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Effect of abscisic acid, Paclobutrazol and Salicylic acid on the growth and Pigment variation in *Solanum Trilobatum* (l)

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Abstract

Solanum trilobatum (Family: Solanaceae) is one of the common Indian medicinal plants and it has been used in traditional medicine for many centuries. This plant is a thorny creeper with bluish violet flower, more commonly available in southern India has been used traditional in Siddha system of medicines to treat various diseases. The roots, leaves, berries and flowers are used for cough. The decoction of entire *Solanum trilobatum* plant is used to treat acute and chronic bronchitis. It has been widely used to treat respiratory disorders. This plant is commonly used to treat asthma, cough, dysonoea, chronic febrile infections and difficult parturition. The constituents of this plant include sobatum, β-solamarine, solanine, solasodine, glycoalkaloid, diosogenin and tomatidine. Plant growth regulators are substance that influences physiological processes of plants at very low concentration. Abscisic acid is a many important plant growth development processed. Paclobutrazol is a triazolic group of fungicide which has plant growth regulating properties. Salicylic acid 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, 90th and 100th days. The groups were treated with respect growth hormones by spraying method. After 10th day, the plants were harvested for further analysis. On over all physical assessment plants treated with paclobutrazol were found to have more whole plant fresh weight, dry weight, root length and stem length followed by abscisic acid and salicylic acid. After the physical evaluation, the leaves were collected from each group for pigment analysis. Chlorophyll, carotenoid, anthocyanin and xanthophylls pigment contents were increased in abscisic acid followed by paclobutrazol and salicylic acid.

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Key words:
Solanum trilobatum (ST), Abscisic acid (ABA), Paclobutrazol(PBZ), Salicylic acid(SA), growth parameter and pigment analysis.

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INTRODUCTION:

Herbal medicine is still the mainstay of about 75% to 80% of the world population, mainly in the developing countries to promote primary health care with better cultural acceptability, human compatibility and lesser side effects [1]. India is a gold mine of treasures with traditional and practical knowledge of herbal medicines [2, 3]. Plants provide basic raw materials for the indigenous pharmaceutical industries such as pharmaceutical, cosmetic, perfumery and food [4]. *Solanum trilobatum* (Solanaceae), a thorny creeper with bluish violet flower, more commonly available in southern India has been used traditionally in Siddha system of medicines to treat various diseases [5]. It has been widely used to treat respiratory disorders, especially bronchial asthma [6]. It was reported *Solanum trilobatum* possess antioxident activity [7], hepatoprotective activity and protects UV induced damage and radiation induced toxicity in mice [8].

The plant hormone Abscisic acid regulators many important plant growth development processes and induced tolerance to different stresses including drought, salinity, and low temperature [9]. ABA regulators process of embryo maturation, seed development and germination, stomatal opening, root development, floral transition. ABA plays a direct role in mediating the photosynthesis and respiration in leaves and also inhibition of lateral root development [10]. Paclobutrazol (2RS. 3RS)-1-(4 Chlorophenyl)- 4, 4-dimethyl-2(1H-1, 2,4-triazole-1-yl-pentan-3-ol) is a triazolic group of fungicide which have plant growth regulating properties. The growth properties of paclobutrazol are mediated by changes in the balance of important plant hormones including the gibberellins, ABA and cytokinins [11]. Paclobutrazol has been proved as an agent in stores amelioration in medicinal plants [12]. Salicylic acid (SA) is a phenolic phytohormones and is formed in plants with role of plant growth and development. SA is involved endogenous signaling mediating in plant defense against pathogens 13. Many studies have indicated that salicylic acid plays an important role in plant defense response against pathogen attack and is essential for the development of SAR [14]. Paclobutrazol treatment increased the chlorophyll and carotenoid content in corn seedlings[15]. *Lolium perenne* [16] ,*Brassica carinata*[17] and *Dianthus caryophyllus*[18]. It may be due to more densely packed chloroplasts in a small leaf area[19]. Enlarged chloroplasts were observed in barley [20]. The increased chlorophyll and carotenoid contents were in tomato [21, 22] and wheat [23] under triazole treatments. Paclobutrozol has proven its effeiciency in the reduction of height and promotion of flowering and fruiting in pear by increasing number of branch. A part from reduction in plant height, Paclobutrozol increases leaf N,P,Ca and Mg content [24]. Paclobutrazol increases number of flowers and their longevity in rose [25].

