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Effect of Iaa on the Growth, Physiological and Biochemical Characteristics in *Catharanthus roseus* (L). G. Don.

S. Muthulakshmi[1], V. Pandiyarajan[2]

[1] Department of Botany with specialization in Plant Biotechnology, The Standard Fireworks Rajaratnam College for Women (Autonomous), Sivakasi, Tamil Nadu, India

[2]Centre for Research and Post Graduate Studies in Botany, Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi, Tamil Nadu, India

Abstract: ***Plant hormones are signal molecules produced within the plant, and occur in extremely low concentrations among the plants and generally considered to modulate many physiological events in higher plants. An experiment was conducted to study the different concentration of IAA (50, 100, 150, and 200ppm) foliar spray on vegetative growth, physiological and biochemical constituents of Chataranthus roseus (L).G.Don. The application of IAA led to significant increase of vegetative growth characters such as shoot and root length, shoot and root fresh weights and dry weights, photosynthetic pigment, non photosynthetic pigment composition and total soluble protein, total soluble glucose, free amino acid, starch, leaf nitrate, NRA and peroxidase activity. On unit fresh weight basis, the total chlorophyll content was found to increase at all concentrations. The optimal concentration for increased overall plants growth was found to be around 150 and 200ppm. Thus the exogenous application of IAA intact Chataranthus seedlings was found to be beneficial in promoting growth and biochemical responses in higher plants.***

Keywords: *Chataranthus roseus*, Vegetative growth, Chlorophyll, Carotenoid, total soluble protein total soluble sugar, free amino acid, starch, leaf nitrate, NRA, peroxidase activity, IAA.

1. Introduction

Plants produce a large and diverse array of organic compounds that appear to have direct functions in growth and development. Secondary metabolites play a major role in the adaptation to the changing environment and in overcoming stress constraints. Plants produce a wide range of compounds of various chemical classes which are effective in their defense against infection, environmental factors including biotic and abiotic stresses.

Medicinal plants have always occupied a pivotal position in human health care. Many of the plants have been an important source of medicine for thousands of years. The World Health organization (WHO) estimates that up to 80% of populations still rely on these traditional remedies. Herbal drugs are preferred over allopathic drugs on account of their efficacy, easy availability and are also said to be free from side effects. It is revealing to know that about 80% of modern drugs are derived from plants. Plant may be considered as famous chemical factory for biosynthesis of a huge array of secondary metabolites and which many of these chemicals are utilized as medicine, scent, high dyes are of commercial importance. (Dixon, 2001).

Secondary metabolites are present only incidentally and of significance to plant life. Plant produce an array of natural products, the so called secondary metabolites or pharmaceuticals, flavors, dyes, oils and resins which are not essential for plant growth and are normally produced in small amounts. These compounds usually have very complicated structures (Hadacek, 2002). Numerous plant secondary metabolites such as alkaloids, anthocyanins, flavanoids quinines, lignin, steroids and terpenoids have found commercial application as drugs, dye, flavor, fragrance, insecticides etc., and many of these compounds are valued for their potential pharmacological activities, industrial or agricultural properties which can be exploited to increase the commercial value of crops *Chataranthus roseus* (L).G.Don (Apocynaceae) derives its economic importance from its highly valued leaf anticancer alkaloids vincristine, vinblastine and its antihypertensive root alkaloid ajmalicine (Treas and Evans, 2002).

Secondary metabolites production from plant has not always been satisfactory because several intrinsic and extrinsic factors affect growth, development. The investigation is going to be carried out whether the phytohormones such as IAA will increase or decrease the production of growth and biochemical composition.

2. Material and Methods

2.1 Cultivation of plants

The seeds of *Catharanthus roseus* were collected from the local stores, Sivakasi. The seeds were surface sterilized in 0.2% $MgCl_2$ solution for 5 minutes and pots were filled with a soil mixture containing red soil, sand and farm yard manure (1:1:1 ratio). The seeds were soaked in running water for 12-18 hours and allowed to germinate in pots.

