INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE AND TECHNOLOGY
© VISHWASHANTI MULTIPURPOSE SOCIETY (Global Peace Multipurpose Society) R. No. MH-659/13(N)
www.vmsindia.org

EFFECT OF EXOGENOUS APPLICATION OF PLANT GROWTH REGULATORS ON POLYPHENOLS AND FLAVONOIDS CONTENT OF *AMARANTHUS GANGETICUS* L. UNDER SALT STRESS

Priyanka P. Jadhav[1] and D. K. Gaikwad[2]
[1]Department of Botany, SSCM, Kolhapur, 416002, (MS) India
[2]Department of Botany, Shivaji University, Kolhapur.416004, (MS) India
jpriyanka0510@gmail.com

Abstract:
Plant growth regulators are widely used to overcome a biotic stresses including salinity stress in plants. *Amaranthus gangeticus* L. belonging to the family Amaranthaceae and it is used as leaf and stem vegetable. The exogenous applications of plant growth regulators like Putrescine, S.A., GABA, and Biotonic under the NaCl salinity stress (50, 100 mM) on the polyphenols and flavonoids content of *A. gangaticus* were studied. It reveals that foliar sprays of these plant growth regulators increases the secondary metabolites as compared to that of untreated plant. This increase in content of polyphenols and flavonoids might be beneficial for increase in antioxidant potential of *A. gangeticus* under saline condition.

Keywords:- Putrisine, Biotonics, Secondary metabolites

Introduction:-

The modern world is facing the problem of ever growing population with decrease in the fertile core of land per capita. Soil salinity is one of the major causes to sustain the productivity of crop plants. The plants which are subjected to the salinity stress can cause disturbance in metabolic process leading to the changes in water balance, photosynthetic, respiratory, carbohydrate, minerals and nitrogen metabolisms as well as it affects the antioxidant metabolism due to the production of reactive oxygen species or free radicals. Thus the foliar application of different plant growth regulators can acts as a signal transducer on messenger to develop the stress tolerance potential. Salinity cause disturbance of metabolic process leading to an increase in phenolics compounds [1]. Flavonoids were highly effective scavengers of oxidizing molecules containing singlet-oxygen and various free radicals [2,3].

Material and Methods:

Salinity treatments and foliar application of plant growth regulators:- *A. gangeticus* Seeds were sown in earthen pots filled with garden soil containing farm yard manure in the proportion of 3:1. Pots were watered twice a week and every care was taken to raise healthy and vigorously growing plants in each pot. 30 days old seedlings were irrigated with equal volume of the saline water (50 and 100 mM NaCl) twice a week alternating with water. After two successive salinity treatments spray of respective plant growth regulators such as SA (50 ppm), Putrescine (10 ppm), GABA (10 ppm) and Biotonic formulation was given. (Biotonic formulation is a compound mixture of amino acids (Cystein, Methionine, Lysine, Valine and GABA), vitamins (Riboflavin B2 and Nicotinic acid B3), Saccharides (Myo-inositol), cytokinin (6BA) and protein (albumin), each compound dissolved separately and then all the compounds were mixed together to make the final volume 100 ml with DW to achieve 100 ppm concentration. This is the stock solution of biotonic formulation. 0.5 ml of stock solution was added in 1000 ml DW and used for foliar application) were applied and spraying was repeated after one week from the first spray. After such two sprays the plants were used for analysis. The weed control of plant was done by hand weeding.

Total Polyphenols

The method of Folin and Denis (1915) was employed for determination of the total polyphenol content in leaf and stem tissue of *A. gangeticus* (from each treatment and control). Five hundred mg leaf and stem tissue was homogenized in 80 % acetone and filtered through Buchner's funnel. The residue was washed several times with 80 % acetone and the final volume was made 50 ml with 80% acetone. Two ml of plant extract along with a series of standard tannic acid (0.1 mg/ml) were taken in separate Nessler's tubes and to each tube 10 ml of 20% Na_2CO_3 and 2 ml of Folin Denis reagent (100 g of sodium tungstate mixed with 20 g Phosphomolybdic acid in about 800 ml distilled water to this 200 ml 25% Phosphoric acid was added and the mixture was refluxed for 2-3 hours to room temperature and volume was made 1000 ml with distilled water) were added. The final volume of reaction mixture was made 50 ml with distilled water. After 20 minutes absorbance was read at 660 nm with reagent blank. Total polyphenols were calculated with the help of std.

curve of tannic acid and expressed as mg 100^{-1} g fresh weight.

