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Influence of indole-3-butyric acid and triazole compounds on the photosynthetic pigments and biochemical constituents of *Withania somnifera* (L.) Dunal

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ABSTRACT

Withania somnifera (L.) Dunal is popularly known as ashwagandha and also called Indian Ginseng and Winter Cherry. It is an important medicinal plant used in Indian System of Medicines like Ayurveda, Siddha, and Unani. The root of this plant has nutritional and medicinal values out of which health foods and herbal tonic are prepared. The root has high demand in pharmaceutical and nutraceutical industries so that it is essential to increase the root yield. This is new phenomenon to increase root yield as well as improve the biochemical constituents by using plant growth regulators. In the present investigation, the influence of indole-3-butyric acid (IBA) and triazole compounds *viz.,* triadimefon (TDM) and propiconazole (PCZ) on the photosynthetic pigments and biochemical constituents 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, and 130 days after sowing (DAS). Plants were analyzed randomly on 60, 100, and 140 DAS and it's parameters like, photosynthetic pigments (total chlorophyll, carotenoids, anthocyanin, and xanthophylls) in leaf and biochemical constituents (starch, protein and amino acids) in leaf, stem and root organs of ashwagandha. It was determined that total chlorophyll, carotenoids, anthocyanin, and xanthophylls, starch, protein and amino acids content were increased in all the treatments. Among the treatments, triazole compounds showed beneficial due to the enhanced the photosynthetic pigments and increased biochemical contents higher level than followed by IBA treatment. From our results, it can be concluded that the triazole shows great significance application at low concentration could be a potential agronomical tool for successfully cultivation of this medicinally important root crops. Triazole compounds enhanced the photo-assimilate to shifting partition from leaves to roots and also alter mineral uptake and plant nutrition, This characters' can be employed to satisfy needs of enhanced the photosynthetic pigments and biochemical constituents in ashwagandha.

KEY WORDS: Ashwagandha, medicinal plant, photosynthetic pigments, biochemical constituents, indole-3-butyric acid, triazole

INTRODUCTION

Medicinal plants have been considered since time immemorial as an alternative source of a wide range of chemical compounds including pharmaceuticals, flavors, fragrance, colors, and insecticides. These compounds are collectively known as secondary metabolites in medicinal and aromatic plants and used as the remedy for health problems (Hussain, 1991; Okigbo *et al.,* 2009). India has been known to be a rich repository of medicinal plants. Several scientific studies conducted throughout the world have revealed and confirmed the dramatic medicinal properties by their inherent nature of containing various phytochemicals such as flavonoids, carotenoids, and alkaloids. In India's progress in improving the cultivation of traditional medicinal crops is a matter of pride for the nation. However, compared to other crops, little attention seems to have been given to medicinal plants which occupy a unique place in the