Carotenoid is essential part of the pigment protein complexes in thylakoids, the regulation of carotenogenesis in green tissues must be linked to the formation of chlorophyll, protein, lipid and chloroplast development itself. This highly regulated process is poorly understood. It is known that light, and its intensity, are involved in the regulation of carotenoid formation in the chloroplast. Although expression of carotenoid genes does occur in etiolated plants, their synthesis is stimulated on transfer to light [26, 27, 28]; *Vaccinium myrtillus* [29]; *Lysimachia minoricensis* [30].

ABA plays a direct role in mediating the photosynthesis to respiration in leaves and also the inhibition of lateral root development as reported in *Arabidopsis* [31]. ABA has been postulated to regulate the formation of xanthophylls in an inducible manner [32]. Treatment with ABA stimulated the accumulation of anthocyanin and phenolics and increased ethylene production as reported in maize leaves [33]. Therefore, it is generally thought that the turnover from xanthophyll precursors to ABA is a limiting factor for ABA biosynthesis. Abscisic acid is a sesquiterpenoid synthesized from Xanthophylls *Camellia sinensis* [34]. Some morphological changes observated in triazole-treated oak plants include the inhibition of plant growth, decreased intermodal elongation, increased chlorophyll levels, enlarged chloroplasts, thicker leaf tissue, increased root to shoot ratio and elevated levels of epcuticalar wax formation [35].

The objectives of the present study are to understand the effect of PGR such as ABA, PBZ and SA on the growth and pigment changes of *Solanum trilobatum* plants under field conditions.

MATERIALS OF METHODS:

Economically important medicinal plant (*Solanum trilobatum L*) belongs to the family solanaceae was selected for the present investigation. Seeds of *Solanum trilobatum* (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.

In the preliminary experiments these concentrations, 10 mgL^{-1} Paclobutrazol, 10μgL^{-1} Absicic acid and 500μgL^{-1} salicylic acid were found to increase the dry weight significantly and the higher concentrations of these compounds slightly decreased the growth and dry weight. Hence 10 mgL^{-1} Paclobutrazol, 10μg L^{-1} Absicic acid and 500 μg L^{-1} were used to determine the effect of these plant growth regulators compound on the growth and metabolism of *Solanum trilobatum*.

Cultivation method:

(a) Nursery bed: *Solanum trilobatum* 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 respect growth hormones by spraying method. After every treatment of the 10th day, the plants were harvested for further analysis.

METHODS:

Root length and stem length were recorded on 80th, 90th 100th and 110th DAS. Below the point of root-stem transition to the tap root and the length of lateral roots were taken as total root length. The length between shoot tip and point of root-stem transition region was taken as stem length. The root length and the stem length are expressed in centimeters per plant. Plants were harvested and separated into leaves, stems and roots and fresh weight was recorded. The samples were dried in an oven at 60 °C until constant dry weight was obtained and dry weight was recorded. The fresh and dry weights are expressed in grams per plant.

Chlorophyll and Carotenoid contents were extracted from the leaves and estimated according to the method of [36]. Xanthophyll contents were estimated by the method of [37]. Anthocyanin content was extracted and estimated by the method of [38].

RESULTS:

Whole plant Fresh Weight: Whole plant fresh weight of the plant increased with the age in the ABA, PBZ and SA treated *Solanum trilobatum* plants. When compared with control plants, the increased fresh weight was found as 239.41percent in PBZ followed by ABA and SA treatment in 173.86 and 150.23 percent over control respectively. Among the treatments the highest growth was recorded in PBZ followed by ABA and SA in all sampling days.

Whole plant Dry Weight: 110 days treatment of PBZ highly increased the dry weight 296.31percent was compared to control plants and also ABA and SA moderately increased and the dry weight was 248.75 and 195.2 percent over control respectively.

Whole Plant Stem Length: When compared with control, *Solanum trilobatum* plants treated with PBZ (161.53%) for 110 days, showed maximum stem length enlargement. While, compared with PBZ, the two treatments ABA and SA lessen the stem length as 143.95 and 129.27 percent over control.

Whole Plant Root Length: Root length was increased as 242.32 percent in the 110 day treatment of PBZ as compared to control. In addition ABA and SA treated plants showed minimal increase as 196.45 and 191.25 percent over control respectively.

