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EFFECT OF AMF (GLOMUS CORONATUM AND GLOMUS ETUNICATUM) ON NUTRIENTS, ENZYMATIC AND NON ENZYMATIC ANTIOXIDANTS OF LESSER PERIWINKLE (VINCA MINOR) GROWING UNDER HIMALAYAN CONDITION

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ABSTRACT

Physiological performance of plants in general, particularly cultured plant productivity and crops quality depend on the rhizosphere characteristic feature, an area of great interest to plants, producers, consumers and environmental health. Among the rhizosphere components, arbuscular mycorrhizae are one of the most common types of symbiotic associations between some rhizosphere microorganisms and plants roots. AM fungi are currently considered key players in agronomic practices as they lead to a reduction in the use of chemical fertilizers and pesticides and are therefore potentially important components for the sustainable management of agricultural ecosystems. Plant growth was affected due to inoculation of AM. There was an increase in enzymatic and non-enzymatic antioxidants indicating that this plant has the ability to scavenge or control the level of cellular ROS and can be grown successfully under AMF inoculated conditions. *Vinca minor*, a medicinal plant, was selected in the present study. The plant was subjected to AMF namely, Glomus coronatum (10%), Glomus etunicatum (10%) Glomus coronatum + Glomus etunicatum (10%) in order to see their impact on nutrients. ezymatic and non enzymatic antioxidants parameters of *Vinca* minor. The study revealed that inoculation of AMF cause significant increase nutrients (Na^+ and $K^{+,}$), CAT, POD, caretenoids, flavonoids, tocopherol, phenol, chlorophyll (a,b) and decreases lipid peroxidiation to great extent.

KEYWORD: Glomus coronatum, Glomus etunicatum, Glomus coronatum + Glomus etunicatum.

INTRODUCTION

Traditional systems of medicine have formed the basis of health care throughout the world since the earliest days of humanity and still continue to be widely practiced globally. Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. Plant derived medicines may become the base for the development of a medicine, a natural architectural plan for the development of new drugs or may be used for the treatment of diseases provide raw materials for pharmaceutical, phytochemical and cosmetic industries.

Analysis of the flora of Himachal Pradesh shows that there are approximately 3,500 species of higher plants. Out of these about 1, 500 species are reported to be of medicinal value (Chauhan, 1999). Moreover, the importance of the mycorrrhizal arbuscular fungi in organic farming and farmers' potential to increase the benefits of AM associations in such systems represented interesting subjects as it was synthesized by (Gosling *et al*., 2006). Promoting sustainable agriculture in developing countries is a key to achieving food security. It is necessary to increase investment in agriculture, broaden access to food, improve governance to global agricultural trade and increase productivity, while conserving the natural resources base (Diouf, 2011). Taking into consideration that one of the main goals of sustainable horticulture is to benefit farmers and consumers and arbuscular mycorrhizae act as biofertilizers and bioprotectants (Shalhevet *et al.,* 2001).

AMF have been found to stimulate growth, improve pathogen resistance, contribute to the formation of proper soil structure as well as influence the level of secondary metabolites in plants (Smith and Read, 2008). Rillig *et al*. (2002) described significant discursive effects of AMF hyphal length on water- stable aggregate (WSA) stabilization via the production of glomalin-related soil protein (GRSP) in a natural grassland system. The presence of these symbiotic soil microorganisms may therefore be crucial for sustainable agriculture (Gianinazzi *et al.,* 2010).

