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Effect of triazoles and indole-3-butyric acid on growth of *Adhatoda vasica* cuttings

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ABSTRACT

In the present investigation, triadimefon (TDM), hexaconazole (HEX), and indole-3-butyric acid (IBA) treatments in *Adhatoda vasica* plants were done to study the comparative effects of traditional as well as non-traditional growth regulators on the rooting behavior. The traditional growth regulator selected was IBA and non-traditional one was HEX and TDM. TDM and HEX treatments decreased the height of the plant, and IBA increased the height of the plant. The leaf area also reduced under triazole treatments, whereas leaf area increased under IBA treatment. The root growth was significantly increased at all stages of growth with TDM, HEX, and IBA treatments. Toward maturity, the whole plant fresh weight increased considerably with TDM, HEX, and IBA treatments in *A. vasica* plants when compared to control. The dry weight also increased in TDM, HEX, and IBA treatments due to the increase in root growth. These preliminary results prove TDM and HEX as potential growth regulators which can be used to enhance the rooting of *A. vasica,* thereby make the propagation easy in this medicinal plant.

KEY WORDS: *Adhatoda vasica, Cutting,* Indole-3-butyric acid*, Triadimefon*

INTRODUCTION

Adhatoda vasica Nees is an evergreen shrub belongs to Acanthaceae originated in India, Nepal, and South Asia. Leaves are elongated and emit an unpleasant odor when crushed (Hati *et al.*, 1990). The *A. vasica* has been used for the treatment of various diseases and disorders. The ayurvedic system of medicine describes the use of this plant for treatment of respiratory ailments, particularly for the treatment of a cough, bronchitis, asthma, and tuberculosis. It is also claimed that it causes thinning of sputum and phlegm in bronchitis and asthma. Alkaloids are secondary metabolites originating generally from amino acids and therefore, contain nitrogen in their molecular structure. Alkaloids are found in 15% of all plants. In the *A. vasica,* alkaloids are vasicine and vasicinone.

The current methods of propagation of *A. vasica* are very cumbersome due to the slow growth and other dormancy problems. The rooting of stem cuttings is one of the best and economically viable methods of vegetative propagation in horticulture and forestry, to get plants of desired genetic types (Hati *et al.*, 1990). The proper formation of adventitious roots at the base of stem cuttings is an important developmental phenomenon in the growth and survival of cuttings, and it involves the initiation of several new meristematic areas in the differentiated tissues of stem cuttings and their subsequent development into mature root structures (Kaur *et al.*, 2002). However, it is very difficult to get plantlets from stem cuttings of *A. vasica*. However, it can be done with the help of plant growth regulator applications. The literature relating to the use of synthetic growth regulators, particularly auxins and indole-3-butyric acid (IBA) in plants like grapevine cutting propagation, is extensive (Krack *et al.*, 1981).

Keeping these in view, triazole compounds *viz.*, Triadimefon (TDM) [1-(4-chlorophenoxy)-3,3-dimethyl–1-(1H-1,2,4–triazole–1-y1)–2 butanone] ($C_{14}H_{16}ClN_3O_3$) M.W. 293.75), Hexaconazole (HEX) (2–(2,4–dichlorophenyl)–1–(1H–1,2,4–triazol–1 y1) hexan 2–0l) ($C_{14}H_{17}Cl_2N_3O$) M.W. 314.2), and IBA (C_6H_4. NH.CH:C$(CH_2)_3$COOH) M.W.203.24) have been chosen for the present study. Since nothing is known regarding the exogenous effect of triazoles and IBA on rooting and sprouting in *A. vasica* cuttings, in the present investigation an attempt was made to achieve the following objectives.

The objectives of this study are to evaluate the effect of TDM, hexaconazole, and IBA on the growth and growth rate of *A. vasica* cuttings under field conditions.

MATERIALS AND METHODS

A. vasica L. belongs to the family Acanthaceae was selected for the present investigation. The cuttings were collected from Sivapuri in Chidambaram, Cuddalore District, Tamil Nadu, India. The stem cuttings of uniform thickness having three nodes were used for planting. Each stem cuttings was 18 cm height and planted in 5 cm inside the polythene bags. The stem cuttings are dipped for 10 min in 1% Bavestin before planting. Each bag contains 1 plant. They are 500 pockets each packet filled with red soil, sand, and FYM in 1:1:1 ratio. The pockets were irrigated immediately after transplantation, and subsequent irrigation was done twice in a week to keep the optimum moisture level required in the soil.