Chlorophyll-"a" content: Among the treatments 110 days old ABA treated plants showed the best chlorophyll-"a"content (242.32%) than control plants. In the same way, PBZ (198.76%) and SA (215.23%) also fainly increased the chlorophyll-"a" content. In the middle of treatments the highest pigment content was recorded in ABA followed by PBZ and SA in all sampling days.

Chlorophyll-"b"content: When compared with control plants, the increased chlorophyll-"b"content was found as 307.84percent in ABA treated plants for 110 day followed by PBZ 256.86 and 215.68 percent over control treatment plants.

Total Chlorophyll content: The 110 days old ABA treated plants the best total chlorophyll content was found in 250.01percent compared with control plants, followed by PBZ 198.77 and 195.51percent over control treatment plants.

Carotenoid content: Increased the carotenoid content was found in 197.50 percent in ABA treated plants for 110 day compared with control plants, followed by PBZ 168.33 and SA 151.00 percent over control treatment plants.

Xanthophyll content: When compared with control plants, the better xanthophylls content was found as 171.39 percent in ABA treated plants for 110 day followed by PBZ 156.41 and SA 148.08 percent over control treatment plants.

Anthocyanin content: Anthocyanin content was greater than 157.10 percent in the 110 day treatment of ABA as compared to control plants, and also PBZ and SA reasonably increased the anthocyanin content as 144.05 and 137.65 percent over control treatment plants.

Discussion:

The main objective of the present investigation is to assess the effect of control and plant growth regulators (PGR) like Absicic acid, Paclobutrazol and Salicylic acid in *Solanum trilobatum* plants. The results obtained on growth parameters and pigment contents discussed here under. On over all physical assessment plants treated with Paclobutrazol were found to have more whole plant fresh weight, dry weight, root length and stem length followed by abscisic acid and salicylic acid. After the, on over all plants treated with Abscisic acid were found to have more pigment analysis content followed by paclobutrazol and salicylic acid.

Paclobutrazol treatment increased root growth when compared to control in all sampling days. Paclobutrazol increased the diameter and length of fibrous roots and enhanced the lateral root formation in tomato plants [39]. Similar results were observed in soybean treated with Paclobutrazol [40]; *Dianthus caryophyllus* [41]; *Lycopersicon esculentum* [42]; wheat [43]. ABA treatment decreases the root growth in all the sampling days. ABA was found to moderately decrease primary root growth, whereas it had no significant effect on the number of lateral roots initiated. The similarity between the responses of roots to exogenously applied ABA is not surprising [44]. Similar results were observed in wheat [45]; soybean [46]; maize [47]; peas [48] and in *Poplar* species [49, 50].

ABA treatment increases the stem length when compared to control and other treatments. Similar results were observed in two contrasting *poplar* species to drought stress and exogenous abscisic acid application [49, 50]. ABA induced growth was resulted from signal transduction at the single-cell level and thereby induces closure of stomata peas [48]; maize [47].

Paclobutrazol treatment decreases the whole plant fresh weight when compared to control in groundnut plants. Similar results were observed in triazole treated plants are a reduction in fresh weight [51]. Triadimefon and paclobutrazol altered the shoot fresh weight in peanut [52] and tomato [53]. ABA treatment decreases the whole plant fresh weight when compared to control in peanut plants. Similar results were observed in two contrasting *poplar* species to drought stress and exogenous abscisic acid application [49, 50].

Paclobutrazol increased the chlorophyll content when compared to control in all the sampling days. Similar results were observed in paclobutrazol treated barley seedlings [54] and tomato [53], retained two times more chlorophyll than control. The reported enhanced chlorophyll synthesis in *Dianthus caryophyllus* treated with Paclobutrazol [55]. Paclobutrazol treated leaves were dark green due to high chlorophyll a and b in *Chrysanthemum* [56]; *Zea mays* [57], cowpea [58] and potato [59]. The reported that the barley seedlings treated with paclobutrazol appeared greener and thicker due increased chlorophyll contents[60].

ABA treatment increases the chlorophyll content when compared to control. Similar results were observed in *Eucalyptus camaldulensis* [61]; tomato [62, 63]; *Kentucky bluegrass* [64], and maize [65]. ABA plays a direct role in mediating the photosynthesis to respiration in leaves and also the inhibition of lateral root development as reported in *Arabidopsis* [66].