The association of arbuscular mycorrhizae with plant species native to the harsh environmental conditions may play a significant role in the re-establishment and conservation of endangered plants (Panwar and Tarafdar, 2006). Plant species exert an important selective force on AMF populations, either directly through their internal root environment or indirectly through their influence on the soil (Johnson *et al*., 2004). The combination of extraradical mycelial network and glomalin secretion is considered to be an important

element for stabilization of soil aggregates, thereby leading to reduced soil erosion and increased soil structural stability and quality (Rillig *et al*., 2006; Bedini *et al*., 2009). AMF play decisive roles in increasing plant growth, nutrient uptake, and ecosystem functioning of sustainable agriculture, as well as enhancing plant tolerance to abiotic stress (Smith and Read, 2008; Gianinazzi *et al*., 2010). AM symbiosis can monitor the responses of plants to temperature stress (Matsubara *et al*., 2004; Zhu *et al*., 2010; Wu and Zou, 2010). AMF, belonging to the phylum Glomeromycota, are considered the oldest group of organisms living in symbiosis with terrestrial plants (Redecker *et al*., 2000). AM symbiosis can enhance the activities of antioxidant enzymes, helping plants to alleviate salt stress (Alguacil *et al*., 2003).

2. MATERIALS AND METHODS

2.1 AM inoculum

The AM fungal species (*Glomus coronatum and glomus etunicatum*) were mass multiplied by using trap plant (*Triticum aestivum*) and were used for inoculation. The starter cultures were procured from Centre for Mycorrhizal Culture Collection, TERI (The energy and resources institute), New Delhi.

2.2 Mass production of AMF

AMF isolated from the soil by Warcup's soil plate method (Warcup, 1950). Air dried soil were placed in sterilized petriplates and 15 ml melted PDA (Potato Dextrose Agar) medium were poured to each petriplate. Then petriplates were incubated at 25±ºC for fungal growth. After inoculation of five to seven days, colonies were identified. These colonies were further purified by sub culturing on PDA medium. After sub culturing, extensive colonies were obtained and further used for mass culturing using wheat bran: saw dust medium. Wheat bran, saw dust and sterilized distilled water were mixed in the ratio of 3:1:4. Mixture will be transferred to conical flasks and sterilized in autoclave at 15 Ib pressure for 30 minutes. After autoclaving the flasks were allowed to cool and fungal colonies grown on petriplates were cut into 8 mm diameter with the help of sterilized cork borer and 5-7 fungal discs were added to each conical flasks containing sterilized medium under aseptic conditions. All the flasks were kept in BOD incubator at 25±2ºC for 7 to 10 days for maximum growth of fungus.

2.3 Preparation of pot mixture

The collected soil were sieved to remove the debris and large organic matter and autoclaved the sieved soil. Three plant cuttings were grown in pots (size 25×25 cm) that depend upon the nature of growth of plants. To each pot 10% inoculum of each AM fungi alone and in

combination were added. Plants will be watered regularly as and when required and nourished with 150ml. Hoagland nutrient solution after every 10 days during the course of experimentation. Three replicates wree utilized for each treatment.

2.4 Different treatments

The purpose of using an AM fungus alone and in combination were to assess which one is better for acclimatization and for various growth parameters. Different combinations of AM fungi were utilized for the experimental work and growth parameters for the selected plant will be evaluated, which are mentioned below;

1. Control (Autoclaved soil without any microbial inoculums)
2. Glomus coronatum
3. Glomus etunicatum
4. G. coronatum + G. etunicatum

2.5 NUTRIENT ANALYSIS

Potassium and sodium in the acid-digest of plant samples were determined using Flame photometer. Weighed 500 mg dried plant sample in 100 ml conical flask. Added 10 ml of conc. HNO3, placed funnel on the flask and kept for about 6-8 hrs or overnight at a covered place for pre-digestion. After pre-digestion when the solid sample was no more visible, added 10 ml of conc. HNO3 and 2-3 ml HClO4. Kept on a hot plate in acid proof chamber having fume exhaust system and heated at about 100°C for first 1 hr and then raised the temperature to 200°C. Continued digestion until the contents became colorless and only white dense fumes appeared. Reduced the acid contents to about 2-3 ml by continuing heating at the same temperature. Filtered through Whattman No. 42 filter paper into a 100 ml volumetric flask. Gave 3-4 washings of 10-15ml portions of distilled water and made the volume to 100 ml. Measured Na+ and K+ concentrations in the filtrate using Flame photometer. Recorded the flame photometer readings for each of the working standards of Na and K after adjusting blank to zero.