The treatments of 15 mg/L TDM and 15 mg/L HEX concentrations were found to increase the dry weight significantly and in higher concentrations they slightly decreased the growth and dry weight. Hence, 15 mg/L TDM and 15 mg/L HEX concentrations were used to determine the effect of these chemicals on the growth and metabolism of *A. vasica*. 1 L of 15 mg/L TDM and 15 mg/L HEX solution per plant was used for the treatment and control was treated with 1 L of irrigation water. 1 L of 0.3 mg/L IBA solution per plant was used for the treatment and control was treated with 1 L of irrigation water. The treatment was given on 15, 30, 45, and 60 days after planting. The average temperature was 32/26°C (maximum and minimum) and relative humidity (RH) varied between 60% and 75% during the experimental period.

TDM has been obtained from Bayer India Ltd., Mumbai, Maharashtra, India and HEX has been obtained from Rallis India Ltd., Mumbai, Maharashtra. IBA (C_6H_4.NH.CH:C(CH_2)$_3$.COOH) M.W.203.24) was obtained from Sigma Chemicals, Bangalore used for this study. The experimental part of this work was carried out in Botanical Garden and Stress Physiology Lab, Department of Botany, Annamalai University, Tamil Nadu.

After washing the plants in the tap water, fresh weight was determined by an electronic balance, and the values were expressed in grams. After taking fresh weight, the plants were dried at 60°C in hot air oven for 48 h. After drying, the weight was measured and the values were expressed in grams.

RESULTS

Root Length

The root length increased with the age of the plants. Triazole treatment increases the root length significantly to a larger extent when compared with control. TDM treatment increased the root length to a higher level when compared to HEX and IBA treated plants (Table 1).

Both the triazole treatment significantly increased the root length. Among the treatments, TDM increased the root length to a larger extent than HEX and IBA. TDM stimulated adventitious root formation in bean hypocotyls (Fletcher and Hofstra, 1988). Paclobutrazol increased the diameter and length of fibrous roots and enhanced the lateral root formation in apple seedlings (Wang and Faust, 1986) and Wheat seedlings (Berova *et al.,* 2002). Triazole treatments increased root elongation in *Plectranthus forskholii* (Lakshmanan *et al.,* 2007), The growth-retarding effect of triazole is caused by the inhibition of GA (Fletcher *et al.,* 2000).

Exogenous application of IBA to the base of cuttings positively impacted rooting percentage, rooting number per cutting as well as rooting length in our study. Our observations were in agreement with previous reports on tropical trees including *Prunus africana* (Tchoundjeu *et al*., 2002).

Number of Branches

The number of branches increased with the age of the plants. Triazole treatment increases the number of branches significantly to a larger extent when compared with control. TDM treatment increased the number of branches followed by the IBA, HEX, and control (Table 2).

Number of Leaves

The number of leaf increased with the age of the plants. Triazole treatment increases the number of leaf significantly to a larger extent when compared with control. IBA treatment increases the number of leaf to a higher level when compared to control plants. Number of leaves was found slightly increased under triazole treatments. Similar results were observed in *P. forskholii* (Lakshmanan *et al.,* 2007) and Cassava (Gomathinayagam *et al.,* 2007).

Table 1: Effect of IBA, TDM, and HEX root length in *Adhatoda vasica*

Growth stages (DAP)	Control	IBA	HEX (15 mg/L)	TDM (15 mg/L)
15	1.87±0.067	2.63±0.094	2.40±0.086	2.55±0.091
30	2.70±0.096	4.22±0.151	3.18±0.109	3.37±0.125
45	4.04±0.139	6.78±0.234	4.10±0.146	4.72±0.169
60	12.40±0.459	21.25±0.731	11.88±0.424	13.16±0.47

Values are mean±SD of four samples expressed in mg/per plant.
IBA: Indole-3-butyric acid, TDM: Triadimefon, HEX: Hexaconazole, DAP: Days after planting, SD: Standard deviation

IBA treatment increases the number of leaf to a higher level when compared to control plants. Plants treated with high exogenous growth regulators flowered earlier and with a lower leaf number (Brookinga and Cohen, 2002). The lack of effect of GA_3 on characteristics of leaves or flowers indicates that GA_3 had no effect on promoting formation or development of vegetative or floral buds on the rhizomes. Several studies revealed the lack of effect of GA_3 on leaf or flower characteristics (Table 3).