2.2 Foliar application of IAA

Indole - 3 Acetic Acid was initially dissolved in 100 ml of ethanol and a stock solution of 5 mM was made up with distilled water containing 0.02% Tween-20 (Polyoxyethylene sorbitan). Selected concentrations viz., 50,

100, 150, and 200ppm were prepared. After 6 months the leaves were sprayed with different concentration of IAA (50, 100, 150, and 200ppm) using sprayer for a continuous period of 30 days care was taken to wet both the surfaces completely to ensure maximum application. The control plants were sprayed with distilled water. The following parameters were analysed after 30 days treatment of various concentrations of IAA.

2.3 Growth parameters

Root length
Plants of both control and treated were randomly selected and uprooted without causing any damage to the root. The length of the root was measured by scale in cm.

Shoot length
Plants of both control and the treated were selected randomly and plucked without causing any damage to the shoot and the length of the shoot was measured by scale in cm.

Fresh weight
The randomly selected plants were harvested without causing any damage to the plants. The fresh weight of the plant was weighed using an electronic balance (Roy, Mumbai)

Dry weight
The selected plants were dried in the hot air oven (NSW, Madurai) at 800c for 1-2 hours. The dry weight of the plant was weighed using an electronic balance (Roy, Mumbai).

Estimation of photosynthetic pigments Photosynthetic pigments such as chlorophyll a, b, a & b and carotenoids were estimated following the method of Wellburn and Lichtenthaler (1984). The absorbance at 662 nm, 645 nm and 470 nm was measured for chlorophyll a, b and carotenoids respectively using an ELICO SL-171 Spectrophotometer.

Non – photosynthetic pigments Anthocyanin
The anthocyanin content was calculated using the formula of Mancinnelli, *et al*., (1975). The absorption of the above solution was read at 530 nm and 657 nm. **Flavonoids**

The flavonoid was estimated by Mirecki and Teramura method (1984). The absorbance was noted at 315 nm using an UV- Visible spectrophotometer (Shimadzu- 1700, Japan).

Estimation of soluble proteins
The total leaf soluble protein was estimated by Lowry's method (1951). The absorbance was read at 650 nm with an ELICO SL-171 Spectrophotometer. The soluble protein content was estimated using Bovin serum albumin (BSA) as standard.

Estimation of Total Soluble Sugar
Total soluble sugar present in leaf was estimated using anthrone reagent (Jayaraman, 1981). The total soluble sugar present in the leaf was estimated from the Standard graph of glucose.

Estimation of free amino acid
Free amino acid was estimated by Ninhydrin method (Jayaraman, 1981). The color developed was measured at 550 nm using proper blank.

Estimation of starch
Starch was estimated by (Dennis and Winfield, 1978). Absorbance of the supernatants were recorded at 600 nm.

Estimation of leaf nitrate
The nitrate content of the leaf tissue was determined by the method of Caralodo *et al*., (1978). The absorbance was measured at 410 nm.

Estimation of In vivo Nitrate Reductase activity
In vivo NR activity was assayed by Jaworski (1971) method. The amount of Nitrite formed per unit time per unit leaf fresh weight was expressed as rate of Nitrate Reductase enzyme activity

Estimation of peroxidase activity
Peroxidase activity was measured by the method of Addy and Goodman, (1972). The absorbance was measured at 420 nm.

3. Result and Discussion

Growth promoting hormones have obviously been used owing to their beneficial effects on growth and yield of plants. Plants are completely dependent on hormonal co-ordination mechanism. Hormones occur in traces and are constantly maintained at low levels. Growth hormones are chemical messengers, which direct cell to carryout various functions of growth and differentiation.

In the present study, it has been observed that IAA had increased the growth characters such as shoot length, root length, fresh weight and dry weight. In the case of biomass attributes, the IAA has appreciably increased the shoot length, root length, plant fresh weight, plant dry weight. The effect was different at various concentrations of IAA.