2.6 ENZYMATIC ANTIOXIDANTS

The enzymatic antioxidants analyzed in the leaves of *Vinca minor* Linn. were POD, CAT.

2.6.1 Peroxidase (POD)

The method proposed by Reddy *et al.* (2005) was adopted for assaying the activity of peroxidase. A 20% homogenate was prepared in 0.1M phosphate buffer (pH 6.5) from the

leaves of the plant, centrifuged and the supernatant was used for the assay. To 3.0ml of pyrogallol solution, 0.1ml of the enzyme extract was added and the spectrophotometer was adjusted to read zero at 430 nm. To the test cuvette, 0.5ml of H2O2 was added and mixed. The change in absorbance was recorded every 30 seconds up to 3 minutes in a spectrophotometer. One unit of peroxidase is defined as the change in absorbance/minute at 430nm.

2.6.2 Catalase (CAT)

Catalase activity was assayed following the method of Luck (1974). Homogenized plant tissue in a blender with M/150 phosphate buffer (assay buffer diluted 10 times) at 1-4°C and centrifuged. Stirred the sediment with cold phosphate buffer, allowed standing in the cold with occasional shaking and then repeating the extraction once or twice. The final volume for the assay mixture was approximately 3 ml. Wavelength at 240nm read against a control cuvette containing enzyme solution as in the experimental cuvette, but containing H2O2-free PO4 buffer (M/15). Pipette into the experimental cuvette 3 ml H2O2-PO4 buffer. Mixed in 0.01-0.04 ml sample with a glass or plastic rod flattened at one end. Noted the time required for a decrease in absorbance from 0.45 to 0.4. This value was used for calculations. One g tissue was homogenized in a total volume of 20ml, diluted 1 to 10 volume with water and taken 0.01 ml for assay. Calculated the concentration of H2O2 using the extinction coefficient 0.036/mmole/ml.

2.7 NON-ENZYMATIC ANTIOXIDANTS

The non-enzymatic antioxidants analyzed were tocopherol, chlorophyll, total carotenoids, total phenols, flavonoids, lipid peroxidation.

2.7.1 Tocopherol

Tocopherol was estimated in the plant samples by the Emmerie-Engel reaction as reported by Rosenberg (1992). The plant sample (2.5g) was homogenized in 50ml of 0.1N sulphuric acid and allowed to stand overnight. The contents of the flask were shaken vigorously and filtered through Whatman No.1 filter paper. Aliquots of the filtrate were used for the estimation. Into 3 stoppered centrifuge tubes, 1.5ml of plant extract, 1.5ml of the standard and1.5ml of water were pipetted out separately. To all the tubes, 1.5ml of ethanol and 1.5ml of xylene were added, mixed well and centrifuged. Xylene (1.0ml) layer was transferred into another stoppered tube. To each tube, 1.0ml of dipyridyl reagent was added and mixed well. Themixture (1.5ml) was pipetted out into a cuvette and the extinction was read at 460nm.

Ferric chloride solution (0.33ml) was added to all the tubes and mixed well. The red colour developed was read after 15 minutes at 520 nm in a spectrophotometer. The concentration of tocopherol in the sample was calculated using the formula,

$$\text{Tocopherol } (\mu g) = \frac{\text{Sample } A_{520} - A_{460}}{\text{Standard } A_{520}} \times 0.29 \times 0.15$$