Total Leaf Area

The leaf area increased with the age in the control and treated plants. Triazole treated plants showed reduced total leaf area when compared to control. TDM treatment inhibited the leaf area to a larger extent when compared to IBA and HEX. The total leaf area was 179.4%, 63.3%, and 80.5% over control in the IBA, HEX, and TDM treated plants, respectively, on 60 DAS (Table 4).

Treatment with triazole compounds significantly affected the leaf growth. Among the treatments, TDM lowered the leaf area to a larger extent than HEX. Triazole treatments reduced the leaf area as observed in soyabean, apple and peech (Davis *et al.,* 1985). The long lag of the IBA effect on growth in the intact plant coincides with that found in excised stem segments of *Khaya anthotheca* (Frimpong *et al*., 2008).

Number of Roots

The number of roots increased with the age of the plants. Triazole treatment increases the number of roots significantly to a larger extent when compared with control. TDM treatment increased the number of root to a higher level when compared to HEX and IBA treated plants (Table 5).

Root Hair

The number of root hair increased with the age of the plants. Triazole treatment decreased the number of root hair significantly to a larger extent when compared with control. TDM treatment decreased the number of root to a higher level when compared to IBA and HEX treated plants (Table 6).

Fresh Weight

Leaf

The fresh weight of the leaf increases with the age of the plants. Triazole treatment decreases the leaf fresh weight significantly to a larger extent when compared with control. TDM treatment decreases the fresh weight of the leaf followed by the HEX, IBA, and control (Table 7).

Stem

The fresh weight of the stem decreased in both triazole treated plant at all stages of growth when compared to control. When compared to those treated plant TDM shoot fresh weight was higher than that of HEX and IBA (Table 8).

Root

The fresh weight of the root increased with the age of the plants. Triazole treatment increases the root fresh weight significantly to a larger extent when compared with control. TDM treatment increased the fresh weight of the root followed by the HEX, IBA, and control (Table 9).

Both the triazole treatments significantly lowered the total stem fresh weight. Among the triazole treatments, HEX inhibited the stem fresh weight to a larger extent when compared to TDM. Triazole treatments reduced the stem elongation, plant height, and decreased the fresh weight in citrus (Mehouachi *et al.,* 1996). The total fresh weight of the leaf decreased significantly in the triazole treated plants. HEX treatment decreased it to a larger extent when

Table 2: Effect of IBA, TDM, and HEX number of branch in *Adhatoda vasica*

Growth stages (DAP)	Control	IBA	HEX (15 mg/L)	TDM (15 mg/L)
15	2±0.071	2±0.069	3±0.107	3±0.103
30	2±0.069	2±0.068	3±0.103	3±0.107
45	3±0.107	3±0.107	4±0.143	6±0.207
60	3±0.103	4±0.143	6±0.207	7±0.25

Values are mean±SD of four samples expressed in mg/per plant.
IBA: Indole-3-butyric acid, TDM: Triadimefon, HEX: Hexaconazole, DAP: Days after planting, SD: Standard deviation

Table 3: Effect of IBA, TDM, and HEX Number of leaves in *Adhatoda vasica*

Growth stages (DAP)	Control	IBA	HEX (15 mg/L)	TDM (15 mg/L)
15	6±0.214	7±0.25	8±0.275	9±0.321
30	8±0.276	10±0.357	11±0.379	12±0.429
45	11±0.393	14±0.5	16±0.571	17±0.607
60	13±0.448	20±0.174	22±0.786	24±0.828

Values are mean±SD of four samples expressed in mg/per plant.
IBA: Indole-3-butyric acid, TDM: Triadimefon, HEX: Hexaconazole, DAP: Days after planting, SD: Standard deviation

Table 4: Effect of IBA, TDM, and HEX total leaf area in *Adhatoda vasica*

Growth stages (DAP)	Control	IBA	HEX (15 mg/L)	TDM (15 mg/L)
15	6.5±0.232	7.75±0.277	5.25±0.188	6.25±0.223
30	29.5±1.054	37.5±1.293	23.5±0.810	27.75±0.991
45	41.5±1.414	64±2.286	29.25±1.083	35.75±1.277
60	66.75±2.472	119.8±4.279	42.25±1.457	53.75±1.919

Values are mean±SD of four samples expressed in cm^2/per plant.
IBA: Indole-3-butyric acid, TDM: Triadimefon, HEX: Hexaconazole, DAP: Days after planting, SD: Standard deviation

compared to TDM. The growth-retarding effect of triazole is caused by the inhibition of gibberellic acid biosynthesis and increased abscissic acid content as observed in barley and radish (Buchenauer and Nath, 1984). Inhibition of leaf growth induced by increased abscissic acid and lowered gibberellin may be the cause for reduced leaf fresh weight.