Indian socio-economy (Jakhar *et al.,* 2003). Therefore, many scientists have repeatedly advocated to undertake applied and fundamental research work on cultivation of medicinal plants, several of which may attain the position of important cash crops for Indian formers in future (Singh and Tyagi, 2004). Ashwagandha (*Withania somnifera* (L.) Dunal, belongs to the family Solanaceae is commonly known as Indian Ginseng and Winter Cherry. It is the most important medicinal plant used in Indian System of Medicines (Ayurveda, Unani, and Siddha) and in fact, it is mentioned as an official plant drug in the Indian Pharmacopocia (Indian Pharmacopocia, 1985). It is described as an herbal tonic and health food in Vedas and considered as "Indian Ginseng." In "Ayurveda" the root drugs are mostly used as an alternative empirical therapy for the treatment and clinical management of male infertility (Gupta *et al.,* 2013; Singh *et al*., 2010; Kokate *et al*., 2005; Puri, 2003; Kapoor, 2001). The plant has also been used as an anticancer, anti-stress, anti-inflammatory and immunomodulator (Rasool and Varalakshmi, 2006; Seartezzini and Sparoni, 2000; Agarwal *et al*., 1999) and natural antioxidant properties (Bhattacharya *et al*., 1997; Palash *et al.,* 2010). Since there is a high demand for the medicinal plants in the pharmaceutical industry, it is essential to increase the commercial cultivation, Furthermore, to ensure proper supply of medicinal plants to the drug industries. It has become necessary that these plants be propagated properly and cultivated scientifically, as well as quality and quantity (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. Plant growth retardants are synthetic compounds, which represent the commercially most important group of plant growth regulators. 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. Triadimefon (TDM) (bayleton) propiconazole (PCZ) (banner), paclobutrazole (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). 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 (ABA), and cytokinins (Fletcher and Hofstra, 1988). The plant hormones are organic substances in low concentrations regulates the growth and development. These substances belong to different classes have different physiological role in plants to modify, regulate and development. The naturally occurring plant growth substances include auxins, gibberellins, cytokinins, abscissic acid, and ethylene (Kakimoto, 2003). Triazoles and IBA have been successfully used to increase the growth and root yield of medicinal plants. Hence, the present study becomes effectual to evaluate the effect of triazole and IBA compounds on the photosynthetic pigments (total chlorophyll, carotenoids anthocyanin, and xanthophylls) and biochemical constituents (starch, protein, and amino acids) content of *W. somnifera*.

MATERIALS AND METHODS

Pot Culture Experiments and Plant Treatments

The seeds of ashwagandha (*W. somnifera* L.) variety "Jawahar Asgandh-20" were obtained from Horticultural College and Research Institute, Department of Medicinal and Aromatic Crops, Tamil Nadu Agricultural University, Coimbatore, India. Indole-3-butyric acid (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 the ratio of 1:1:1 and each pot were filed with the 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 root 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 one litter of tap water. Each pot has three plants and the treatments were given on 50th, 90th, and 130th days after sowing (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 and 140th DAS and they were used for the determination of photosynthetic pigments and biochemical constituents of *W. somnifera*.

Pigments Analysis

Total chlorophyll and carotenoid contents were extracted from the leaves and estimated by the method of Arnon (1949). Anthocyanin content was estimated following the method of Beggs and Wellmann (1985) and xanthophylls content was estimated by the method of Neogy *et al.* (2001). The results were expressed in milligram per gram fresh weight.

Biochemical Analysis

Starch content was extracted from the leaf, stem and root and estimated by the method of Clegg (1956), protein content was estimated according to Bradford (1976) and amino acid content was estimated followed by the method of Moore and Stein (1948). The results were expressed in milligrams per gram fresh weight.

Statistical Analysis

The experiment was conducted by CRBD. Statistical analysis was performed using one-way analysis of variance followed by Duncan's multiple range test. The values were expressed in mean ± standard deviation for six samples in each group $P \geq 0.5$ values were considered as significant.

RESULTS AND DISCUSSION

Influence of IBA, TDM, and PCZ on the Photosynthetic Pigments Content of Ashwagandha

Triazole and IBA treatments significantly enhanced the total chlorophyll content in leaves of *W. somnifera*. Among the triazole, PCZ, and TDM treatments increased the chlorophyll content to a higher level when compared to IBA treated plants [Table 1]. TDM and hexaconazole treatments increased the chlorophyll content in radish (Sridharan *et al.*, 2006b). TDM treatment increased the chlorophyll content in *Cucumis sativus* seedlings (Feng *et al.*, 2003) and in banana (Galal *et al*., 2011). Paclobutrazol treated barley seedlings were increased total chlorophyll content (Sunitha *et al.*, 2004), carrot (Gopi *et al.*, 2007) and tomato (Still and Pill, 2004). Paclobutrazol treated leaves were dark green due to high chlorophyll a and b content in potato (Tekalign *et al.*, 2005). Sebastian *et al.* (2002) reported enhanced chlorophyll synthesis in *Dianthiis caryophyllus* treated with paclobutrazol. Similar results were observed in PCZ treated *Amorphophallus campanulatus* (Gopi *et al.*, 2005). The increased chlorophyll content with the PCZ and TDM treatment may be due to the ability of triazole to enhance cytokinin production, which stimulates the chlorophyll biosynthesis in *W. somnifera* leaves. IBA treatment significantly enhanced the chlorophyll a & b and total chlorophyll content in grapevine cuttings (Kaur *et al.*, 2002). The total chlorophyll content increased in IBA treated *Berberis thunbergii* (Pacholczak, 2006) and also in *Pisum sativum* (El-Shraiy and Hegazi, 2009). The effect of IBA on the chlorophyll concentration has been mentioned by several workers (Ludwig-Muller, 2000; El-Wahed *et al.*, 2006).

