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EFFECT OF FOLIAR APPLICATIONS OF PLANT GROWTH REGULATORS ON YIELD PARAMETERS AND SENNOSIDE CONTENTS OF *CASSIA ANGUSTIFOLIA* VAHL.

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ABSTRACT

The uses of plant growth regulators have emerged as an important tool in improving agricultural production. The study was conducted to compare the effects of three PGRs on the yield parameters and sennoside (a and b) contents from leaves and pods of medicinal plant *Cassia angustifolia.*The PGRs viz., Gibberellic acid (GA_3), Indole acetic acid (IAA) and the growth retardant Abscisic acid (ABA) were applied at the concentrations of 25, 50 and 100mg/l as foliar spray. Performances of different PGRs in *C. angustifolia* indicated that all the PGRs used have caused statistically significant increase in yield attributes and sennoside contents over control. Amongst the different treatments of PGRs, IAA and ABA (100mg/l) had shown maximum positive influence on yield parameters in *C angustifolia*. While for enhancing sennoside a and b contents from leaves and pods, IAA and ABA (50mg/l) emerged best treatments over control.

Key words: senna, foliar spray, plant growth regulators, HPLC.

INTRODUCTION

Different strategies like, use of dormancy breaking agrochemicals, proper irrigation and fertilizers, hybrid seeds, plant growth regulators (PGRs) etc. are generally used to achieve vigorous growth and to enhance the flowering, fruiting, yield and production of commercially important secondary metabolites. Amongst these, use of PGRs is proven and widely used technique for different crops including medicinal plant to achieve these objectives.

The term plant growth regulating substances (PGRS) include both naturally occurring and synthetic growth substances, and include both growth promoters and growth retardants. They control and regulate the growth, metabolic processes and developments in plants by physiological manipulation. The use of PGRs have emerged as an important tool in improving agricultural production and to help in removing many of the barriers imposed by heredity and/or environmental stress. The major types of plant bio-regulators are auxins, gibberellins, cytokinins, abscisic acid, and ethylene.

Auxins is the first hormone to be discovered in plants, which regulate polar translocation, apical bud dominance, root initiation, delay in abscission, vascular differentiation, floral bud formation and fruit development. Gibberellins (GAs) are commonly used as growth enhancers because they stimulate cell division, cell growth or increase wall plasticity and the transcription of genes for α-amylase synthesis (Jones and Mac

Millan, 1984). Gibberellins stimulate stem growth by promoting both cell elongation and cell division. Growth retardants are also used in crop improvement programs. These induce variety of morphological and biochemical responses in plants, including retarded shoot elongation, stimulated rooting and protection from various environmental stresses. Hence the study was conducted to compare the effects of three PGRs on the yield parameters and sennoside (a and b) contents from leaves and pods of medicinal plant *Cassia angustifolia* under natural condition.

Cassia angustifolia (Family: Caesalpiniaceae), popularly known as senna, is a valuable plant drug in ayurvedic and modern system of medicine for the treatment of constipation and is used to cure a large number of intestinal diseases (Aktar et al. 2008). Sennoside A and B are the two anthraquinone glycosides that are responsible for purgative action of senna. However, the performance of this plant in salinity stress environments, and the effect of these stresses on its sennoside production have not been studied well. The objective of this research was to evaluate the effect of foliar application of PGRs yield parameters and sennoside contents of senna.

MATERIAL AND METHOD

Plant material

Authentic seeds of *Cassia angustifolia* Vahl were obtained from National Research Centre for Medicinal and Aromatic Plants, Boriavi, Gujarat. Field culture experiments were conducted in the research field of Department of Botany, University of Pune, from October 2008 to May 2009 to determine the effect of foliar application of PGRs on the, yield parameters and secondary metabolites content i.e. sennoside a and b in senna.

Field Experimental

All experiments were conducted in a complete randomized design. The pre-soaked seeds of senna were sown in ridges and furrows cover with thin layer of soil. The distance between two plants was 30 cm and the distance between two rows was 40 cm. Each treatment had 3 replications and each replication had 10 seedlings. Light irrigation was immediately given after seed sowing. Standard inter cultivation practices were used throughout the experiment. Temperatures during the experiment were in the range of 28-30^{0}C during day and 19-21^{0}C at night.

