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SECONDARY METABOLITES CONTENTVARIATIONS IN *SOLANUM TRILOBATUM* (L.) UNDER TREATMENT WITH PLANT GROWTH REGULATORS

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ABSTRACT

Solanumtrilobatum Linn (Solanaceae) is a thorny shrub widely distributed in Bengal, Uttar Pradesh, Southern India and Srilanka in moist places. This plant is well known in Ayurveda and Siddha system as 'Alarka and Tuduvelai', respectively. Leaves, roots, berries and flowers are used for cough. The constituents such as solasodine and β-solamarine have been isolated from whole plant. Plant growth regulators are substance that influences physiological processes of plants at very low concentration. Abscisic acid (ABA) is a many important plant growth development processed. Paclobutrazol (PBZ) is a triazolic group of fungicide which has plant growth regulating properties. Salicylic acid (SA) is phenolic phytohormones and is formed in plants with role of plant growth and development. The given treatments were started at 70th day followed by 80th 90 and 100th days. The groups were treated with respective growth hormones by spraying method to ABA 10µg/L, PBZ 10mg/L and SA 500µg/L. After 10th day, the plants were harvested and analysed on over all analytical values assessment plant treated with PBZ followed by ABA, SA, Control plants. Qualitative on secondary metabolites on alkaloids, terpenoids, flavonoids, saponin, tannins and phenol content in present, but gum & mucilage, fixed oils& fats , phytosterol absences. Quantitative of secondary metaboliteson over all metabolites values assessment plant treated with ABA followed by PBZ, SA and Control plants.

Key Words:- Abscisic acid (ABA),Paclobutrazol (PBZ),Salicylic acid (SA),*Solanumtrilobatum* Linn and phytochemical studies.

INTRODUCTION

Solanumtrilobatum Linn (Family: Solanaceae) a thorny creeper with bluish white flower, widely distributed throughout India and has long been used in siddha system of medicines to treat various diseases. Solasodine and sobatum isolated from Solanumtrilobatum plant has been shown to possess anti-inflammatory activity. Solanumtrilobatum L. a potential rejuvenator drug and nutraceuticalvegetable, occurs in Southern India and has been used traditionallyin Siddha system of medicines to treat various diseases (Mohanan PV*et al*., 1998). This plantis well known in Ayurveda and Siddha system as 'Alarka' and'Tuduvelai'. Previous pharmacological studies confirm that it possessantibacterial, antifungal, antioxidant, anti-tumor, anti-asthmatic,anti-ulcerogenic, anti-inflammatory, analgesic, counteracts snakepoison and cures lung disease (Pandurangana*et al*., 2008). Plant growth regulators can be defined as either natural or synthetic compounds that modify the plant growth and development pattern by exerting profound influence on many physiological processes and thereby increasing the productivity of crops (Kakimoto,2003). The plant hormone abscisic acid (ABA) exerts profound effects on

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fundamental processes of plant growth and development (Xie*et al*., 2006). The plant growth regulator paclobutrozol (PBZ) reduces plant growth, which may reduce the need for mechanical pruning of shade trees in urban settings and utility rights of way (Mann *et al*., 1995), although tree species can vary in their sensitivity to this growth regulator (Bai*et al*., 2004). Salicylic acid is an endogenous plant growth regulator. It in involved in various physiological processes of plant growth and development (Klessing& Malamy,1994).The use of elicitor such as Salicylic acid (SA) will also be incorporated into this approach as an effective strategy to increase the production of important alkaloids in cell and organ cultures (Pitta- Alvarez *et al*., 2000; Spollansky*et al*., 2000). Secondary metabolites are important defences of plants to insects and pathogens, and their concentration can be mediated by resource based allocation trade- offs between growth and other functions (Herma and Mattson 1992; Herms and Mattson 1997; Herms 2002). The growth differentiation balance hypothesis predicts that environmental factors that reduce plant growth with little effect on photosynthesis should increase the availability of resources allocated to the production of secondary metabolites, thereby increasing resistance to insects and other herbivores (Herms and Mattson 1992; Glyn *et al*., 2007). The medicinal value of thesis plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Dhandapani and Sabna. 2008).In present studies on phytochemical studies on the leaves part of *solanumtrilobatum.*

MATERIALS AND METHODS

Economically important medicinal plant (*Solanumtrilobatum L*) belongs to the family Solanaceae was selected for the present investigation. Seeds of *Solanumtrilobatum* (Thuthuvallai) were obtained from the Horticulture Department in Annamalai University, Chidambaram in Tamil Nadu, India. Paclobutrazol is a triazolic group of fungicide having plant growth regulating properties obtained as CULTAR 25% w/v from Zeneca ICI Agrochemical Ltd., Mumbai, India. Absicic acid and Salicylic acid from Himedia chemicals, Pondicherry were used in the present study. The experiments were conducted at the Botanical Garden and Plant Growth Regulation Laboratory, Department of Botany, Annamalai University, Tamil Nadu, India.