The amount of carotenoid content of the leaves of *W. somnifera* increased with all the stages of growth. Triazole and IBA treatments increased significantly the carotenoid content when compared to control plant. Among the treatments, PCZ and TDM increased the carotenoid content larger extent when compared to IBA [Table 1]. TDM treatment induced higher level of carotenoid content in cucumber seedlings (Feng *et al.*, 2003), cowpea (Gopi *et al.*, 1999), *Catharanthus roseus* (Jaleel *et al.*, 2008) and also in maize plants (Kaya *et al.*, 2006). Paclobutrazol treatment increased the carotenoid content in *Raphanus sativus* (Sankari *et al.*, 2006). Similar results were observed in carrot (Gopi *et al.*, 2007), *Solenostemon rotundifolius* (Kishorekumar *et al.*, 2007) and barley seedlings (Sarkar *et al.*, 2004). Sunitha *et al.* (2004) reported that the barley seedlings treated with paclobutrazol appeared greener and thicker due to increased pigment contents. Similar results

Table 1: Influence of IBA, TDM and PCZ on the total chlorophyll, carotenoid, anthocyanin and xanthophyll contents of ashwagandha leaf

Photosynthetic pigments	Growth stages (DAS)	Control	IBA	TDM	PCZ
Total chlorophyll (mg/g FW)	60	1.261±0.096[a]	1.4167±0.107[b]	1.516±0.116[bc]	1.558±0.118[c]
	100	1.630±0.125[a]	1.863±0.143[b]	2.00±0.152[bc]	2.068±0.158[c]
	140	1.900±0.143[a]	2.190±0.170[b]	2.395±0.183[bc]	2.450±0.188[c]
Carotenoids (mg/g FW)	60	1.086±0.080[a]	1.213±0.092[b]	1.305±0.098[bc]	1.3333±0.100[c]
	100	1.390±0.107[a]	1.580±0.120[b]	1.748±0.132[bc]	1.791±0.136[c]
	140	1.655±0.125[a]	1.860±0.143[b]	1.935±0.147[b]	1.978±0.149[b]
Anthocyanin (mg/g FW)	60	0.930±0.067[a]	1.030±0.076[b]	1.100±0.085[bc]	1.130±0.085[c]
	100	1.210±0.094[a]	1.370±0.102[b]	1.501±0.114[c]	1.541±0.118[c]
	140	1.498±0.114[a]	1.748±0.132[b]	1.900±0.143[bc]	1.930±0.143[c]
Xynthophyll (mg/g FW)	60	0.693±0.053[a]	0.760±0.058[ab]	0.821±0.060[bc]	0.840±0.067[c]
	100	0.921±0.069[a]	1.031±0.078[b]	1.120±0.085[bc]	1.140±0.085[c]
	140	1.296±0.098[a]	1.466±0.112[b]	1.600±0.120[bc]	1.631±0.123[c]