PGRs treatments

The plants were sprayed four times at 45, 60, 75 and 90 days after sowing with freshly prepared solutions of Gibberellic acid (GA_3), Indole acetic acid (IAA) and the growth retardant abscisic acid (ABA) in the concentration of 25, 50 and 100mg/l using 1.5, 2.5 and 3.5 litter of solution respectively for each experimental plot. PGRs were dissolved in 95% ethanol and then brought to final concentration using distilled water. The treatment was continued up to flowering stage (90DS) at the interval of 15 days. DW spread plants were considered as control.

Yield parameters analysis

Yield attributes like number of flowers per plant, number of pods per plant, pods fresh and dry weight, 100 seed weight were measured. Pod formation began at 90 DAS and therefore sampling of pods was done at 105 and 120 DAS. The fresh and dry weight of the whole plant was measured at post flowering stage (120DAS). For this randomly selected healthy plants were uprooted from each treatment and control at the stages mentioned above. After cleaning the roots fresh weight of ten plants was measured immediately with the help of electronic top pan balance and expressed in gram per plant and from this average fresh weight of each plant was determined. This plant after taking the fresh weight was kept in oven at 40^{0}C still the constant weight was obtained. The constant weight was recorded as dry weight of each plant.

For analysis of sennoside a and b contents ten randomly selected third leaf from top of each plants from control and treatment was selected at 60 DAS and green pods at 120 DAS while, kept in oven at 60^0C for 48 h and used for HPLC analysis.

HPLC analysis of Sennoside content

HPLC analysis was done at 60DAS from leaves and 120DAS from pods. All the chemicals used were of AR grade. Methanol and water were obtained from Merck (Mumbai). The standard for the experiment was obtained from Mehta Pharmaceuticals Pvt. Ltd. (Mumbai).The dried leaves (1.0 gm.) were finely powered and extracted with hexane (3 X 25 mL). The hexane extract was discarded and 25 ml of methanol:water (70:30, v/v) was added to mark, the suspension left overnight at room temperature (25^0C) and then extracted with the methanol:water mixture (2 X 25 mL). The extract was made up to 100mL with methanol: water and 10 µL samples were subjected to HPLC analysis. The extraction of pods was performed exactly as described above for leaf sample. The solution containing known concentration of range 10-100 µg/ml of sennoside was prepared in methanol, and used as standard for HPLC analysis.

HPLC analysis was performed using a Water modular system consisting of two model 501 pumps, an automated gradient controller, a model U6K injector, an in-line solvent degasser, a model 996 photodiode array detector and Millennium 2010 chromatography management software. A Symmetry C_{18} column (150 X 406mm) was used for analysis, and spectral acquisition was performed at 285nm after scanning the standards. The solvent system consist of (A) methanol: water: acetic acid (80:20:0.1v/v/v; pH 4.0). The flow rate was maintained at 0.6ml/min for the first 20 min, while at 30 min it was 1.0ml/min.

The HPLC data including height and area of the sennoside peak at particular retention time, of standard sennoside sample (with known concentration) was recorded. This data of standard sennoside sample was compared with the data obtained from plant samples and content of sennoside in the respective plant samples was calculated.

Statistical analysis

The data was presented as arithmetic means of three replicates ±standard deviation. The significance of the mean differences was explored through one-way-ANOVA statistics followed by DMRT (Duncan's multiple range test) at p=0.05 as a post hoc test. SPSS for Windows ver. 11.5 and Microsoft Excel 2003 were used to carry out statistical analyses and graphical data presentations.

RESULTS

Number of flowers

The influence of different PGRs on various yield parameters in *Cassia angustifolia* clearly indicated that the yield parameters had shown positive increase over control. The increase in number of flowers per plant was maximum in IAA 100mg/l (23.59%) recorded in Fig1. The next better treatment was GA_3 100mg/l (20.53%). However the treatment of ABA 100mg/l was comparatively less effective (18.53%).