Cultivation method

(a)Nursery bed: *Solanumtrilobatum* seeds were surface sterilized with 0.2% mercuric chloride solution for 2 min and rinsed thoroughly with distilled water. The seeds were soaked for 3 hours in conical flask before sowing. The Nursery bed is prepared with clay, red loam soil and FYM in 1:1:1 ratio. Then seeds were spread on the Nursery bed. The plants were allowed to grow till 40 days with regular irrigation. The seedlings were selected with 10-12cm height and develop 6 leaves for even growth conditions.

(b)Field: The field is laid out exactly as for ridged,irrigated sufficiently and, after ploughing twice, is watered heavily and ploughed again. Farm yard manure (FYM) and neem cake will give as fertilizers. In the initial period, irrigation is done once in a week and then in later stages as per requirement. The selected plants were transplanted to field. The given treatments were started at 70th day followed by 80th, 90th and 100th days. The groups were treated with respective growth hormones by spraying method to ABA 10µg/L, PBZ 10mg/L and SA500µg/L. The groups were treated with respect growth hormones by spraying method. After every treatment of the 10th day, the plants were harvested for further analysis.

PHYTOCHEMICAL STUDIES

Preparation of powder: (Harborne, 1973): The leaves parts *solanumtrilobatum* were collected and dried under shade. These dried materials were mechanically powder & heaved using 80 meshes and stored in an airtight container. These powdered materials were used for further analysis.

Analytical methods: - The procedures recommended in Indian Pharmacopoeia (Anonymous, 1996) were following for the determination of total ash, water-soluble ash, acid-insoluble ash, sulphated ash and loss on drying at 110° C.

Total ash value: 5g of plant powder was ignited in an electric furnace at 600° C in silica crucible until the sample reaches a constant weight.

Water soluble ash value: Total ash obtained was heated up to 600° C with addition of 25ml of water for 10 minutes. If was filtered in an ash less fillterpaper (What man No 41) and the residue was ignited in the furnace to get a constant weight.

Acid-insoluble ash value: Total ash obtained was heated with addition of 25ml dilHcl for 10 minutes. It was filtered in ash less filter paper (What man No.41) and the residue was ignited in the furnace to get a constant weight.

Sulphated ash value: 1g of plant powder was ignited in an electric furnace until the drug gets charred.The crucible was cooled and the residue was moistened with 1 ml of H2SO4, heated gently until the white fumes were no longer evolved and ignited at 800° C+25° C until all black particles disappear. The crucible was allowed to cool; few

drops of H2SO4 was added and again heated. The ignition was carried as before, allowed to cool and then weighted. This was repeated until the sample reaches a constant weight.

Loss on drying

Prepared air dried material (2gm) was placed on a tarred evaporating dish and dried at 105°Cfor 1hour and weighed. The drying was continued until two successive reading matches each other or the difference between two successive weighing was not more than 0.25%. Constant weight was reached when two consecutive weighing was reached when two consecutive weighing after drying for 30 minutes in a desiccators; Showed not more than 0.01g difference.

Qualitative Phytochemical Analysis

Preparation of plant extracts: 5g of powdered material with 50% alcohol (50ml alcohol with 50 ml water) was shaken well occasionally for 6 hours and kept undisturbed for 18 hours. The liquefied extract thus obtained was concentrated in a vacuum pump and the percentage was calculated with the weight of the extract obtained. The extract was stored in a refrigerator and used for the present study. Qualitative phytochemical analysis was done using the procedures of (Kokate, 1994). Alkaloids, carbohydrates, tannins and phenols, flavonoids, gums and mucilage, fixed oils and fats, saponinsphytosterol were qualitative analysed.

Alkaloids: Small portion were dissolved in dil. H_2SO_4 and filtered. The filtrate was treated with Mayer's Dragendroffe's, Hager's and Wagner's reagents separately. Appearance of cream, orange brown, yellow and reddish brown precipitates in response to the above regents respectively indicate the presence of alkaloids.