Expressed values are 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

were observed in growth regulators treated *Catharanthus* plants (Jaleel *et al.,* 2006). Uniconazole and paclobutrazol treatment induced higher level of carotenoid content in wheat seedlings (Fletcher and Hofstra, 1988; Berova *et al.,* 2003). Increased level of uniconazole treatment and thus increased zeatin might be responsible for the increased synthesis of carotenoid in the plants (Grossmann *et al.,* 1994). Triazole increase the active oxygen species, thus delaying the senescence of wheat and prolonging the duration of flag leaf photosynthesis *Triticum aestivum* and *Didymella exitialis* (Bertelsen *et al.,* 2001; Cromey *et al.,* 2004). IBA increased the vegetative growth and pigments concentration in Maize (Kaya *et al.,* 2006). Similar result was also observed in grapevine cuttings (Sukhwant *et al.,* 2002). Growth hormones have been shown to play an important role in regulating the amount and distribution of assimilates in plants (Galston and Davies, 1969). The increased photosynthetic content in leaves increased in IBA treated cutting that these might have altered the synthesis and translocation of assimilates (Kaur *et al.,* 2002). IBA increased photosynthetic pigments in garlic and these results are agreement with Bideshki and Arvin (2013), onion (Amin *et al.,* 2007) and pea (El-Shraiy and Hegazi, 2009). The IBA increasing photosynthetic pigments increased growth parameters and bulb yield in garlic and increasing in growth parameters and yield was reported at onion (Amin *et al,* 2006; 2007).

PCZ, TDM, and IBA treatments increased the anthocyanin content of ashwagandha leaves. Among the treatments, PCZ and TDM increased larger extent when compared to IBA [Table 1]. TDM treatment increased the chlorophyll and anthocyanin content in radish (Lichtenthaler, 1979; Sridharan *et al.,* 2006b). Triazole greatly increases anthocyanin accumulation in carrot through tissue culture (Husen and Dougall, 1992). Tetraconazole increased the anthocyanin content in maize (Angela *et al,* 1997). Similar results were observed in hexaconazole and paclobutrazol treated in carrot (Gopi *et al,* 2007). Paclobutrazol treated plant leaves were dark green due to high chlorophyll content in *D. caryophyllus* (Sebastian *et al.,* 2002) and in potato (Tekalign and Hammes, 2004; Tekalign *et al.,* 2005). Triazole induced a transient raise in abscisic acid content in bean (Asare-Boamah and Fletcher, 1986). The increase in ABA content induced by triazole might be the cause for the increased anthocyanin content of ashwagandha.

Triazole and IBA treated plants showed increased xanthophyll content at all stages of growth. Among those, PCZ increased it to a higher level than TDM when compared to IBA treated *W. somnifera* leaves [Table 1]. Triazole compounds increased photosynthetic pigments and induced a variety of morphological, physiological, biochemical responses in *A. campanulatus* (Gopi *et al.,* 2005). Triazole treatment increased the xanthophyll content to a higher level in cucumber (Feng *et al,* 2003) and carrot (Gopi *et al,* 2007). TDM treatment increased the chlorophyll, carotenoid and xanthophyll content in the leaves of barley (Forster, 1978). The unsaturated C_{40} hydrocarbons not only give color to fruits and flowers but also have multiple functions in photosynthesis. They participate in light harvesting in photosynthetic membranes and protect the photosynthetic apparatus from excessive light energy by quenching triplet chlorophylls and singlet oxygen (Siefermann-Harms, 1987). PBZ increased the chlorophyll content to a larger extent when compared to the control plants in *rosea* and *alba* varieties of *C. roseus* (Jaleel *et al.,* 2008).

Auxins are another group of endogenous plant growth substances. The major site of cytokinin biosynthesis in higher plants is the root, and then transported to the aerial portions of the plant through the xylem. These hormones have potent effects on plant physiology and are intimately involved in the regulation of cell division, apical dominance, chloroplast development, anthocyanin production and maintenance of the source-sink relationship (Hutchinson and Kieber, 2002). Growth hormones have been shown to play an increasingly important role in regulating the amount and distribution of assimilates in plants (Galston and Davies, 1969). IBA might have altered the synthesis and translocation of assimilates. This may be due to alterations in the rate of photosynthesis and outflow of assimilates from leaves. So, triazole and IBA treated ashwagandha plants increased the chlorophyll, anthocyanin, and xanthophylls content.