The total fresh weight of the root increased significantly in both triazole and IBA treated plants. Triazole compounds, such as TDM, paclobutrazol, and uniconazole, have promoted rooting in various plants such as *Chrysanthemum*, Bean (Davis *et al.,* 1988), English Ivy (Geneve, 1990), and apple (Wang and Faust, 1986).

Dry Weight

Leaf

The leaf dry weight increased with the age of the plants. Triazole treatment decreases the leaf dry weight significantly to a larger extent when compared with control. TDM treatment decreases the dry weight of the leaf to a higher level when compared to HEX and IBA treated plants (Table 10).

Stem

The dry weight of the shoot increased with the age in the control and treated plants. Triazole treated plants slightly lowered the shoot dry weight at all stages of growth (Table 11).

Root

The root dry weight increased with the age of the plants. Triazole treatment increases the root dry weight significantly to a larger extent when compared with control. TDM treatment increased the dry weight of the root to a higher level when compared to HEX and IBA treated plants (Table 12).

The total stem dry weight decreased with triazole treatments. HEX treatment decreased the stem dry weight significantly when compared to TDM. Triazoles reduce the cell number, length, and width of the xylem cells (Fletcher *et al.,* 2000). Triazole induced a marked reduction in stem dry weight, and this may be attributed to the reduced GA level by triazole treatment. Triazole treatment decreased the total dry weight of the leaf significantly in tapioca. TDM, triadimenol, paclobutrazol, and S-3307 reduced the height and dry weight of shoot in *Phaseolus vulgaris* (Fletcher and Arnold, 1986), and wheat seedlings (Berova *et al.,* 2002).

In tapioca total dry weight of the root marginally increased with triazole treatments. Among the treatments,

Table 5: Effect of IBA, TDM, and HEX number of root in *Adhatoda vasica*

Growth stages (DAP)	Control	IBA	HEX (15 mg/L)	TDM (15 mg/L)
15	14±0.5	17±0.607	18±0.621	20±0.714
30	18±0.621	25±0.862	28±0.966	33±1.179
45	20±0.714	28±0.966	34±1.214	38±1.310
60	23±0.793	33±1.179	41±1.414	47±1.161

Values are mean±SD of four samples expressed in mg/per plant.
IBA: Indole-3-butyric acid, TDM: Triadimefon, HEX: Hexaconazole, DAP: Days after planting, SD: Standard deviation

Table 6: Effect of IBA, TDM, and HEX number of root hair in *Adhatoda vasica*

Growth stages (DAP)	Control	IBA	HEX (15 mg/L)	TDM (15 mg/L)
15	23±0.821	41±1.464	22±0.759	26±0.929
30	40±1.429	76±2.621	49±1.75	55±1.897
45	67±2.310	136±4.857	94±3.357	103±3.679
60	94±3.357	204±7.034	148±5.481	168±5.793

Values are mean±SD of four samples expressed in mg/per plant.
IBA: Indole-3-butyric acid, TDM: Triadimefon, HEX: Hexaconazole, DAP: Days after planting, SD: Standard deviation

Table 7: Effect of IBA, TDM, and HEX on leaf fresh weight *Adhatoda vasica*

Growth stages (DAP)	Control	IBA	HEX (15 mg/L)	TDM (15 mg/L)
15	0.47±0.017	0.51±0.018	0.42±0.014	0.046±0.017
30	1.21±0.042	1.34±0.046	0.99±0.037	1.04±0.037
45	1.96±0.073	2.56±0.088	1.43±0.049	1.57±0.054
60	2.34±0.084	3.25±0.116	1.62±0.058	1.74±0.06

Values are mean±SD of four samples expressed in mg/per plant.
IBA: Indole-3-butyric acid, TDM: Triadimefon, HEX: Hexaconazole, DAP: Days after planting, SD: Standard deviation

Table 8: Effect of IBA, TDM, and HEX on shoot fresh weight *Adhatoda vasica*

Growth stages (DAP)	Control	IBA	HEX (15 mg/L)	TDM (15 mg/L)
15	5.21±0.186	6.33±0.234	4.51±0.161	5.13±0.177
30	6.79±0.234	9.32±0.332	5.56±0.192	6.27±0.216
45	7.24±0.268	11.3±0.404	5.67±0.203	6.47±0.231
60	8.36±0.288	15.01±0.536	5.98±0.214	7.07±0.253