Total Flavonoids

Total flavonoids were estimated according to the method of Luximon-Ramma *et al.*, (2002). Five hundred milligrams of leaf and stem material from the different treatments and control were extracted in 80 % acetone in cold mortar and pestle. The homogenate was filtered through Buchner's funnel using Whatman No. 1 filter paper. Final volume of the filtrate was made 50 ml with 80 % acetone. The reaction mixture contained 1.5 ml of the plant extract and 1.5 ml, 2% Methanolic Aluminum Chloride (2 g Aluminium chloride dissolved in 100 ml pure methanol). Blank was prepared with distilled water in place of sample. The absorbance of the reaction mixture was measured at 368 nm on a Shimadzu, UV-190 double beam spectrophotometer. Total flavonoid contents were calculated with help of standard curve of quercetine (0.3 mg/ml) and values were expressed as mg 100^{-1} g of fresh weight.

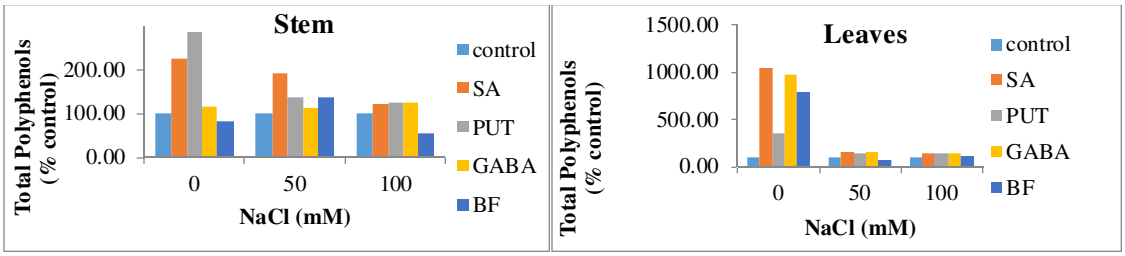

Figure 1: Effect of foliar application of plant growth regulators on total Polyphenols content of the leaves and stem of *A. gangeticus* L. grown under NaCl salinity stress.