Terpenoids: Five ml of each extracts was mixed in 2ml of chloroform, and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoid.

Flavonoids: The extract mixed with few ml of alcohol was heated with magnesium and then concentration Hcl was added under cooling. Appearance of pink colour indicates the presence of flavonoids. The extract was treated with few ml of aqueous NaOH2. Appearance of yellow and change to colourless with Hcl indicate the presence of flavonoids.

Tannins and phenols: Small quantity of 50% alcoholic extract was dissolved in water and 5% ferric chloride solution or 1% Gelatine solution or 10% lead acetate solution was added. Appearance of blue colour with ferric chloride or precipitation with other reagent indicates the presence of tannins and phenols.

Saponins: About 1ml of the extract was dissolved in 20ml of water and shade in graduated cylinder for 15 minutes. Formations of one cm layer of foam indicate presence of saponins.

Phytosterol: The extract was treated with Lieberman Bur chard under suitable condition. Appearance of blue emerald green indicates the presence of phytosterol and terpenes.

Gum and Mucilage: About 10ml of the extract was slowly added to 25ml of absolute alcohol under constant stirring. Precipitation indicates the presence of gum and mucilage.

Fixed oils and Fats: A drop of concentrated extract was pressed in between two filter papers and kept undisturbed. Oil stain on the paper indicates the presence of oils and fats.

Carbohydrates: 300mg of 50% alcoholic extracts were dissolved in water and filtered. The filtrate was treated with con H2So4 and then with Molisch's regent. Appearance of pink or violet colour indicates the presence of carbohydrates. The filtrated was boiled with Fehling's and Benedict solution. Formation of brick red precipitate in Fehling's and Benedict's solution is the positive result for reducing sugars and non- reducing sugars respectively.

QUANTITATIVE PHYTOCHEMICAL STUDIES

Estimation of total Alkaloids (Harbornne, 1973)

5g of the sample was weighted into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4h. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide is the alkaloid, which was dried and weighed.

Estimation of total Terpenoids(Ferguson, 1956)

100g of plant powder were taken separately and soaked in 250ml alcohol for 24 hours. Then filtered, the filtrate was extracted with petroleum ether, the ether extracts was treated as total terpenoids.

Estimation of total Flavonoids (Bohm and KocipaiAbyazan, 1994)

10g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through what man filter paper No 42(125nm). The filtrate was later

transferred into a crucible and evaporated into dryness over a water bath and weighted to a constant weight.

Estimation of total Tannins (Van-Burden and Robinson, 1994)

500mg of the sample was weighed into a 50ml plastic bottle. 50ml of distilled water was added and shaken for 1hr in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filtered was pipette out into a test tube and mixed with 2ml of 0.1 M $FeCl_3$ in 0.1 N Hcl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120nm within 10min.

Estimation of total phenols by Spectrophotometric method

The fat free sample was boiled with 50ml of ether for the extraction of the phenolic compound for 15 min. 5ml of the extract was pipette into a 50ml flask, then 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. This was measured at 505nm.

Estimation of totalSaponin(Obadoni and Ochuko,2001)

The method used was that of Obadoni and Ochuko (2001). The samples were ground and 20g of each were put into a conical flask and 100ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4hr with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90°C. The contra rate was transferred into a 250 ml separators funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60ml of n- butanol was added. The combined n- butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

RESULTS

Analytical values: On over all analytical values assessment plant treated with Paclobutrazol were found to have more total ash value, water soluble ash value, acid-insoluble ash value, sulphated ash value and loss on drying followed by abscisic acid salicylic acid and control plants are given in Figures : 1-5.

Qualitative phytochemical: Methanolextracts of the plants sample show presence ofAlkaloids, Terpenoids, Flavonoids, Saponin and Total phenol. The plant sample shows absence of Fixed oil & fats, Gums & mucilage and Phytosterol. The given in the Table-1.

Quantitative of secondary metabolites: On over all Quantitative secondary metabolites values assessment plant treated with abscisic acid were found to have more total alkaloids, total terpenoids, total flavonoids, total saponin and total phenol followed by paclobutrazol, salicylic acid and control plants are given in Table: 2-6.