Influence of IBA, TDM, and PCZ on the Biochemical Contents of Ashwagandha

In ashwagandha plants, the starch contents were increased with all stages of growth in the control and treated plants. The starch contents increased in all the plant parts by the triazole and IBA treatments. Among the treatments, PCZ and TDM caused a profound effect in the increase of starch content when compared to IBA. Among the plant parts, roots had higher level accumulation of biochemical contents when compared to leaves and stem in PCZ treatments [Table 2]. Triazole exhibited a higher level of accumulation of starch content in root at all stages of growth when compared to IBA treated plants. Triazole compounds are known to alter the carbohydrate status in various plants like sweet orange (Vu and Yelenosky, 1992) and potato (Kapur *et al.,* 1993). TDM and paclobutrazol increased the nonstructural carbohydrates in mature tuber of *Dioscorea* (Jaleel *et al.,* 2007) and *S. rotundifolius* (Kishorekumar *et al.*, 2007). Triazole compounds are known for their altering effect of carbohydrate metabolism in many plants (Fletcher *et al.,* 2000). The increased starch content in triazole treated plants may be due to decrease in starch hydrolysis as reported in treated Bean (Steffens *et al.,* 1983; Upadhyaya *et al.,* 1986). Starch is the predominant carbohydrate reserve in many plants and is found in both the photosynthetic tissues (Slattery *et al.*, 2000). Triazole inhibited the gibberellins biosynthesis while increasing the cytokinins. The increased cytokinin content induced by TDM and PCZ might have increased the starch content in roots of ashwgandha. IBA and NAA concentration increased the major reserve compounds such as starch and soluble carbohydrates content in *Aechmea blanchetiana* (Chu *et al.,* 2010). IBA treatment significantly increased the total carbohydrate content in *P. sativum* (El-Shraiy and Hegazi, 2009). Similar results have observed in *Zea mays* (Amin *et al.,* 2006). IBA caused a gradual increase in the total carbohydrate in *Thunbergia grandiflora* and *Beaumontia grandiflora* (Hussein, 2008, 2003).

In all the treatments significantly increased the protein content in the leaves and in roots of *W. somnifera*. Among the treatments, PCZ and TDM caused higher level of accumulation of protein content of leaf and root at all stages of growth when compared to IBA. Among the plant parts storage roots had higher content when compared to leaves and stem [Figure 1]. The biochemical content increased, while protein, amino acid, were increased due to TDM treatment and lead to early sprouting in *Dioscorea rotundata* (Jaleel *et al.,* 2007). TDM treatment increased the protein content in cucumber seedling (Feng *et al.,* 2003). Difenoconazole caused higher level of protein accumulation in all parts of *Mentha piperita* (Kavina *et al.,* 2011). TDM and PCZ treatment increased the protein content in *R. sativus* (Sridharan *et al.,* 2006a). Ketaconazole treatment increased the protein content in cowpea (Gopi *et al.,* 1999; Somasundaram *et al.,* 2005). TDM treatment increased the protein content in *C. roseus* (Jaleel *et al.,* 2008). Esashi and Leopold (1968) correlated the increased soluble protein synthesis with the enhanced cytokinin content of the tubers of *Begonia evansiana.* The increased cytokinin content induced by TDM and PCZ treatments might have increased the protein content in the leaf, stem and root of *W. somnifera*. The protein content was significantly higher in both IBA and NAA treated