2.7.2 Chlorophyll

Cholorophyll extraction was done by using dimethyl sulphoxide (DMSO) Chlorophyll extraction technique of Hiscox & Israelstam (1979). For the extractions, glass centrifuge vials containing 7 ml DMSO were preheated to 65°C in a water bath. Chlorophyll was extracted from three disks (each 3.038 cm2; approx. 100 mg f. wt total) from each leaf sample. In preliminary trials, we found that extraction at 65°C was complete within 15–20 min and no loss of Chlorophyll occurred in the heated DMSO during the first hour; therefore ran extractions for 30 min. When the extractions were complete, samples were removed from the water bath and each graduated vial was topped up to 10 ml with DMSO using a Pasteur pipette; 3 ml of each extract were then transferred to disposable polystyrene cuvettes. The spectrophotometer was calibrated to zero absorbance using a blank of pure DMSO. Absorbance of both blank and sample were measured at 645 and 663 nm. The elapsed time between removal from the water bath and completion of spectrophotometer measurements was in the order of 20 min. Hiscox & Israelstam (1979) recommended the use of Arnon‟s (1949) equations:

Chla (g l−1) = 0.0127 A663 – 0.00269 A645;

Chlb (g l−1) = 0.0229 A645– 0.00468 A663;

Total Chl (g l−1) = 0.0202 A645+0.00802 A663.

The Chlorophyll (Chl) concentration of the extract calculated from these equations was then converted to leaf Chl content (mg Chl cm^{-2} leaf area).

2.7.3 Carotenoids

Total carotenoids were estimated by the method described by Zakaria *et al.* (1979). The experiment was carried out in the dark to avoid photolysis of carotenoids once the saponification was complete. The sample (0.5g) was homogenized and saponified with 2.5ml of 12% alcoholic potassium hydroxide in a water bath at 60°C for 30 minutes. The saponified extract was transferred to a separating funnel containing 10-15ml of petroleum ether and

mixed well. The lower aqueous layer was then transferred to another separating funnel and the upper petroleum ether layer containing the carotenoids was collected. The extraction was repeated until the aqueous layer became colourless. A small amount of anhydrous sodium sulphate was added to the petroleum ether extract to remove excess moisture. The final volume of the petroleum ether extract was noted. The absorbance of the yellow colour was read in a spectrophotometer at 450 nm and 503 nm using petroleum ether as blank. The amount of total carotenoids was calculated using the formulae,

$$\text{Amount of total carotenoids} = \frac{A_{450} \times \text{Volume of the sample} \times 100 \times 4}{\text{Weight of the sample}}$$

The total carotenoids were expressed as mg/g of the sample.

2.7.4 Phenols

Phenol content was estimated by Malick and Singh (1980). Weighed 0.5 to 1.0g of the sample and grind it with a pestle and mortar in 10-time volume of 80% ethanol. Centrifuged the homogenate at 10,000rpm for 20 min. Saved the supernatant. Re-extracted the residue with five times the volume of 80% ethanol, centrifuged and pooled the supernatants. Evaporated the supernatant to dryness. Dissolved the residue in a known volume of distilled water (5 ml). Pipette out different aliquots (0.2 to 2 ml) into test tubes. Made up the volume in each tube to 3mL with water.Added 0.5 ml of Folin-Ciocalteau reagent. After 3 min, added 2 ml of 20% Na_2CO_3 solution to each tube. Mixed thoroughly. Placed the tubes in a boiling water bath for exactly one min, cooled and measured the absorbance at 650 nm against a reagent blank. Prepared a standard curve using different concentrations of gallic acid. From the standard curve the concentration of phenols in the test sample was determined and expressed as mg phenols/100g material.

2.7.5 Flavonoids

The samples were homogenized at the rate of 0.1 g per ml of 80% methanol. The methanolic extract (250 µl) was mixed with 1.25 ml of distilled water and 75 µl of a 5% $NaNO_2$ solution. After 5 min, 150 µl of a 10% $AlCl_3.H_2O$ solution was added and filtered for 6 min. About 500 µl of 1M NaOH and 275µl of distilled water were added to the mixture well and the intensity of pink colour was measured at 510 nm. The level of total flavonoids concentration was calculated using quercetin (QU) as a standard (Cameron *et al.,* 1943). The results were expressed as mg of quercetin/g fresh weight of plant sample.