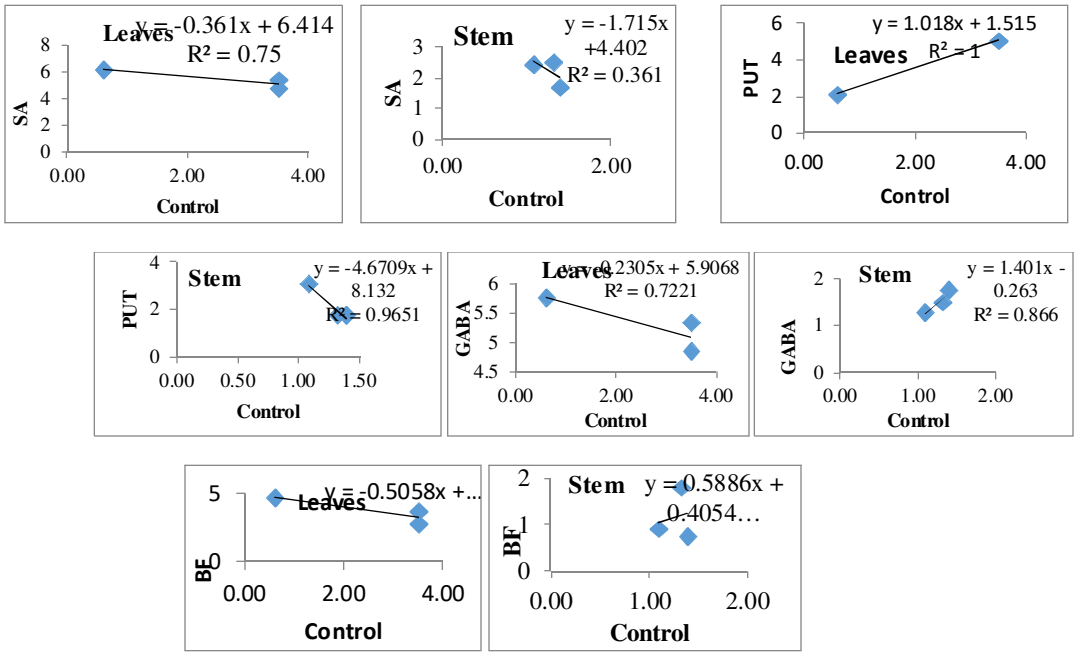

Figure 2: Relationship between plant growth regulators and different salinity treatment on total Polyphenol content of the leaves and stem of *A. gangeticus* L.

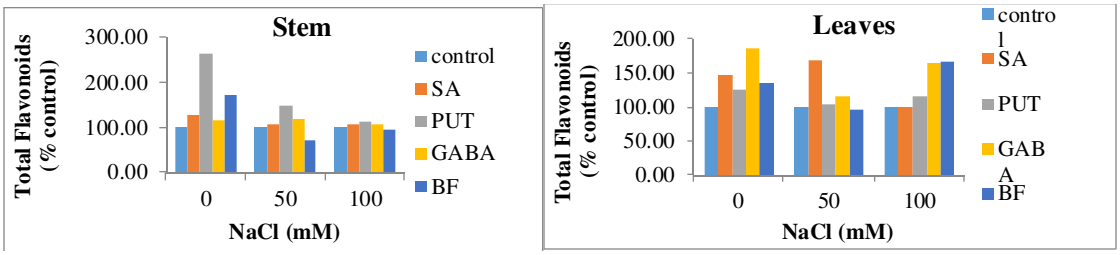

Figure 3: Effect of foliar application of plant growth regulators on total Flavonoids content of the leaves and stem of *A. gangeticus* L. grown under NaCl salinity stress.

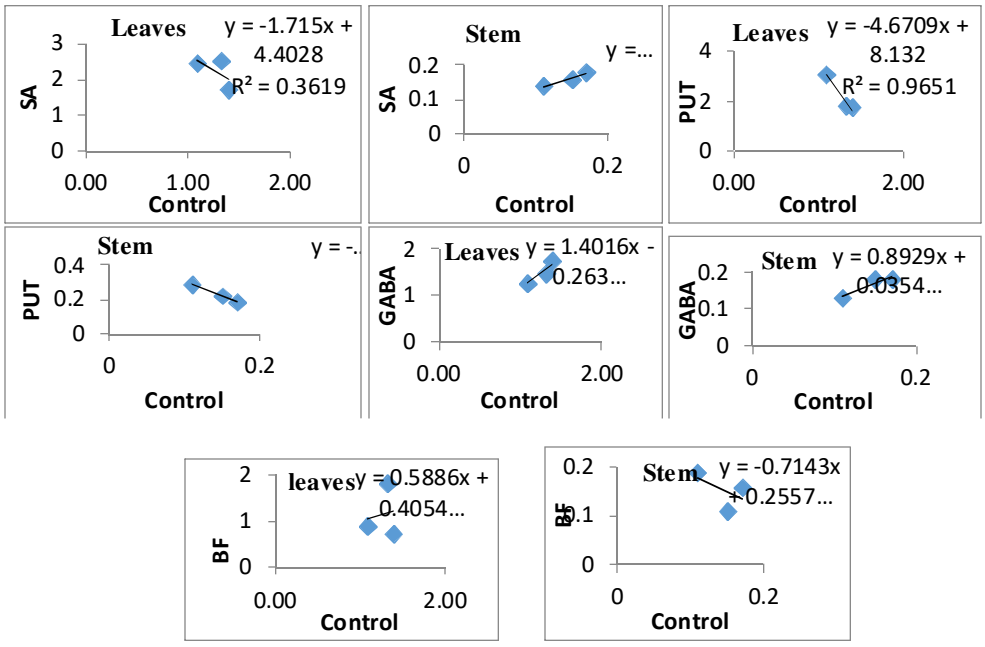

Figure 4: Relationship between plant growth regulators and different salinity treatment on total Flavonoid content of the leaves and stem of *A. gangeticus* L.