Analytical Methods Studies

Figure 1. Effect of Abscisic acid (ABA), Paclobutrazol (PBZ) and Salicylic acid (SA) treatments induced changes on percentage of total ash value (% w/w)

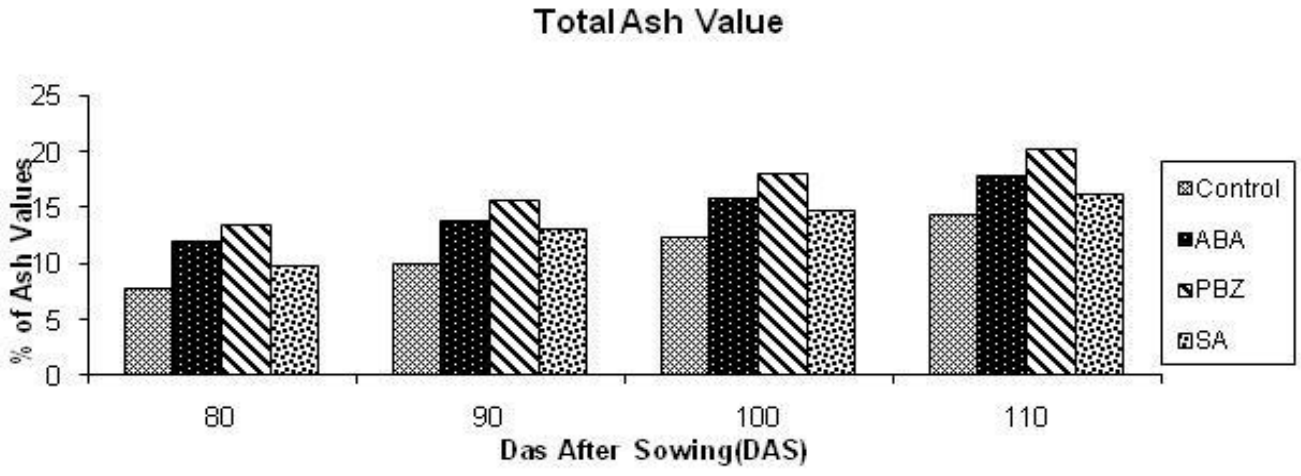

Figure 2. Effect of Abscisic acid (ABA), Paclobutrazol (PBZ) and Salicylic acid (SA) treatments induced changes on percentage of water- soluble ash value (% w/w)

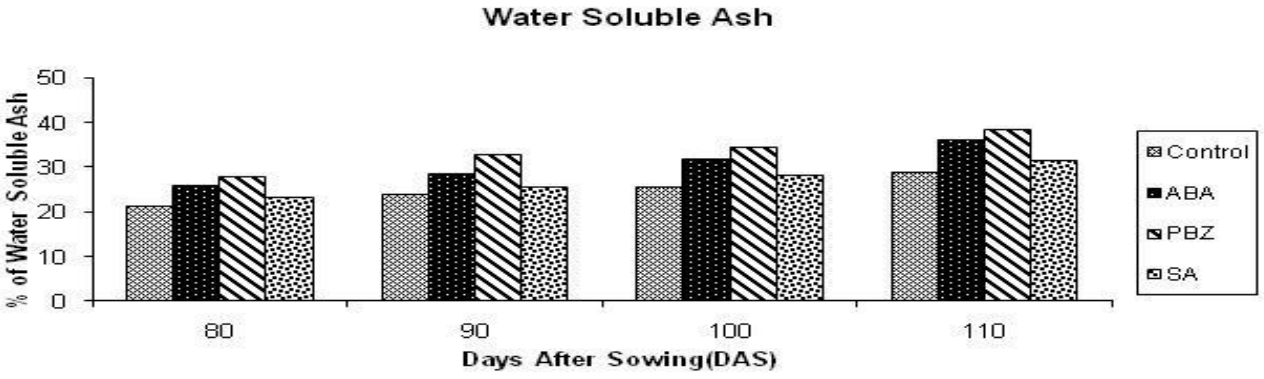

Figure 3. Effect of Abscisic acid (ABA), Paclobutrazol (PBZ) and Salicylic acid (SA) treatments induced changes on percentage of acid- insoluble ash value (% w/w)