2.7.6 Lipid Peroxidation

The extent of lipid peroxidation was estimated according to the method of Dhindsa *et al.* (1981). Lipid peroxidation was estimated from the accumulated malondialdehyde (MDA) following the method given by Dhindsa *et al.* (1981). In brief, the plant tissue (approx. 200 mg) was homogenised with 0.1% trichloroacetic acid (TCA) (2ml). The homogenate was centrifuged at 10,000 rpm for 10 min. and supernatant collected. The supernatant (2 ml) was reacted with 4 ml of 20% TCA containing 0.5% thiobarbituric acid (TBA). The mixture was then heated at 95ºC for 45 min. and rapidly cooled in an ice bath for 5 min. Absorbance was read at 532 nm. Measurements were corrected for unspecific turbidity by subtracting the absorbance at 600 nm.

3. STATISTICAL ANALYSIS

The data was analysed statistically using GraphPad Prism® 5.2. Mean values were calculated from measurements of six replicates and the standard error of means were determined. Oneway and two-way analysis of variance (ANOVA) was applied to determine the significance of results between different treatments and Tukey's multiple comparison test and Bonferroni's post tests were performed at the significance level of $P<0.05$.

4. RESULTS AND DISCUSSION

Analysis of the present investigation showed that soil inoculated with AM fungi increased Nutrients, enzymatic and non enzymatic antioxidants defense system of *Vinca minor* a medicinal plant.

4.1 NUTRIENT ANALYSIS

Sodium and potassium nutrients were analysed in the leaves of *V. minor* after 45 and 90 days and results are presented in (Fig. 4.1(A)). All AM Fungi applied (*G. coronatum, G. etunicatum* and *G. coronatum+G. etunicatum*) affected the Na+ content significantly. AMFungi (*G.coronatum, G. etunicatum* and *G. coronatum + G. etunicatum* showed an increase in Na+ content in appropriate manner. After 45 days, it increased by 25% in 10% *G. coronatum*; by 37% in 10% *G. etunicatum*; by 55% in 10% *G. coronatum + G. etunicatum*. After 90 days of AM Fungi treatment again it increased by 25% in 10% *G. coronatum*; by 35% in 10% *G. etunicatum*; by 55% in 10% *G. coronatum + G. etunicatum* (fig 4.1 (A)). After 45 days of incubation the degree of total Na+ content was minimum in 10% *G. coronatum* (0.16mmolg-1DW) and maximum in 10% *G. coronatum+ G. etunicatum* (0.39mmolg-1DW) as against 0.11mmolg^{-1}DW total the Na+content in control. After 90

days of incubation the degree of increases in total Na+ content was minimum in 10% *G. coronatum* (0.33mmolg-1DW) and maximum in 10% *G. coronatum*+ *G. etunicatum* (0.55mmolg^{-1}DW) as against 0.14mmolg^{-1}DW total Na+ content in control. Data analysis was done by Two-way analysis of variance (ANOVA) and Bonferroni multiple comparison post test showed significant differences in total Na+ content after 45 and 90 days between treated and untreated. It is concluded that AMF inoculation treatment had significant impact in the total Na+ content of plant.