In general, phytohormones are having their own morphological, physiological and biochemical role in plants. Based on their role, the effect was different in *Catharanthus roseus*. The obtained results were supported by several researchers.

Effect of IAA on growth characteristics

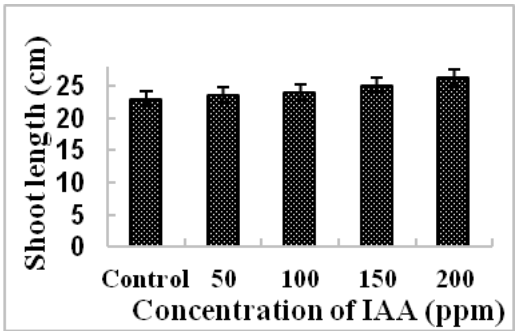
The overall vegetative growth was measured in terms of shoot and root length, root and shoot fresh weight, dry weight and leaf area. The exogenous application of IAA increases the shoot length, root length, shoot fresh weight, root fresh weight and leaf area.

There are several reports that suggest the growth promoting activity of IAA. It has been reported that foliar application of NAA at the concentration of 50ppm caused significant improvement in the vegetative growth and yield in garlic (Das *et al*., 1996) as has been found in the present investigation.

Increase in plant height with the application of NAA was also reported by Lakshmamma and Subba Rao (1996) in wheat which is consistent with the results of our present study. Effect of IAA on cell division and elongation of cells might have resulted in the increased plant height.

Application of IAA increased germination percentage, plant height, number of branches and leaves, total chlorophyll content and dry weight in *Lens culinaris* Naeem *et al*. (2004). This coincides with our findings. Similar to the change in shoot dry weight, root dry weight and leaf area also increased under hormone treatment. Leaf area is generally considered as an index of plant growth. Therefore, increase in leaf area is a confirmatory evidence to show that the phytohormones used in the studies are effective.

Application of GA_3 showed remarkable increase in the number of compound leaves and the length of shoot and the application of IAA resulted in an increase in the number of compound leaves. GA_3 and IAA had regulatory effect to enhance the plant height, number of branches, number of leaves as compared to other plant growth regulators and control in soybean (Sarkar *et al*., 2002). Ashraf *et al*., (2006) observed that IAA is successful in enhancing the plant growth and yield of barley cultivars and alleviated the adverse effect of water stress. In our study all concentration of IAA increases the shoot fresh weight and dry weight. The changes in shoot dry weight are a clear representation of the vegetative growth.



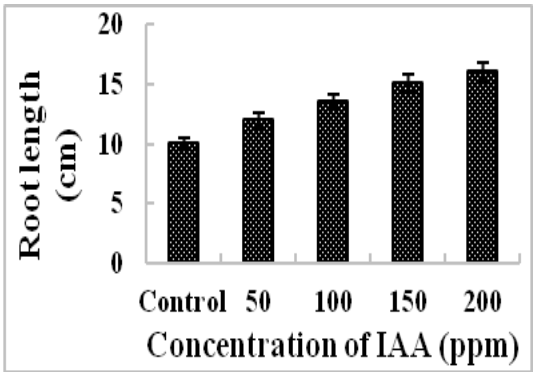
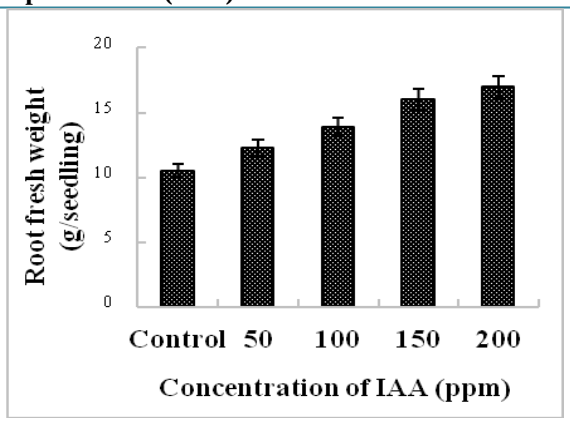


Figure 1: Typical changes in shoot length and root length of *Catharanthus* treated with various concentrations of IAA. The values are an average of 5 independent measurements. Mean ± SE, n=5.