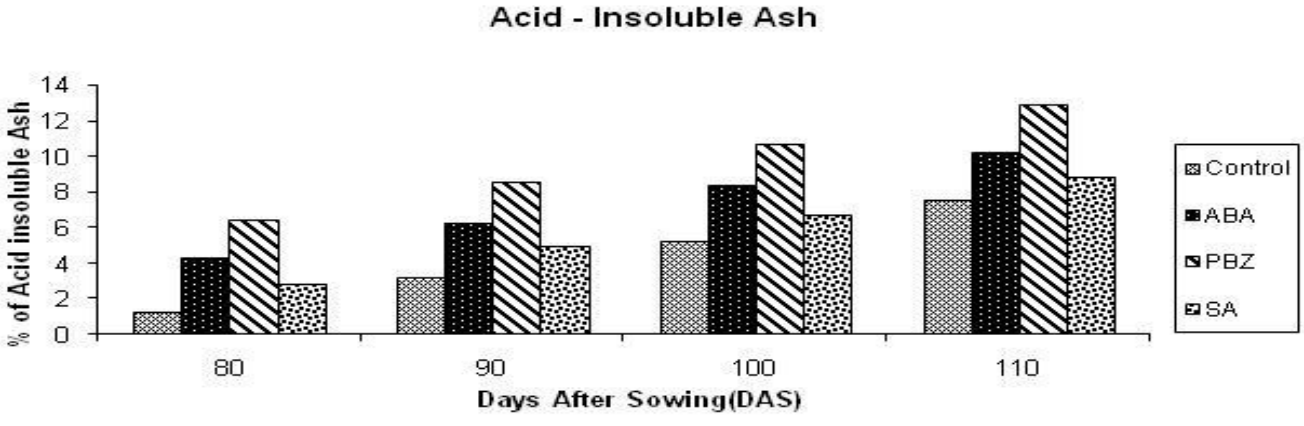

Figure 4. Effect of Abscisic acid (ABA), Paclobutrazol (PBZ) and Salicylic acid (SA) treatments induced changes on percentage of sulphated ash value (% w/w)

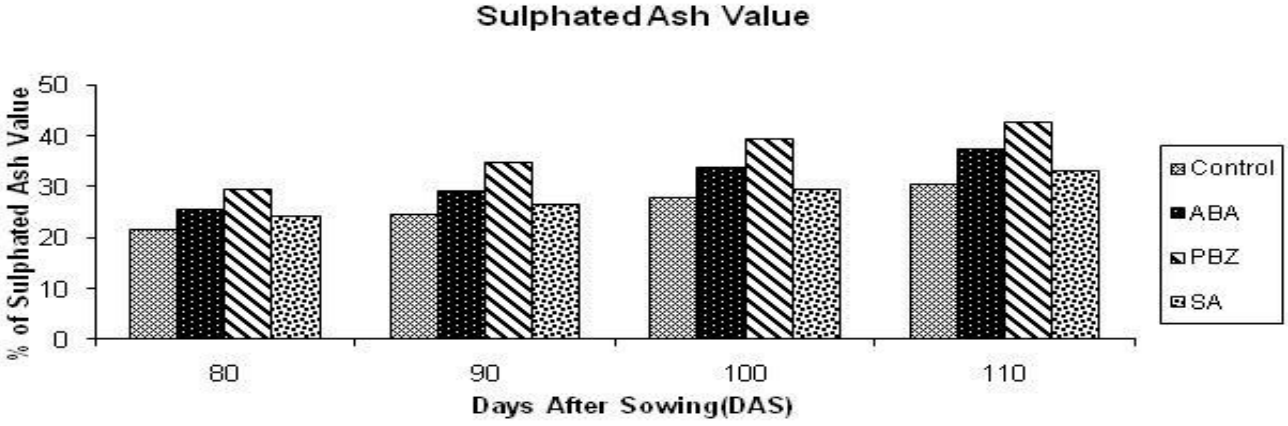

Figure 5. Effect of Abscisic acid (ABA), Paclobutrazol (PBZ) and Salicylic acid (SA) treatments induced changes on percentage of loss on drying (% w/w)