Number of pods

The impact of various growth regulators on number of pods per plant recorded in Fig 1, illustrated that all the treatments had favoured the positive increase in number of pods per plant over control. The percentage increase was ranging from 21.78% to 41.26%. The impact of ABA (100mg/l) was highly significant, as it has caused maximum increase in number of pods per plant by 41.26%. Amongst the remaining treatments IAA and GA_3 with 100mg/l were following the previous one. They have caused 32.56% and 25.56% increase in number of pods per plant over control.

Pods fresh weight

The results on pods fresh weight per plant recorded in Fig 2 revealed that GA_3, IAA and ABA had positively influenced the pods fresh weight per plant over control. The percentage increase over control was in the range of 24.53% to 41.56%. The maximum increase in pods fresh weight was 41.56% in ABA 100mg/l. The next better treatment was IAA 100mg/l, which had caused 37.59% increase in pod fresh weight. The GA_3 100mg/g had caused 32.56% increase over control.

Pods dry weight

The impact of various growth regulators on dry weight of pods per plant recorded in Fig 2 revealed that all growth hormones used in the present studies had increased the fresh as well as dry weight of pods per plant. However the increase in yield was more significant in dry weight of pods. The increase in dry weight during field experiment was 43.26% in ABA 100mg/l. It was the highest increase over control and other treatments. While IAA 100 mg/l has caused 42.53% increase in pod dry weight and emerged as second better treatment. Even the performance of GA_3 (100mg/l) was very promising over control, because it has also caused the positive increase in pods dry weight by 36.15% over control.

Dry weight of seeds

The influence of various plant growth regulators on 100 seeds' dry weight presented in Fig 3 revealed that the 100 seeds dry weight was positively increased by all the treatments of PGRs over the control. The percentage increase in dry weight of seeds was in the range of 11.53% to 34.26%. The treatment of IAA emerged as the best treatment for enhancing dry weight of seeds (34.26%). It was followed by ABA 100 mg/l (29.42%). The GA_3 treatment had not shown so promising effect on dry weight of seeds.

Fresh weight per plant

The effects of PGRs treatment on fresh weight per plant for field grown plants of *Cassia angustifolia* are presented in Fig 4. From the results it was clearly seen that all the PGRs used in the present investigation had significantly and positively caused the enhancement in fresh biomass of treated plants over control. The highest increase in fresh weight per plant was recorded in ABA 100mg/g. (50.23 %). The next best treatment was GA_3 100 mg/l (40.23 %). The remaining treatment i.e., IAA (100mg/l) registered 23.56 % increase over control

Dry weight per plant

The results on dry weight per plant influenced by various PGRs treatments during field experiments in Cassia angustifolia presented in Fig 4 revealed that dry weight per plant was increased in the range of 24.26% to 76.23% in field experiment. ABA 100mg/l was more effective than other treatments and control for causing the accumulation of dry matter (76.23%); it was followed by GA_3 50 mg/g (51.26%). The IAA (100mg/g) treatment was less effective as compared to other treatments (30.21%).

Sennoside content

From the figure 5 and 6; it is seen that all the plant growth regulator treatments resulted in to increased sennoside content as compared to control. Amongst all the treatments, IAA (50mg/l) had a pronounced effect on the sennoside content from leaves and pods which was followed by ABA (50mg/l). The treatments of GA_3 were also quite effective over control for enhancing the sennoside content (Figure No.5 and 6). The impact of GA_3 on sennoside content might be due to enhanced biosynthetic pathway of sennoside. The treatments of IAA (50mg/l) emerged as best treatments for enhancing the sennoside content from leaves and pods by 37.15 % and 51.23% respectively over control.

The overall results on various yield parameters such as number of flowers per plant, number of

pods per plant, fresh and dry weight of pods and even dry weight of seeds were positively influenced by the treatments of GA_3, IAA and ABA. Amongst the different treatments of PGRs, IAA and ABA (100mg/l) had shown maximum positive influence on yield parameters in *Cassia angustifolia*. While for enhancing sennoside content from leaves and pods, IAA and ABA (50mg/l) emerged best treatments over control.