Table 2: Influence of IBA, TDM and PCZ on the starch content of ashwagandha

Plant parts	Growth stages (DAS)	Control	IBA	TDM	PCZ
Starch (mg/g fr. wt.)					
Leaf	60	13.661 ± 1.039^{a}	14.731 ± 1.124^{ab}	15.320 ± 1.167^{c}	15.430 ± 1.175^{c}
	100	15.006 ± 1.148^{a}	16.495 ± 1.262^{ab}	17.190 ± 1.316^{c}	17.310 ± 1.325^{c}
	140	16.370 ± 1.247^{a}	18.023 ± 1.370^{ab}	19.003 ± 1.447^{c}	19.123 ± 1.455^{c}
Stem	60	12.038 ± 0.918^{a}	12.931 ± 0.981^{ab}	13.623 ± 1.037^{c}	13.745 ± 1.046^{c}
	100	13.711 ± 1.050^{a}	14.816 ± 1.130^{ab}	15.813 ± 1.211^{c}	15.911 ± 1.220^{c}
	140	15.103 ± 1.151^{a}	16.803 ± 1.276^{b}	17.713 ± 1.348^{c}	17.831 ± 1.359^{c}
Root	60	16.223 ± 1.236^{a}	17.673 ± 1.344^{ab}	18.763 ± 1.429^{c}	18.820 ± 1.433^{c}
	100	18.033 ± 1.381^{a}	19.853 ± 1.518^{ab}	21.283 ± 1.630^{c}	21.511 ± 1.645^{c}
	140	20.013 ± 1.523^{a}	22.376 ± 1.704^{b}	24.336 ± 1.852^{c}	24.513 ± 1.868^{c}

Expressed values are mean±SD of six replicates in each group. Values that are not sharing a common superscript (a, b, c) differ significantly at $P\leq0.05$. IBA: Indole-3-butyric acid, TDM: Triadimefon, PCZ: Propiconazole, DAS: Days after sowing, SD: Standard deviation

Aechmea blanchetiana (Chu *et al.*, 2010). IBA treatment also increases the proteins in maize plants (El-Wahed *et al.*, 2006), *Dalbergia sissoo* (Husen, 2008) and grapevine (Kaur *et al.*, 2002).

In ashwagandha plants, the amino acid content increased with the age in control and treated plants in all growth stages. In root and leaves, a maximum increase was noted on 140 DAS in triazole when compared to IBA treatments. Among the organs, leaf tissue accumulated higher level of amino acids than stem and root tissue of the *W.somnifera* with triazole treatments [Figure 2]. Similarly, TDM treatment increased the amino acid content in *Catharanthus* in all parts at all growth stage (Jaleel *et al.,* 2007). Hexaconazole and paclobutrazol treatments increase in amino acid content was reported in carrot plants by Gopi *et al.* (2007) and TDM and PCZ treated in *R. sativus* (Sridharan *et al.,* 2006a). Uniconazole and ABA treatment were found to increase the concentration of amino acid, protein and proline in *Phaseolus vulgaris* (Mackay *et al.,* 1990). Similarly, Penconazole induced a moderate increase in amino acid in higher plants (Radice and Pesci, 1991). TDM increased