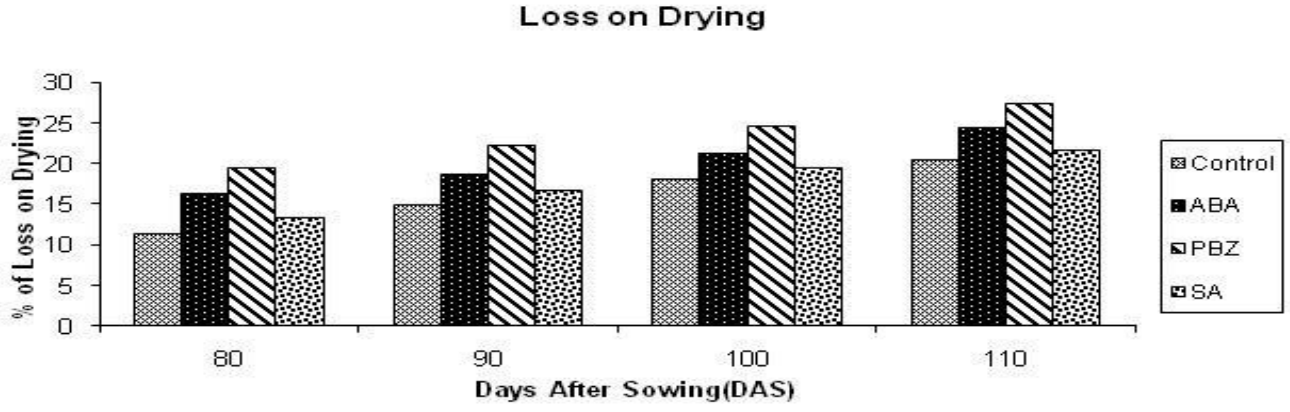

Table1. Qualitative phytochemical screening of methanol extracts of leaves parts of *Solanumtrilobatum*L.

Compound Test	Reagent used	100% Methanol
Alkaloids	Dragendraff's	+
	Wagner's	+
	Hager's	+
	Mayer's	+
Terpenoids	Salkowski test	+
Flavonoids	Na OH + Hcl	+
Saponinnis	Foam Test	+
Tannins& Phenols	10% Lead acetate	+
Gum&Mucilage	Alcoholics Precipitation	-
Fixed oils& Fats	Spot Test	-
Phytosterol	LB Test	-
Carbohydrates	Fehling's	-
	Molish's	-

Quantitative phytochemical studies

Table2. Effect of Abscisic acid (ABA), Paclobutrazol (PBZ) and Salicylic acid (SA) treatments induced changes on percentage of crude alkaloids

Days	Control	ABA	PBZ	SA
80	0.502±0.035	1.280±0.048(254.94)	1.142±0.060(227.49)	0.792±0.350(157.76)
90	0.655± 0.047	1.983±0.420(302.74)	1.570±0.244(239.69)	1.112±0.434(169.77)
100	0.813± 0.033	2.605±0.369(320.41)	1.985±0.044(244.15)	1.455±0.597(178.96)
110	0.976 ±0.036	3.377±0.045(346.01)	2.432±0.547(249.18)	1.798±0.635(184.22)

(Values are mean ±S.D of 4 samples)Values given in brackets indicate % over control

Table3. Effect of Abscisic acid (ABA), Paclobutrazol (PBZ) and Salicylic acid (SA) treatments induced changes on percentage of crude terpenoids

Days	Control	ABA	PBZ	SA
80	0.765±0.038	1.377±0.030(180.00)	1.095±0.026(143.13)	0.939±0.037(122.74)
90	0.870±0.0365	1.682±0.263(193.33)	1.375±0.341(158.04)	1.134±0.454(130.34)
100	0.980± 0.294	1.987±0.547(202.75)	1.657±0.056(169.08)	1.389±0.043(141.73)
110	1.075± 0.038	2.292±0.042(213.20)	1.945±0.264(180.93)	1.645±0.051(153.02)

(Values are mean ±S.D of 4 samples)Values given in brackets indicate % over control

Table4. Effect of Abscisic acid (ABA), Paclobutrazol (PBZ) and Salicylic acid (SA) treatments induced changes on percentage of crude flavonoids

Days	Control	ABA	PBZ	SA
80	0.392± 0.039	1.180±0.040(301.02)	0.750±0.031(191.32)	0.625±0.038(159.43)
90	0.567± 0.043	1.760±0.043(310.40)	1.267±0.034(223.45)	0.950±0.029(167.54)
100	0.747 ±0.047	2.362±0.025(316.19)	1.785±0.056(238.95)	1.280±0.0391(171.35)
110	0.927± 0.031	2.987±0.025(322.22)	2.330±0.050(251.95)	1.625± 0.034(175.29)

(Values are mean ±S.D of 4 samples)Values given in brackets indicate % over control

Table5. Effect of Abscisic acid (ABA), Paclobutrazol (PBZ) and Salicylic acid (SA) treatments induced changes on percentage of crude saponin