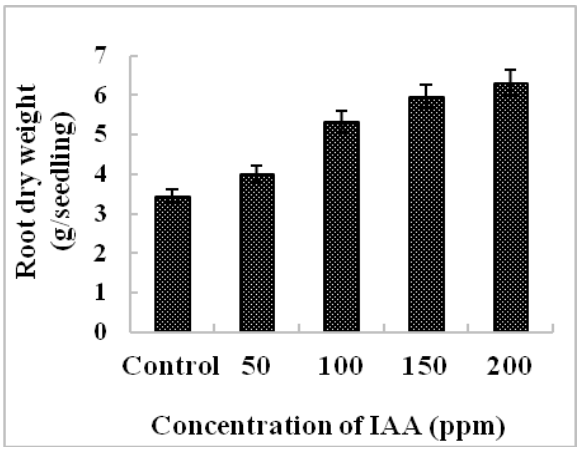
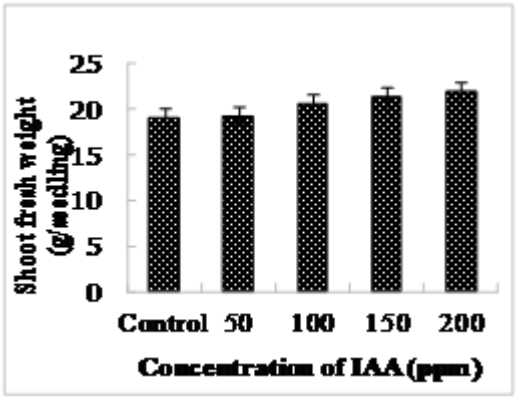


Figure 2: Typical changes in shoot and root fresh weight of *Catharanthus* treated with various concentrations of IAA. The values are an average of 5 independent measurements. Mean ± SE, n=5



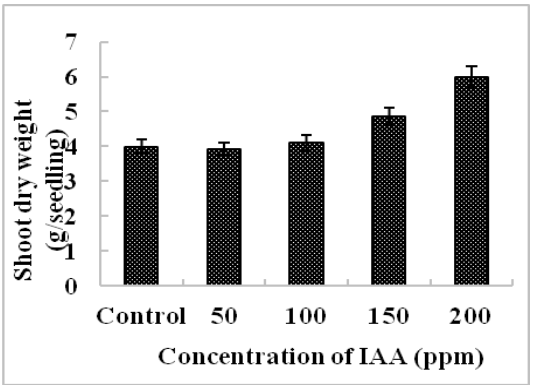


Figure 3: Typical changes in shoot and root dry weight of *Catharanthus roseus* treated with various concentrations of IAA. The values are an average of 5 independent measurements. Mean ± SE, n=5.

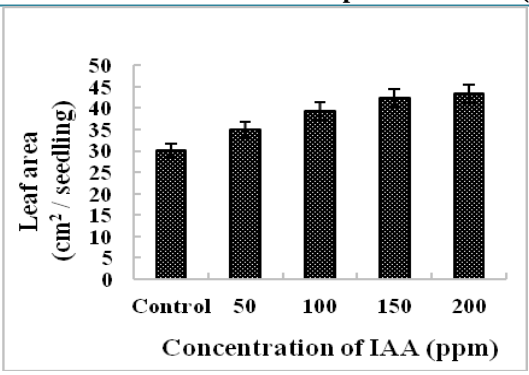


Figure 4: Typical changes in leaf area of *Catharanthus roseus* treated with various concentrations of IAA. The values are an average of 5 independent measurements. Mean ± SE, n=5.