DISCUSSION

Total number of flowers present on a plant is one of the important yields contributing character in various plants. The correlation between number of flowers per plant and economic yield has been well established. Singh et al. (2003) noted early flowering with IAA (100ppm) and maximum number of flowers with GA_3 (100ppm) in *Tagetes patula*. Choudhuri and Chatterjee (1979) reported that the GA_3 was responsible for inducing increase in number of flowers per plant in *Solanum khasianum*. Farooqui et al. (1999) had recorded the significant increase in number of flowers in GA_3 treated *Chrysanthymum cinerariae*. The work of Gowda et al. (1986) in *S. viarum* treated with GA_3 (1000 ppm) reported enhanced and earlier flowering. Flower induction with GA_3 was observed by Warm (1980) in *Hyoscyamus niger*. The earliest flowering in *Coriandrum sativum* due to GA_3 (50 ppm) was noted by Verma and Sen (2003). Similarly Verma et al. (2003) had also reported maximum number of flowers in *Prunus salicina* with GA_3 (100 ppm) treatment. Prasad et al. (2003) registered maximum number of flowers heads due to GA_3 (300 ppm) in *Artemisia pallens.*

The positive effect of growth retardant cycocel for improving the fruit number was recorded in *Solanum khasianum* (Hazarika, 1985; Haleem and Thimmaraju 1983), *Trichosanthes dioica* (Singh et al. 2003). Most of the workers like Chakarvarty and Basu (1973), Chaudhary and Chatterjee (1979), Borse and Dhumal (2001), Barua and Hazarika (1982) have reported that growth retardant CCC was more superior than GA_3, auxin and kinetin for increase in fresh and dry weight of fruits in *S. khasianum*.

Several investigators also reported an increase in yield of different plants by application of auxins (Mandurah, 1984; Rao and Narayanan, 1997). Foliar spray of IAA increased number and weight of pods as well as number and weight of seeds in cowpea (Saeid et al. 2010). Similarly Kumar and Singh (2003) recorded maximum seed weight due to IAA (50mg/l) in *Linum usitatissimum.* The maximum grain weight per plant was recorded in chickpea (Zarrin et al. Several investigators also reported an increase in yield of different plants by application of 2008) with ABA (foliar spray).

The enhanced fresh and dry weight due to growth retardants was reported by various workers in different plants like *Morus alba* (Mishra et al. 2001), potato (Prakash et al. 2001), *Solanum khasianum* (Shobhane et al. 2003), *Datura metal* (Gupta and Madan 1976), *Sida acuta* (Seal and Gupta 2001). The results of the present investigation in conformity with above results, indicating the suitability of PGRs for enhancing the fresh as well as dry weight of plants, which directly or indirectly caused the improvement in yield and productivity.

Sharma et al. (2001) claimed improved growth, biomass, chlorophylls, photosynthetic rate, leaf area are responsible to enhance the production of various secondary metabolites. The results of present investigation are in agreement with the above findings.

Similar results have reported an improvement in secondary metabolites through treatment of auxins in different medicinal plants. In *Solanum nigrum*, the positive influence of IAA on alkaloid production was recorded by Bhatt et al. (1983). Reports in *Solanum jaminoides* (Sahoo et al. 1999), *Hyoscyamus muticus* (Gamasy et al. 1978) *Solanum khasianum* (Borse et al. 2000),

Artemisia annua (Yaseen and Tajuddin 1998), *Andrographis paniculata* (Gudhate and Dhumal 2008) showed positive influence of IAA on secondary metabolite production. The influence of GA_3 on biosynthesis and accumulation of alkaloid content was recorded by various workers in *Solanum khasianum* (Borse et al. 2000, Borse and Dhumal, 2001; Gowda, 1986). In the present studies the treatment of growth retardant ABA found to be the second best treatment for enhancing the sennoside content. Several investigators also reported an increase in secondary metabolites content by application of plant growth retardants in *Solanum khasianum* (Gowda et al. 1986), *S. laciniatum* (Antably et al. 1975; Eid et al. 1974), *Datura metal* (Gupta and Madan 1976), turmeric (Jirali et al. 2001), *Catharanthus roseus* (Choudhary and Gupta 1996).