Result and Discussion:-

Effect of foliar application of SA, Putrescine, GABA and Biotonic formulation on polyphenols content of the leaf and stem tissue of *A. gangeticus* grown under saline condition is shown in Figure 1. It is evident from the results that the content of polyphenols is increased with increasing salinity treatments in leaf and stem tissue of *A. gangeticus* as compared to unsprayed unstressed control. While the foliar application of SA, Putrescine, GABA and Biotonic formulation results in an elevation of the polyphenols content in the leaf and stem tissue than unsprayed stressed control and sprayed unstressed control.

Phenpolic compound are most effective antioxidants present in edible and no edible plant material including fruits, vegetables, herbs, cereals, tree material plant sprouts and seeds[6]. Many workers reported that polyphenols content where positively correlated with antioxidant potential in different antioxidant assay DPPH radical scavenging assay, FRAP assay, LPO assay, H_2O_2 radical scavenging assay and OH radical scavenging assay [7, 8]. Application of SA increases total phenolics compounds in wheat seedlings [9]. Foliar application of SA increases polyphenols content in *Simarouba glauca* [10]. Foliar application of Putrescine and Spermine. increases polyphenols content in Cucumber [11].

Effect of foliar application of SA, Putrescine, GABA and Biotonic formulation on flavonoids content of the leaf and stem tissue of *A. gangeticus* grown under saline condition is shown in Figure 3. It is evident from the results that the content of flavonoids is increased with increasing salinity treatments in leaf and stem tissue of *A. gangeticus* as compared to unstressed control. While the foliar application of SA, Putrescine, GABA and Biotonic formulation results in elevation in the flavonoids content in leaf and stem tissue than unsprayed stressed control and sprayed unstressed control. Flavonoids are phenolics compounds and they are present naturally in the plant tissue, in different members of plant kingdom more than 4000 flavonoids have been detected and this number continuously increasing with analysis of different species [12]. Flavonoids content increased with increasing salinity in roots and shoot of barley plant [13]. Application of SA. increases flavonoids content in *Taraxcum officinale* [14]. Exogenous application of SA showed increase in flavonoid content in soybean plant under salt stress than control [15].Foliar application of Putrescine enhanced the flavonoid content in root, shoot and flower of *Chamomilla recutita* and *Origanum majprana* under salinity stress [16]

The linear regression analysis method of determining the R^2 (0.915) on polyphenols content Fig. 2. indicates that Putrescine and GABA exhibits a positive correlation under different salinity stress treatment in the stem of *A. gangeticus*. In present study the content of polyphenols was significantly stimulated due to salt stress as well as in the salt stressed foliar

sprayed plants and this increase was more pronounced due to application of the SA in leaf tissue and Putrescine in stem tissue. This increase in content of polyphenols might be beneficial for increase in antioxidant potential of *A. gangeticus* under saline condition.

The linear regression analysis method of determining the R^2 (0.937) on flavonoids content Fig.4. indicates that Putrescine and GABA displayed a positive correlation with different salinity stress treatment in leaves and SA, Putrescine and GABA in stem of *A. gangeticus*. In present study the content of flavonoids was significantly increased due to salt stress as well as in the salt stressed foliar sprayed plants and this increase was more pronounced due to application of the GABA in leaf tissue and Putrescine in stem tissue. An increased content of flavonoids under stressed condition might be effective in scavenging of oxidizing molecule containing singlet oxygen and various free radicals and also play an important role in physiological processes.

Conclusions:-

The content of polyphenols was significantly stimulated due to unsprayed salt stressed as well as in the foliar sprayed salt stressed plants and this increase was more pronounced due to application of the SA in leaf tissue and Putrescine in stem tissue. This increase in content of polyphenols might be beneficial for increase in antioxidant potential of *A. gangeticus* under saline condition.

The content of flavonoids was significantly increased due to salt stress as well as in the salt stressed foliar sprayed plants and this increase was more pronounced due to application of the GABA in leaf tissue and Putrescine in stem tissue. The increased content of flavonoids under stressed condition might be effective in scavenging of oxidizing molecule containing singlet oxygen and various free radicals.

