diameter and fresh and dry weight of roots, but decreased the shoot growth. Whereas triazoles enhance the antioxidants in like AA, α-toc and TP contents both in root and shoots. But IBA treatment increased all the parameters when compared to control. Among the treatments, triazole compounds like PCZ showed great significance for cultivation of this root crop, which is helpful to meet the needs of improvement in root growth and enhanced antioxidant contents in Ashwagandha followed by TDM and IBA, respectively.

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