CONCLUSION

The comparative account regarding the performance of different PGRs in C. angustifolia indicated that all the PGRs used have caused statistically significant increase in yield and sennoside content over control, but the effect of IAA and ABA was more significant and hence use of these PGRs can be recommended for commercial purpose after studying the cost benefit ratio. All the results on yield parameters and sennoside content were showing positive and significant correlations.

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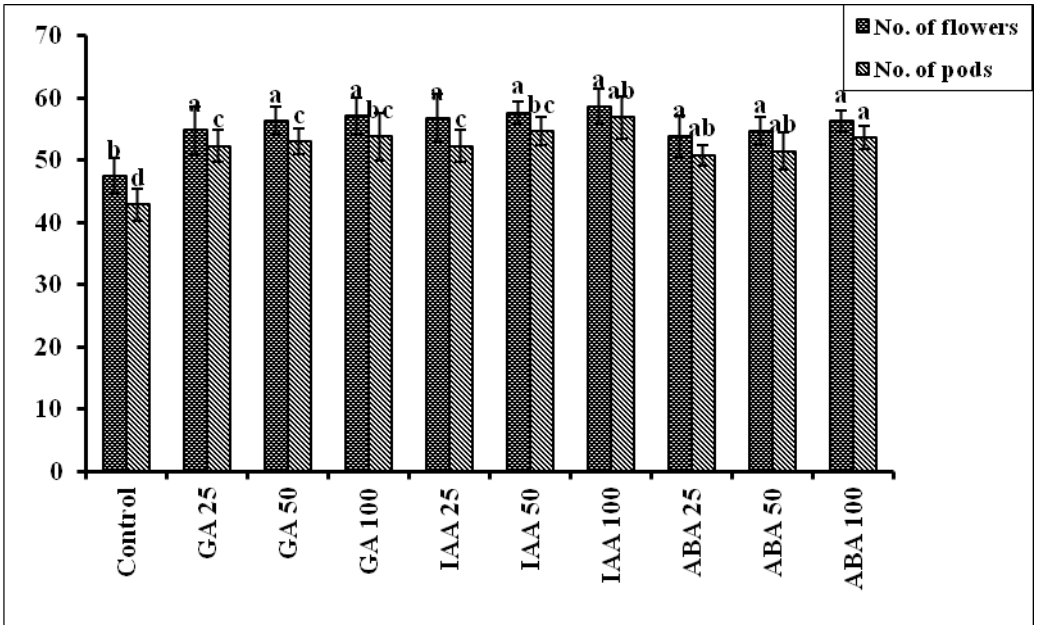