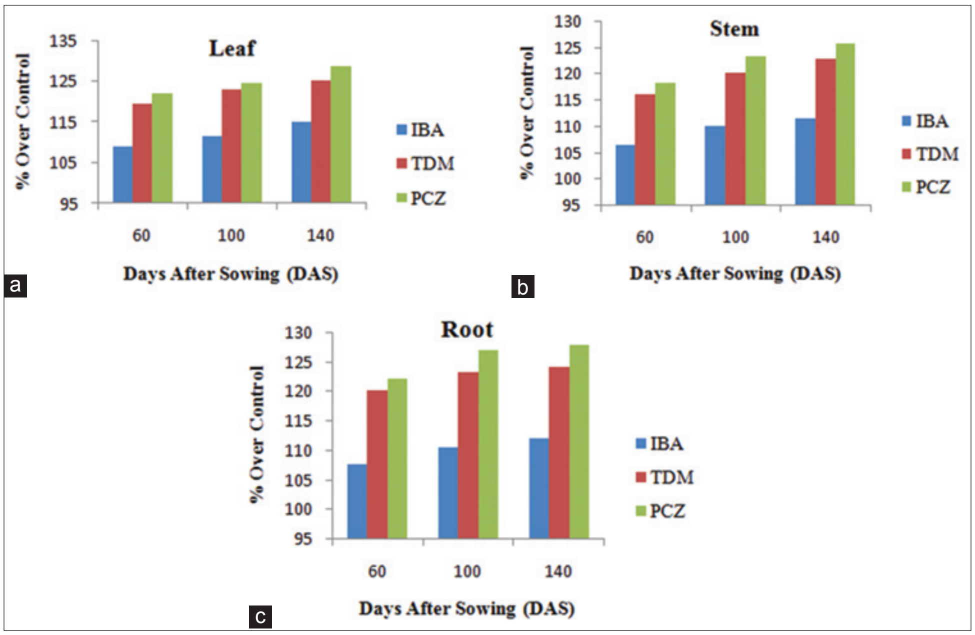

Figure 1: (a-c) Influence of indole-3-butyric acid, triadimefon and propiconazole on the protein content of ashwagandha

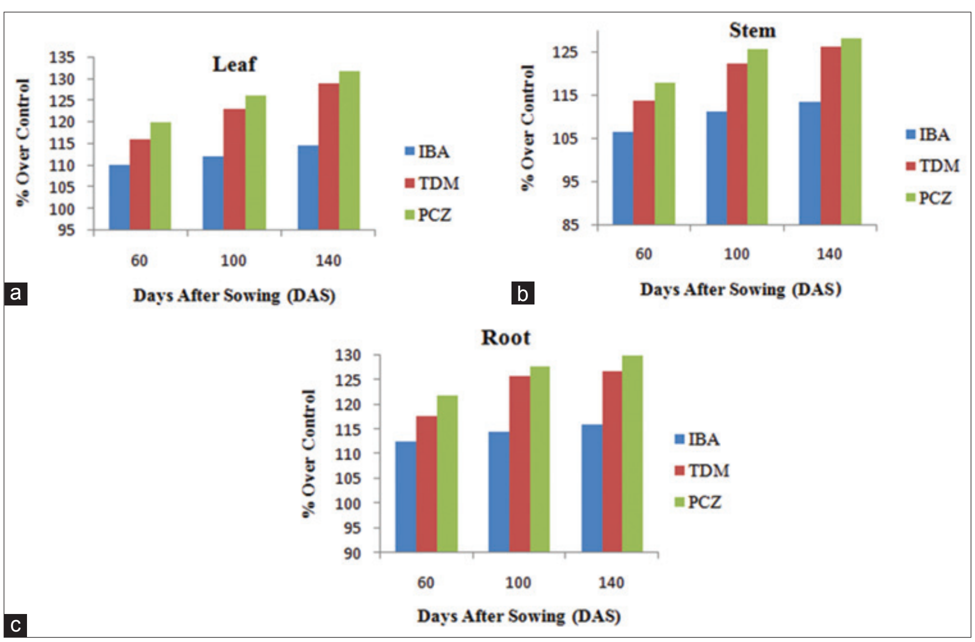

Figure 2: (a-c) Influence of indole-3-butyric acid, triadimefon, and propiconazole on the amino acid content of ashwagandha

the amino acid content in soybean (Panneerselvam *et al.,* 1998) and radish (Muthukumarasamy *et al.,* 2000). IBA treated pea plant significantly increased the amino acid content (El-Shraiy and Hegazi, 2009) and garlic (Bideshki and Arvin, 2013).

CONCLUSION

All the treatments significantly increased photosynthetic pigments and biochemicals constituents of leaf, stem and root in *W. somnifera*. Among the treatments, PCZ and TDM caused a pronounced effects on increased the photosynthetic pigments (total chlorophyll, carotenoids anthocyanin, and xanthophylls) and biochemical constituents (starch, protein, and amino acids) content when compared to IBA. Triazole treated plants typically appear dark greener and this has been correlated with higher level of chlorophyll content in ashwagandha leaves. Triazole compounds Viz., PCZ and TDM act as growth regulators or retardants to enhanced the biochemical constituents such as., starch, protein and amino acids contents, in starch more in roots, which is helpful to satisfy the needs of improve the root growth and enhance the biochemicals of medicinally important root crops of ashwagandha.

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