Paclobutrazol and ABA treatments increased the chlorophyll 'b' content. The higher chlorophyll content in triazole treated radish may be related to the influence of triazole on endogenous cytokinin levels. It has been proposed that triazoles stimulate cytokinin synthesis that enhances chloroplast differentiation, chlorophyll biosynthesis and prevents chlorophyll degradation [67]. ABA treated plants increase the chlorophyll content. Similar results were observed in tomato [62, 63]; *Kentucky bluegrass* [64] and maize [65].

Paclobutrazol treatment increased the total chlorophyll content when compared to control. Similar results were observed in paclobutrazol treated barley seedlings [54]; and tomato [53]. Paclobutrazol treated leaves were dark green due to high chlorophyll a and b in *Chrysanthemum* [56]; *Zea mays* [57], cowpea [58] and potato [59]. The reported that the barley seedlings treated with paclobutrazol appeared greener and thicker due increased chlorophyll contents [60].

ABA treatment increases the total chlorophyll content when compared to control. Similar observation was made in *Arabidopsis* [66]; *Eucalyptus camaldulensis* [61]; tomato [62, 63]; *Kentucky bluegrass* [64] and maize [65].

The treatment with paclobutrazol increased the carotenoid content. Similar results were observed in paclobutrazol treated barley seedlings [54]; and tomato [53]. Enhanced carotenoid content synthesis in *Dianthus caryophyllus* treated with Paclobutrazol [55]. Similar results were coincides in *Zea mays* [57]; *Brassica ampestris* [68]; cowpea [58] and potato [59]. [60] Reported that the barley seedlings treated with paclobutrazol appeared greener and thicker due to increased pigment contents. Similar results were observed in growth regulators treated *Catharanthus* plants [69]. Triadimefon treatment increased the carotenoid content to a higher level in cucumber [70]. Paclobutrazol treatment increased the carotenoid content in *Raphanus sativus* plants [71].

ABA treatment increases the carotenoid content when compared to control. Similar results were observed in *Eucalyptus camaldulensis* [61]; tomato [62, 63]; *Kentucky bluegrass* [64] *and* maize [65]. ABA plays a direct role in mediating the photosynthesis to respiration in leaves and also the inhibition of lateral root development as reported in *Arabidopsis* [66]. Triazole treatment increased the xanthophyll content to a higher level in cucumber [70] and carrot [72].

Treatment with ABA increased anthocyanin accumulation in strawberry fruits [73]. Triazoles induced a transient raise in abscisic acid content in bean [74]. This increased ABA content induced by triazole might be the cause for the increased anthocyanin content.

A similar effect of SA on stomatal closure was observed also for barley [75, 76] and for maize [77]. SA increased shoot length, root length and biomass under optimal conditions. Application of α-tocopherol, GB or SA did not alter rice phenology. This differs from findings of [78, 79], which state that SA application induces early flowering in duckweeds (*Lemna gibba* L.) and Chena millet (*Panicum miliaceum* L.). Salicylic acid induces flowering in plants by acting as a chelating agent [80].

It is well documented that many phenolic compounds play an essential role in the regulation of different physiological processes, including plant growth and development, ion uptake and photosynthesis. Phenolic molecules produced by plant roots are essential for germination and plant development [81]. The first indication for a physiological effect of SA was the discovery of flower-inducing action and bud formation in tobacco cell cultures [82]. The stimulatory effect of SA on flowering was latter demonstrated in other plant species and this was ground for suggesting that SA functions as an endogenous regulator of flowering [83]. The SA effect was not specific and it promoted flowering in combination with other plant regulators (e.g. gibberellins). Besides flowering, SA also affected multiplication rate, anthocyanin and chlorophyll contents in *Spirodella polyrrhiza*. High concentration of SA (greater than 10-6 M) retarded the growth of fronds. SA treated fronds became gibbous with large air-chambers. Maximum gibbosity was observed in the 5´10-5 M SA-treated fronds of *Spirodela* [84].

Acknowledgement:

My special thanks to facilities for my work, the experiments were conducted at the Botanical Garden and Plant Growth Regulation Laboratory, Department of Botany, Annamalai University, Tamil Nadu, India.