Fig.1. Effect of PGRs on no. of flowers and no. of pods per plant

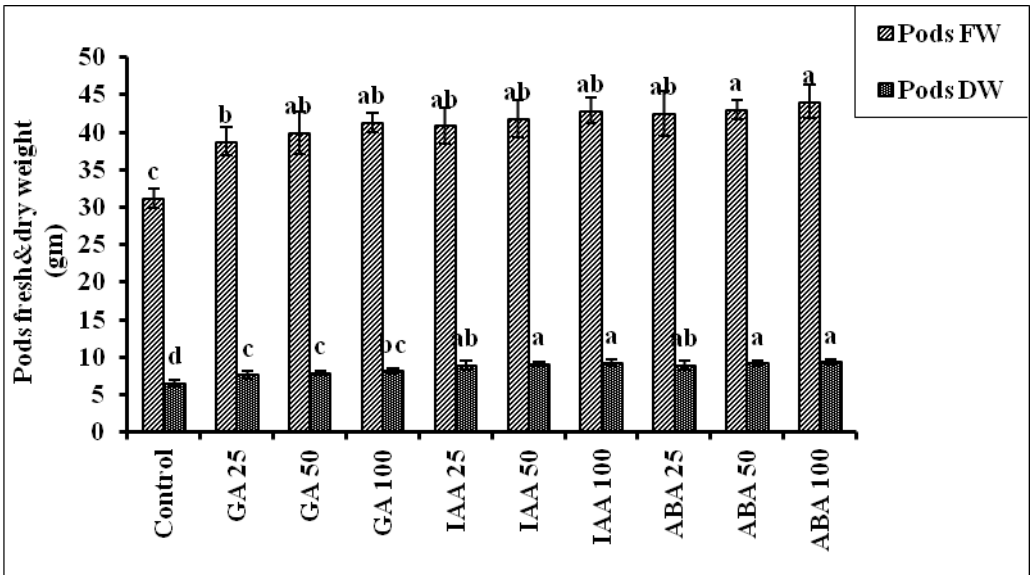

Fig. 2 . Effect of PGRs on pods fresh weight and pod dry weight per plant

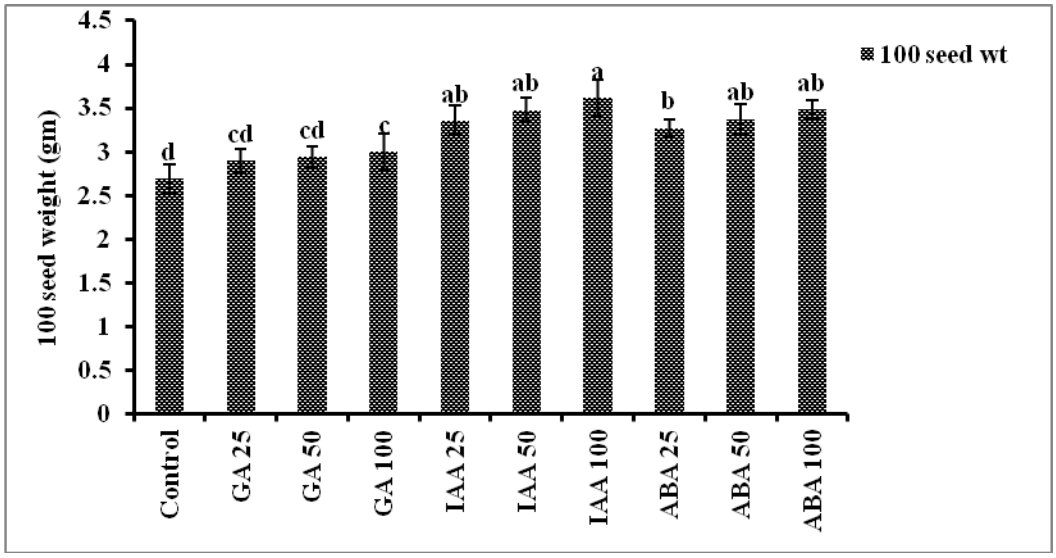

Fig.3 Effect of PGRs on 100 seed weight

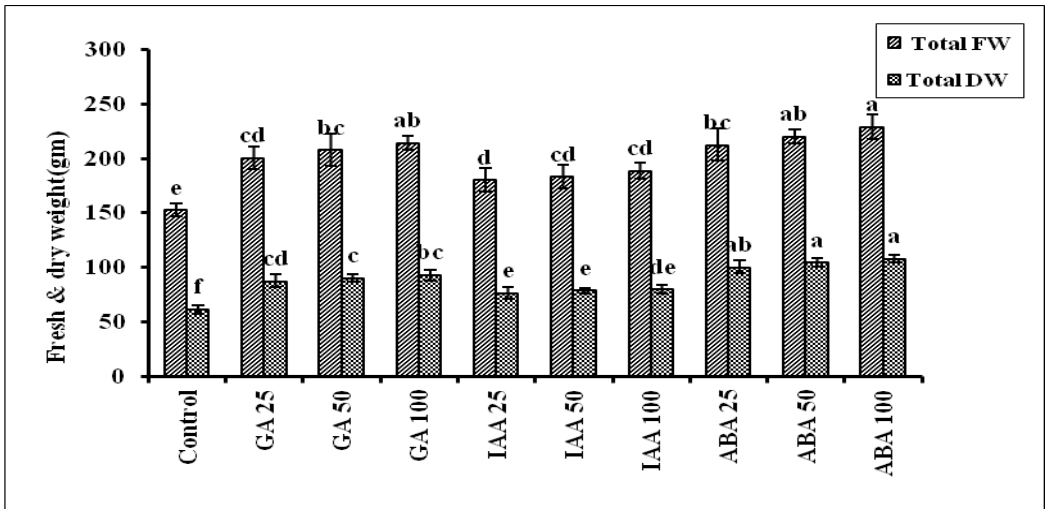