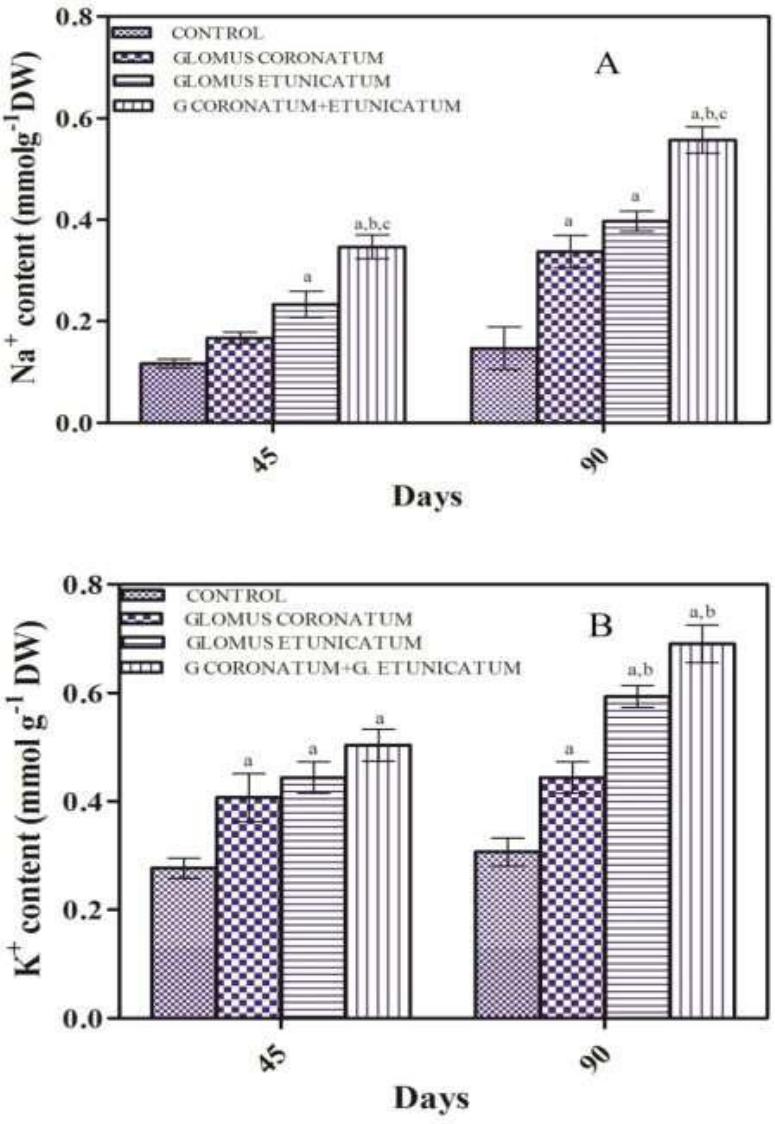

Figure 4.1: Effects of *G.coronatum, G.etunicatum, G. coronatum*+ *G.etunicatum* Total Na+ content (mmolg-1DW) (A), K+ (mmolg-1DW) (B), of *Vinca minor.* Values are mean±SE; N=3 ap<0.05 Vs control, bp<0.05 Vs 10% *G. coronatum*cp<0.05 Vs 10% *G etunicatum*, dp<0.05 Vs*G coronatum*+*G. etunicatum*; analysed by Two-way ANOVA followed by Bonferroni's multiple comparison test.

All AM Fungi applied (*G. coronatum*, *G. etunicatum* and *G. coronatum* + *G. etunicatum*) affected the K+ content significantly. AMFungi (*G. coronatum*, *G. etunicatum* and *G. coronatum* + *G. etunicatum* showed an increase in K+ content in appropriate manner. After 45 days It was increased by 54% in 10% *G. coronatum*; by 60% in 10% *G. etunicatum*; by 67% in 10% *G. coronatum* + *G. etunicatum*. After 90 days of AM Fungi treatment again it increased by 43% in 10% *G. coronatum*; by 50.8% in 10% *G.etunicatum*; by 65% in 10% *G. coronatum* + *G. etunicatum* (fig 4.1(B)). After 45 days of incubation the degree of K+ content was minimum in 10% *G. coronatum* (0.40mmolg-1DW) and maximum in 10% *G. coronatum* + *G. etunicatum* (0.50mmolg-1DW) as against 0.27mmolg-1DW total the K+ content in control. After 90 days of incubation the degree of increases in total K+ content was minimum in 10% *G. coronatum* (0.44mmolg-1DW) and maximum in 10% *G. coronatum* + *G. etunicatum* (0.69mmolg-1DW) as against 0.30mmolg-1DW K+ content in control. Data analysis was done by Two-way analysis of variance (ANOVA) and Bonferroni multiple comparison post test showed significant differences in K+ content after 45 and 90 days between treated and untreated. It is concluded that AMF inoculation treatment had significant impact in the K+ content of plant.