Reference:-

1. Dhingra, H. R. and Varghese, T. M. (1985). Effect of salt stress on viability,germination and endogenous levels of some metabolites and ions in maiz (*Zea may L.*) pollen. *Ann. bot.*, **55**:415-420.

2. Rice-Evans, C.A. and Miller, N. J. (1998). Structure-antioxidant activity relationships of flavonoids and isoflavonoids. In: Rice-Evans C.A., Packet I. (eds.): Flavonoids in Health and Disease. Marcel Dekker Inc., NewYork: 199–220.

3. Ali, R.M. and Abbas, H.M. (2003). Response of salt stressed barley seedlings to phenylurea. *Plant Soil Environ.*, **4**: 158–162.

4. Luximon-Ramma, A., Bahorum, T., Soobratee, M.A. and Aruoma, O.I. (2002). Antioxidant activities of phenolic proanthocyanidin and flavonoid components in extracts of *Cassia fistula*. *Journal of Agriculture and food chemistry.*,**50**(18): 5042-5047

5. Folin, O. and Denis, W. (1915). A colourimetric estimation of phenol and phenol derivatives in urine. *J. Biol. Chem.,* **22:**305-308.

6. Kahkonen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S. and Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.*, **47**: 3954-3962.

7. Dudonne, S., Vitrac, X., Coutiere, P., Woillez, M. and Merillon, J. M. (2009). Comparative Study of Antioxidant Properties and Total Phenolic Content of 30 Plant Extracts of Industrial Interest Using DPPH, ABTS, FRAP, SOD, and ORAC Assays. *J. Agric.Food Chem.*, **57**: 1768–1774.

8. Anwar, F., Jamil, A., Iqbal, S. and Sheikh, M. A. (2006). Antioxidant activity of various plant extracts under ambient and accelerated storage of sunflower oil. *Grasas Y. Aceites.*, **57**(2): 189-197.

9. Sari, E. and Etebarian, H. R. (2009). Concentration-dependent effect of salicylic acid application on wheat seedling resistance to take-all fungus, *Gaeumannomyces graminis* var. tritici. *Phytoparasitica.*, **37(**1): 67-76.

10. Patil, M. and Gaikwad, D. K. (2013).Effect of PGRs on secondary metabolites of medicinal plant Simarouba glauca dc. *International Journal of Pharmaceutical Research and Development.,* **5**(9): 115–119.

11. Radhakrishanan, R., Shin, J., Choo, Y.S., Kim, J. G. and Lee I. J. (2014). Studies on effect of nitrogenous compounds putrescine and Spermine on cucumber plant growth. *Journal of environmental biology.*, **35**: 247-251.

12. Croteau, R., Kutchan, T.M. and Lewis, N.G. (2000). Natural Products (Secondary metabolites). In: Biochemistry and Molecular Biology of Plants. (eds.) Buchnan, B., Gruissem, W. and Jones, R. (Publ.) American Society of Plant Physiologists: pp- 1250-1318.

13. Kim, Y. H., Hamayun, M., Khan, A. L., Na, C. I., Kang, S. M.., Han, H. H. and Lee, I. J. (2009). Exogenous application of plant growth regulators increased the total flavonoid content in *Taraxacum officinale* Wigg. *African Journal of Biotechnology*., **8**(21): 5727-5732.

14. Simaei, M., Khavari-Nejad, R. A. and Bernard, F. (2012). Exogenous Application of Salicylic Acid and Nitric Oxide on the Ionic Contents and Enzymatic Activities in NaCl- Stressed Soybean Plants. *American Journal of Plant Sciences*., **3:** 1495-1503**.**

15. Simaei, M., Khavari-Nejad, R. A. and Bernard, F. (2012). Exogenous Application of Salicylic Acid and Nitric Oxide on the Ionic Contents and Enzymatic Activities in NaCl- Stressed Soybean Plants. *American Journal of Plant Sciences*., **3:** 1495-1503**.**

16. Ali, M. B., Hahn, E. J. and Paek, K. Y.(2007). Methyl jasmonate and salicylic acid induced oxidative stress and accumulation of phenolics in *Panax ginseng* bioreactor root suspension cultures. *Molecules.,* **12**: 607-621.