Fig.4 Effect of PGRs on total fresh weight and total dry weight

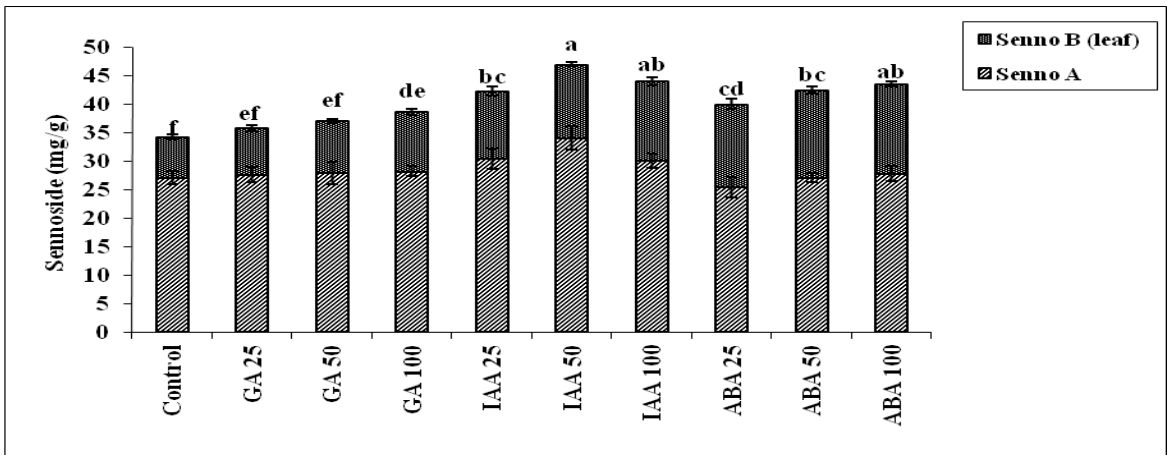

Fig.5 Effect of PGRs on sennoside content a and b from leaves (60 DAS)

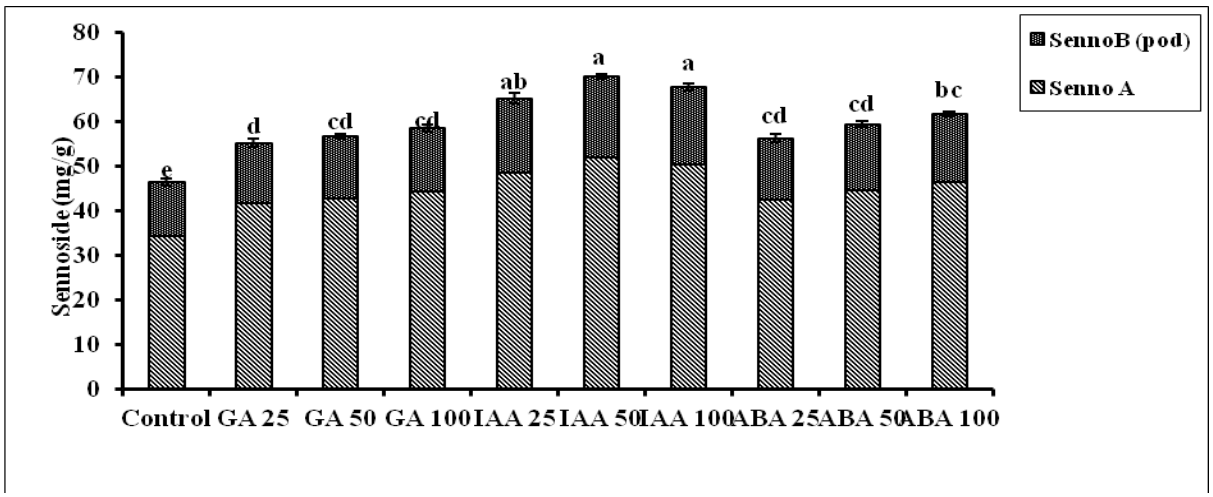

Fig. 6 Effect of PGRs on sennoside content a and b from pods