Effect of IAA on biochemical constituents

In the present study, it has been noted that the *Catharanthus* seedling treated with different concentration of IAA substance increased the biochemical characters such as photosynthetic pigments, non photosynthetic pigments, protein, free amino acid, sugar, and starch and NR activity over the control plant.

The chlorophyll *a* and chlorophyll *b* were found to increase with increase in concentration of IAA in *C. roseus*. The level of chlorophyll *b* which was high under hormone treatment indicates changes in storichiometry of PS II and PS I. As Chl *b* is associated more with PS II, any significant change in Chl *b* levels, would indirectly affect the efficiency of PS II rather than PS I.

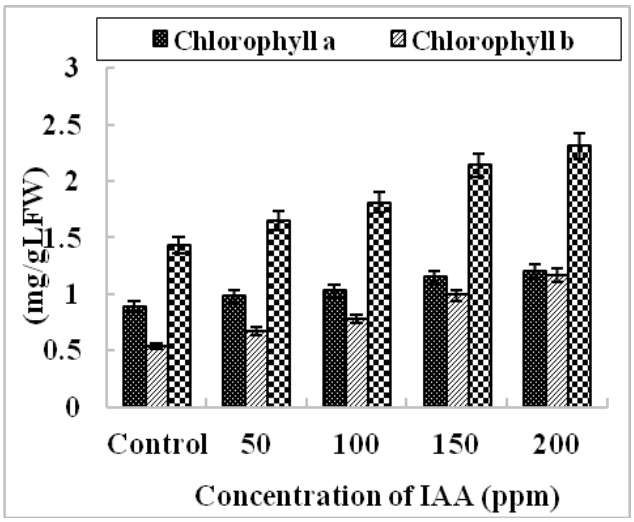
IAA treated plants exhibited higher values of dry weight and chlorophylls content than the control (Abdel-Latef, 2003; Afroz *et al*., 2005; Abou Al-Hamd, 2007). Enhanced germination and seedling growth by plant growth regulators may be mediated through changes in the activities of carbohydrate metabolism enzymes (Kaur *et al*., 2000). The increase in the dry matter due to soaking in GA_3 and IAA solution might be attributed to rapid increase in cell division, cell enlargement and accumulation of building units that accompanied by greater saccharine content than those of untreated plants (Abdel-Latef, 2003; Abdel-Latef *et al*., 2009).

Application of IAA increases protein synthesis in *Catharanthus*. It may be substantiated by the active participation of an enzyme activity nitrate reductase (reduction of nitrate to nitrite and then to aminoacids) and increase in the polyribosome and protein synthesis. The changes in leaf nitrate content and *in vivo* nitrate reductase activity reveals the low concentration of IAA favored the accumulation of may be due to enhancement of nitrogen or nitrate uptake by plants. Muthuchelian *et al*., (1994) have proved that the positive effect of salicyclic acid on nirate reductase activity was due to its possible role in the activation of inactivate nitrate reductase protein and prevention of enzyme degradation by proteolysis. This might also be involved in the enhancement of enzyme synthesis or its maintenance in active form and thus has a protective role on nitrate reductase activity (Rane *et al*., 1995). In a legume tree (*Parkia javanika*) showed significant increase in the nitrate reductase activity after treatment for indole acetic acid (Premabatidevi, 1998) .

NAA (10^{-6} M) enhanced *in vivo* nitrate reductase activity and in vitro NRA was increased in chicory roots. NAA may control nitrate reductase by a phosphorylation mechanism underscored by higher sensitivity of *in vitro* NRA to magnesium (Vuylsterker, *et al*., 1997). The auxins increases ammonium assimilating potential of maize leaves (Awasthi and Garg 2007). .Which coincides with our findigs

Application of GA_3 and IAA increased accumulation of protein content and carbohydrates content. This accumulation of carbohydrates due to GA_3 and IAA treatment might be linked with the efficiency of photosynthetic apparatus, which leads to increase in plant productivity and dry matter production (Azooz *et al*., 2004). IAA did not influence the nitrate reductase activity in *Hordeum vulgare* (Kumar *et al*., 1993).