Figure-1: Effect of abscisic acid (ABA), Paclobutrazol (PBZ), and Salicylic acid (SA) treatments induced changes on Whole plant fresh weight.

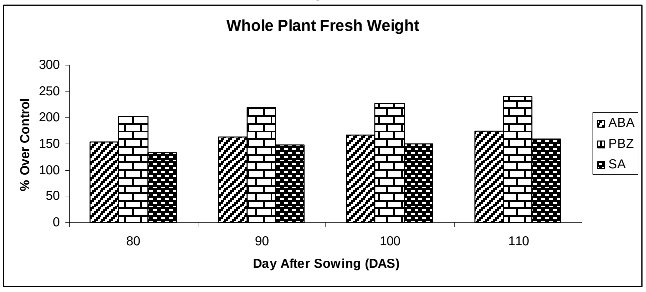


Table - 2: Effect of abscisic acid (ABA), Paclobutrazol (PBZ), and Salicylic acid (SA) treatments induced changes on Whole plant dry weight.

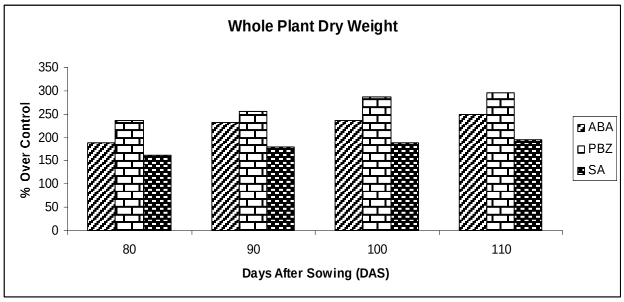


Figure-3: Effect of abscisic acid (ABA), Paclobutrazol (PBZ), and Salicylic acid (SA) treatments induced changes on stem length.

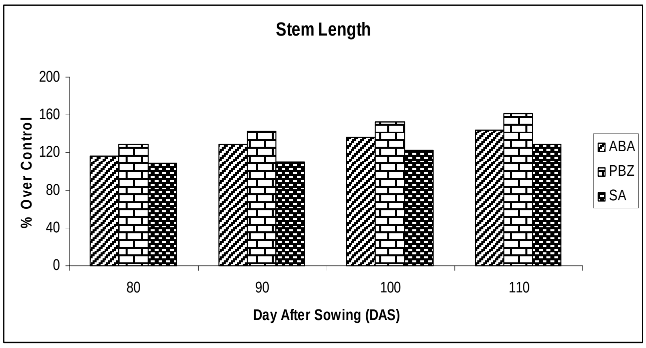


Figure-4: Effect of abscisic acid (ABA), Paclobutrazol (PBZ), and Salicylic acid (SA) treatments induced changes on root length.

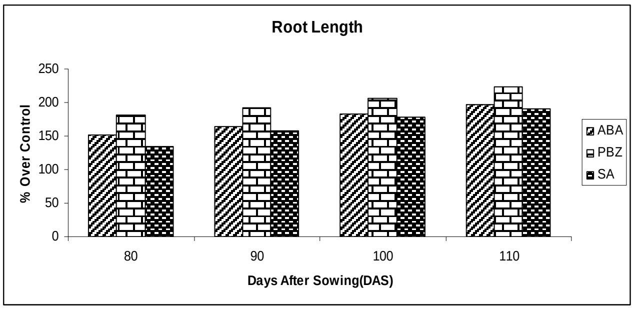


Figure-5: Effect of abscisic acid (ABA), Paclobutrazol (PBZ), and Salicylic acid (SA) treatments induced changes on Chlorophyll 'a'.

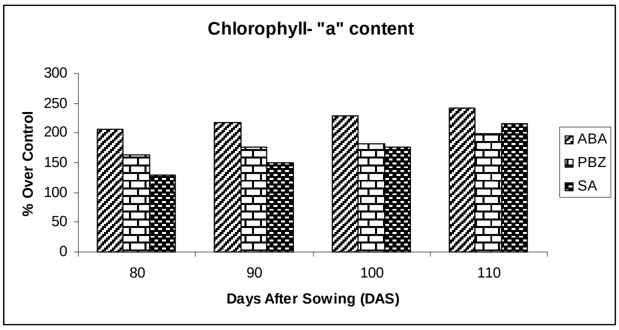


Figure-6: Effect of abscisic acid (ABA), Paclobutrazol (PBZ), and Salicylic acid (SA) treatments induced changes on Chlorophyll 'b'.