Values are mean±SD of four samples expressed in mg/per plant.
IBA: Indole-3-butyric acid, TDM: Triadimefon, HEX: Hexaconazole, DAP: Days after planting, SD: Standard deviation

Table 9: Effect of IBA, TDM, and HEX on root fresh weight *Adhatoda vasica*

Growth stages (DAP)	Control	IBA	HEX (15 mg/L)	TDM (15 mg/L)
15	0.23±0.008	0.27±0.009	0.31±0.011	0.35±0.013
30	0.79±0.028	1.03±0.038	1.19±0.043	1.27±0.045
45	0.99±0.036	1.4±0.048	1.61±0.057	1.83±0.063
60	1.12±0.04	1.74±0.062	2.02±0.072	2.21±0.076

Values are mean±SD of four samples expressed in mg/per plant.
IBA: Indole-3-butyric acid, TDM: Triadimefon, HEX: Hexaconazole, DAP: Days after planting, SD: Standard deviation

TDM increased the root dry weight higher level than HEX treatment. Triazole compounds such as TDM, paclobutrazol, and uniconazole has promoted rooting in various plants such as *Chrysanthemum*, bean (Davis *et al.,* 1985; Fletcher and Hofstra, 1988) English ivy (Geneve, 1990), apple (Wang and Faust, 1986) soybean (Davis *et al.,* 1985) citrus (Bausher and Yelenosky, 1987), and radish (Muthukumarasamy and Pannerselvam, 1997a).

It was apparent that treating plants with IBA increased height of the plant over the control, which may be attributed to the growth promotion effect of IBA in stimulating and accelerating cell division, increasing cell elongation and enlargement or both (Hartman *et al.,* 1990) which in turn increased the dry weight of the plants.

CONCLUSION

TDM and HEX treatments decreased the height of the plant, and IBA increased the height of the plant. The leaf area also reduced under triazole treatments, whereas leaf area increased under IBA treatment. The root growth was significantly increased at all stages of growth with TDM, HEX, and IBA treatments. Toward maturity, the whole plant fresh weight increased considerably with TDM, HEX, and IBA treatments in *A. vasica* plants when compared to control. The dry weight also increased in TDM, HEX, and IBA treatments due to the increase in root growth.

Table 10: Effect of IBA, TDM, and HEX leaf dry weight *Adhatoda vasica*

Growth stages (DAP)	Control	IBA	HEX (15 mg/L)	TDM (15 mg/L)
15	0.10±0.004	0.11±0.004	0.08±0.003	0.09±0.003
30	0.25±0.009	0.31±0.011	0.19±0.007	0.21±0.008
45	0.39±0.014	0.51±0.018	0.28±0.01	0.31±0.011
60	0.47±0.017	0.64±0.023	0.32±0.011	0.35±0.012

Values are mean±SD of four samples expressed in mg/per plant.
IBA: Indole-3-butyric acid, TDM: Triadimefon, HEX: Hexaconazole, DAP: Days after planting, SD: Standard deviation

Table 11: Effect of IBA, TDM, and HEX shoot dry weight *Adhatoda vasica*

Growth stages (DAP)	Control	IBA	HEX (15 mg/L)	TDM (15 mg/L)
15	1.04±0.037	1.24±0.043	0.9±0.032	1.01±0.035
30	1.26±0.043	1.65±0.059	1.06±0.04	1.21±0.043
45	1.47±0.051	2.1±0.072	1.13±0.039	1.3±0.046
60	1.68±0.062	2.8±0.104	1.21±0.045	1.41±0.049

Values are mean±SD of four samples expressed in mg/per plant.
IBA: Indole-3-butyric acid, TDM: Triadimefon, HEX: Hexaconazole, DAP: Days after planting, SD: Standard deviation

Table 12: Effect of IBA, TDM, and HEX root dry weight *Adhatoda vasica*

Growth stages (DAP)	Control	IBA	HEX (15 mg/L)	TDM (15 mg/L)
15	0.07±0.003	0.08±0.003	0.10±0.004	0.11±0.004
30	0.16±0.006	0.21±0.007	0.24±0.009	0.26±0.009
45	0.20±0.007	0.28±0.01	0.31±0.011	0.34±0.012
60	0.25±0.009	0.36±0.013	0.40±0.014	0.45±0.016

Values are mean±SD of four samples expressed in mg/per plant.
IBA: Indole-3-butyric acid, TDM: Triadimefon, HEX: Hexaconazole, DAP: Days after planting, SD: Standard deviation

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