Treatment with IAA increasesd anthocyanin and flavonoid content. The anthocyanin and flavonoids are non-photosynthetic pigments taking part in plant defense mechanisms. The effect of these non-photosynthetic pigments depends on the environmental factors like light temperature, drought, radiation stress *etc*. Have suggested that the concentration of surface flavonoids decrease with leaf age in all plants. Both anthocyanins and flavonoids tend to accumulate more in foliar tissues at times of abiotic stresses. High concentrations of the phytohormones lead to the development of these pigments in order to protect the seedlings against the action of IAA oxidase. IAA treated plants in our study showed increases in peroxidase activity at all the concentration of IAA. The higher level of endogenous auxins could also lead to early sprouting of leaves. The high rate of peroxidase activity may be due to enhanced auxin catabolism triggering the root intitation process (Kochhar *et al*., 2005). While IAA oxidase seems to be involved only in triggering and initiating the root / shoot primordia peroxidase is involved in both root initiation and elongation processes and oxidation products of auxin catabolism may be involved in the initiation of roots.



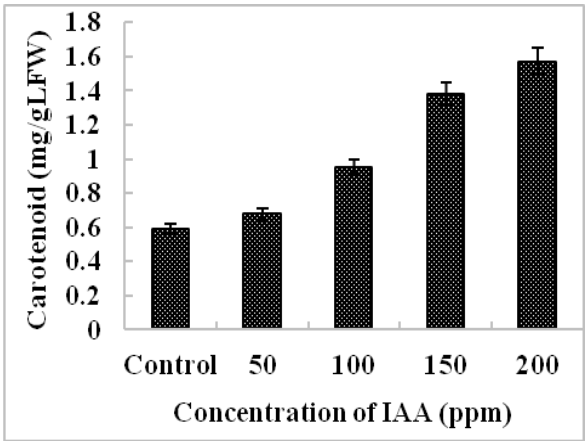
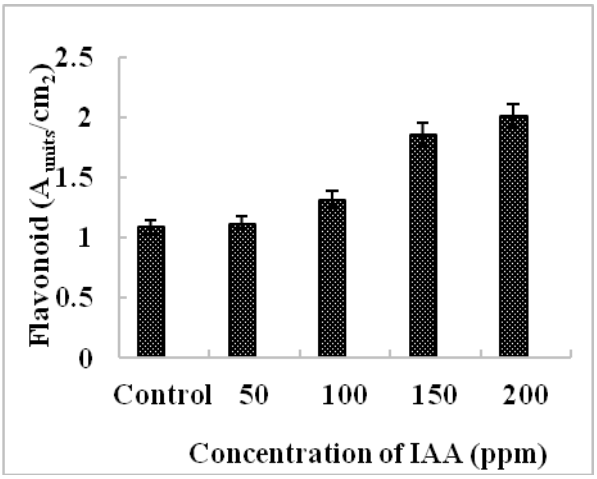


Figure 5: Typical changes in chlorophyll *a*, *b*, total chlorophyll and carotenoid content of *Catharanthus* treated with various concentrations of IAA. The values are an average of 5 independent measurements. Mean ± SE, n=5.