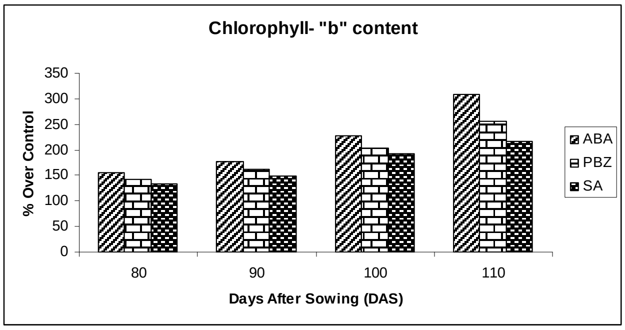


Figure-7: Effect of abscisic acid (ABA), Paclobutrazol (PBZ), and Salicylic acid (SA) treatments induced changes on Total Chlorophyll.

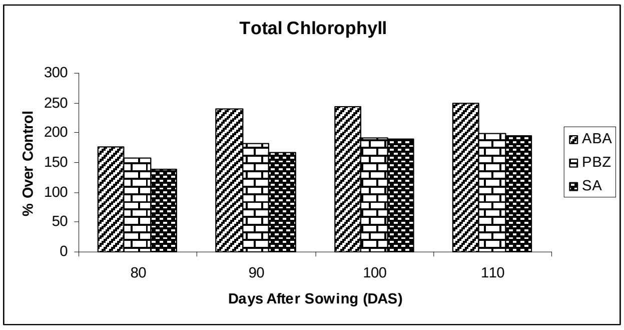


Figure-8: Effect of abscisic acid (ABA), Paclobutrazol (PBZ), and Salicylic acid (SA) treatments induced changes on Carotenoid.

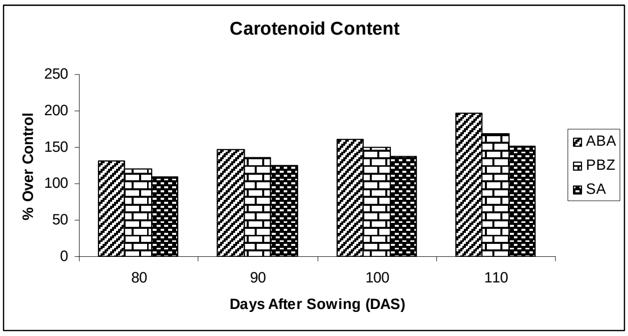


Figure- 9: Effect of abscisic acid (ABA), Paclobutrazol (PBZ), and Salicylic acid (SA) treatments induced changes on Xanthophyll.

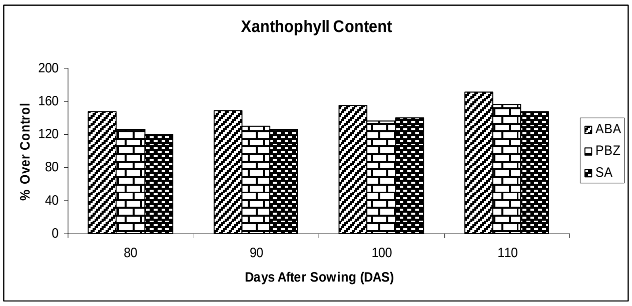
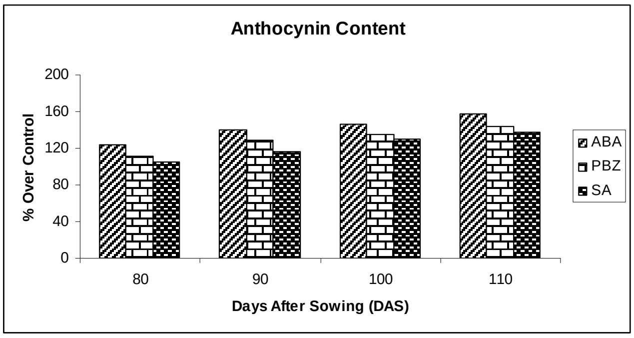


Figure-10: Effect of abscisic acid (ABA), Paclobutrazol (PBZ), and Salicylic acid (SA) treatments induced changes on Anthocyanine.



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