Days	Control	ABA	PBZ	SA
80	6.270 ±0.039	9.330±0.029(148.80)	8.785±0.047(140.11)	7.527±0.036(120.04)
90	7.475± 0.050	12.890±0.052(152.09)	10.753±0.039(143.85)	9.517±0.068(127.31)
100	8.982 ±0.045	14.327±0.037(159.54)	3.405± 0.047(149.27)	11.972±0.070(133.31)
110	10.485±0.036	16.980±0.069(161.99)	16.057±0.034(153.14)	4.427± 0.066(137.59)

(Values are mean ±S.D of 4 samples)Values given in brackets indicate % over control

Table6. Effect of Abscisic acid (ABA), Paclobutrazol (PBZ) and Salicylic acid (SA) treatments induced changes on percentage of crude total phenol

Days	Control	ABA	PBZ	SA
80	4.350± 0.039	9.265±0.042(212.98)	7.902±0.080(181.65)	6.072±0.042(139.58)
90	6.267 ±0.499	13.766±0.042(219.65)	1.792± 0.58(188.16)	8.930±0.036(142.49)
100	8.184± 0.022	8.267± 0.055(223.20)	15.982±0.040(195.28)	11.788±0.043(144.03)
110	11.012 ±0.585	24.863±0.087(225.78)	21.998±0.028(199.76)	16.500±0.391(149.83)

(Values are mean ±S.D of 4 samples)Values given in brackets indicate % over control

DISCUSSION

The main objective of the present investigation is to assess the effect of control and plant growth regulators (PGR) likeAbscisic acid, Paclobutrazol and Salicylic acid in *Solanumtrilobatum*plants. The results obtained on analytical values contents discussed hereunder. On over all analytical values assessment plants treated with Paclobutrazol to have followed by abscisic acid and salicylic acid. After the, on over all plants treated with Abscisic acid were found to have quantitative content followed by paclobutrazol and salicylic acid.

The strong and rapidly stimulating effect of elicitor on plant secondary metabolism in medicinal plants attracts considerable attentions and research efforts Jaleel*et al*., 2009 and Karthikeyan B *et al*., 2007. The reasons responsible for the diverse stimulating effects of elicitors are complicated and could be related to the interactions between elicitors and plant cells, elicitor signal transduction and plant defense responses Karthikeyan B *et al*., 2008. The objectives of the present study are to understand the effect of plant growth promoters (GA and *Pseudomonas fluorescence* elicitors) and retardant (PBZ) on the anatomical characteristics changes of *C. roseus*plants under field conditions.

Paclobutrazol decreases plant growth by inhibiting gibberellins biosynthesis (Rademacher 2000) without diminishing photosynthetic rate (Wieland and Wample 1985; Archbold and Houtz 1988; Vu and Yelenosky 1988; Yim*et al*., 1997). It reduces plant growth without directly interfering with secondary metabolite biosynthetic pathways, as it inhibits gibberellins synthesis downstream in the chain of reactions leading to the production of secondary metabolites, i.e., the biosynthesis of tannins, phenolic compounds, and terpenoids (Rademacher 2000).

The use ofelicitor such as Salicylic Acid (SA) will also beincorporated into this approach as an effective strategy toincrease the production of important alkaloids in celland organ cultures (Pitta-Alvarez *et al*., 2000; Spollansky*et al*., 2000).

Plant derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are richest bioresource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube*et al*., 2008). The phytochemical screening and quantitative estimation of the percentage

crude yields of chemical constituents of the plants studied showed that the leaves and roots were rich in alkaloids, phenol and tannins. The presence of phenolic compounds in the plants indicates that these plants may be anti-microbial agent. This agreed with the findings of Ofokansi*et al*., 2005. Tannins have stringent hasten the healing of wounds and inflamed mucous membranes. Apart from tannin and phenolic compounds, other secondary metabolite constituents of all the five plants detected include the alkaloids, saponin and flavonoids. Flavonoids on the other hand are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage, have strong anticancer activity (Salah *et al*., 1995; Del-Rio *et al*., 1997; Okwn, 2004). Saponin has the property of precipitating and coagulating red blood cells (Sadipo*et al*., 2000; Okwe, 2004). Therefore, the data generated from these experiments have provided the chemical basis for the wide use of this plant as therapeutic agent for treating various ailments. However, there is need to further carry out advanced hyphenated spectroscopic structure of these compounds. Furthermore, this data may be handy in probing of biochemistry of this plant in the future.

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