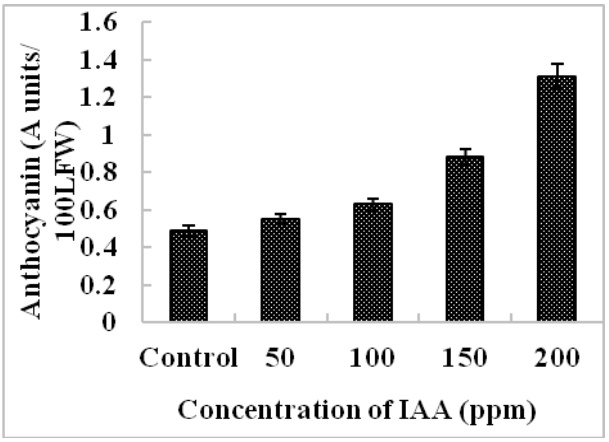
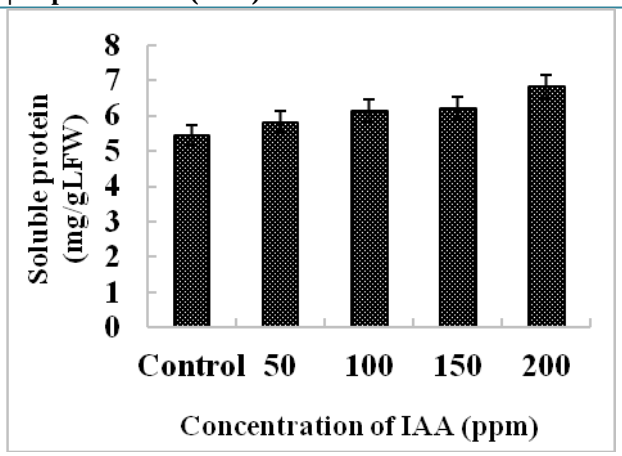


Figure 6: Typical changes in flavonoid and anthocyanin content of *Catharanthus* treated with various concentrations of IAA. The values are an average of 5 independent measurements. Mean ± SE, n=5.



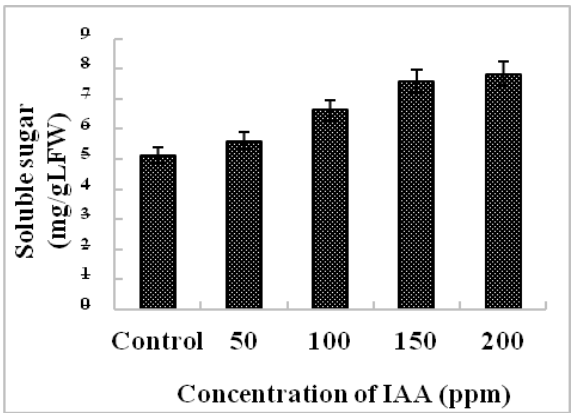
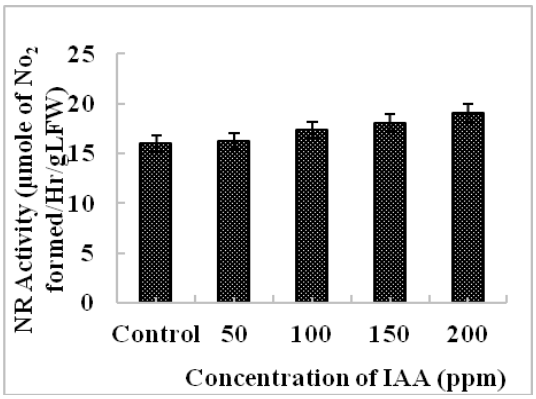


Figure 7: Typical changes in soluble protein and sugar content of *Catharanthus* treated with various concentrations of IAA. The values are an average of 5 independent measurements. Mean ± SE, n=5.



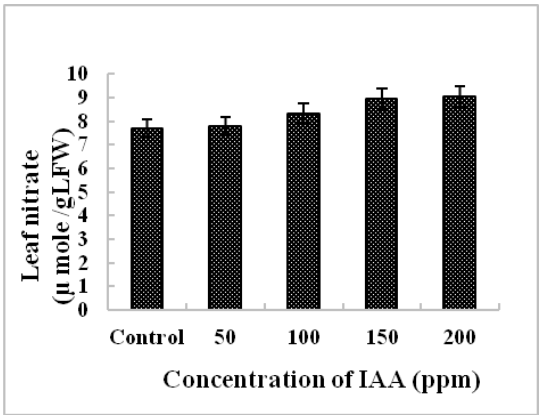


Figure 8: Typical changes in NR activity and leaf nitrate content of *Catharanthus* treated with various concentrations

of IAA. The valued are an average of 5 independent measurements. Mean ± SE, n=5.

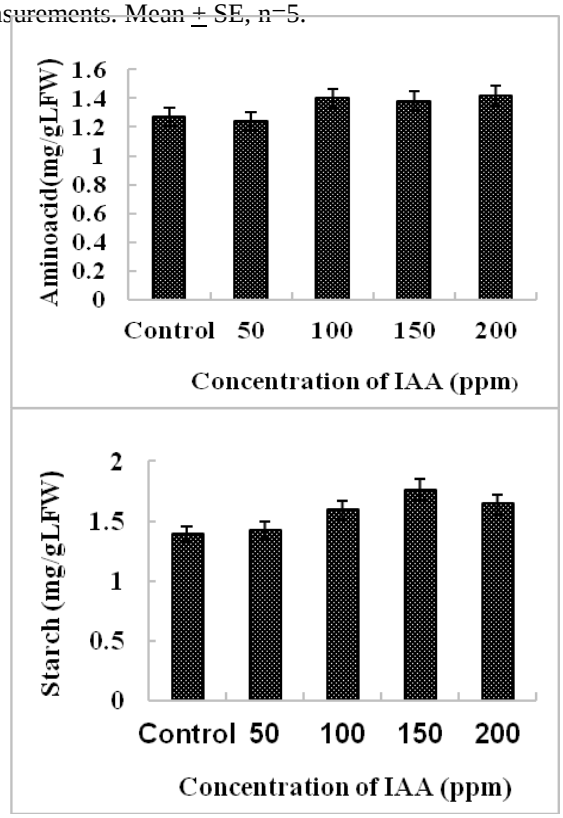


Figure 9: Typical changes in free aminoacid and starch content of *Catharanthus* treated with various concentrations of IAA. The values are an average of 5 independent measurements. Mean ± SE, n=5.

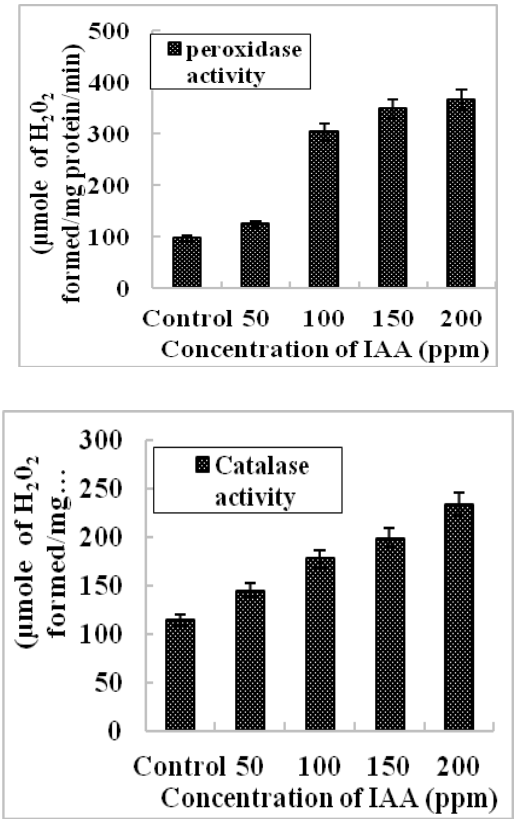


Figure 10: Typical changes in peroxidase activity and catalase activity of *Catharanthus* treated with various concentrations of IAA. The values are an average of 5 independent measurements. Mean ± SE, n=5.

4. Conclusion

IAA has a good potential in imporving accumulation of biochemical composition. Treating seeds of *Catharanthus roseus* IAA is found to be more effective in improving alkaloid content. The concentrations of 150 and 200ppm of IAA give the best results. So, the farmers may be advised to make up of IAA for improving biomass and alkaloid content in *Catharanthus roseus*.

5. Acknowledgement

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