4.2 Enzymatic Antioxidants

The enzymatic antioxidants analyzed in the leaves of *V. minor* were, CAT, POD

4.2.1. Catalase (CAT)

Catalase activity of *V. minor* leaves was measured after 45 and 90 days of sowing and these results obtained are given in Fig. 4.2(A). AMF concentrations increased the catalase activity. The increase was variable in different treatments. All AM Fungi applied (*G. coronatum*, *G. etunicatum* and *G. coronatum* + *G. etunicatum*) affected the catalase activity significantly. AM Fungi (*G. coronatum*, *G. etunicatum* and *G. coronatum* + *G. etunicatum* showed an increase in catalase activity in appropriate manner. After 45 days, it increased by 20% in 10% *G. coronatum*; by 25% in 10% *G. etunicatum*; by 38% in 10% *G. coronatum*+*G. etunicatum*. After 90 days of AM Fungi treatment again it increased by 40% in 10% *G.coronatum*; by 54% in 10% *G.etunicatum*; by 72% in 10% *G. coronatum*+*G. etunicatum*. After 45 days of incubation the degree of catalase activity was minimum in 10% *G. coronatum* (3.94 µ/mg) and maximum in 10% *G. coronatum*+ *G. etunicatum* (7.40 µ/mg) as against 1.50µ/mg total the catalase activity in control. After 90 days of incubation the degree of increases in total catalase activity was minimum in 10% *G. coronatum* (6.07µ/mg) and maximum in 10% *G.*

coronatum+ *G. etunicatum* (10.81µ/mg) as against 4.41µ/mg in control. Data analysis was done by Two-way analysis of variance (ANOVA) and Bonferroni multiple comparison post test showed significant differences in catalase activity after 45 and 90 days between treated and untreated. It is concluded that AMF inoculation treatment had significant impact in the catalase activity of plant.

4.2.2 Peroxidase (POD)

Total peroxidase content of *V. minor* leaves was measured after 45 and 90 days of sowing and the results obtained are given in (fig. 4.2(B)). AMF concentrations increased the total peroxidase content. All AM Fungi applied (*G. coronatum, G. etunicatum* and *G. coronatum* + *G. etunicatum*) affected the total peroxidase content significantly. AM Fungi (*G. coronatum, G. etunicatum* and *G. coronatum*+ *G. etunicatum* showed an increase in total peroxidase content in appropriate manner. After 45 days, it increased by 46% in 10% *G. coronatum*; by 55% in 10% *G.etunicatum*; by 67% in 10% *G. coronatum*+*G. etunicatum*. After 90 days of AM Fungi treatment again it increased by 37% in 10% *G. coronatum*; by 50% in 10% *G.etunicatum*; by 68% in 10% *G. coronatum*+*G. etunicatum*. After 45 days of incubation the degree of total peroxidase content was minimum in 10% *G.coronatum* (4.52 µ/mg) and maximum in 10% *G. coronatum*+ *G. etunicatum* (6.73 µ/mg) as against 3.11 u/mg total peroxidase content in control. After 90 days of incubation the degree of increases in total peroxidase content was minimum in 10% *G. coronatum*(5.02 µ/mg) and maximum in 10% *G. coronatum*+ *G. etunicatum* (8.53 µ/mg) as against 3.24 µ/mg in control. Data analysis was done by Two-way analysis of variance (ANOVA) and Bonferroni multiple comparison post test showed significant differences in total peroxidase content after 45 and 90 days between treated and untreated. It is concluded that AMF inoculation treatment had significant impact in the total peroxidase content of plant.

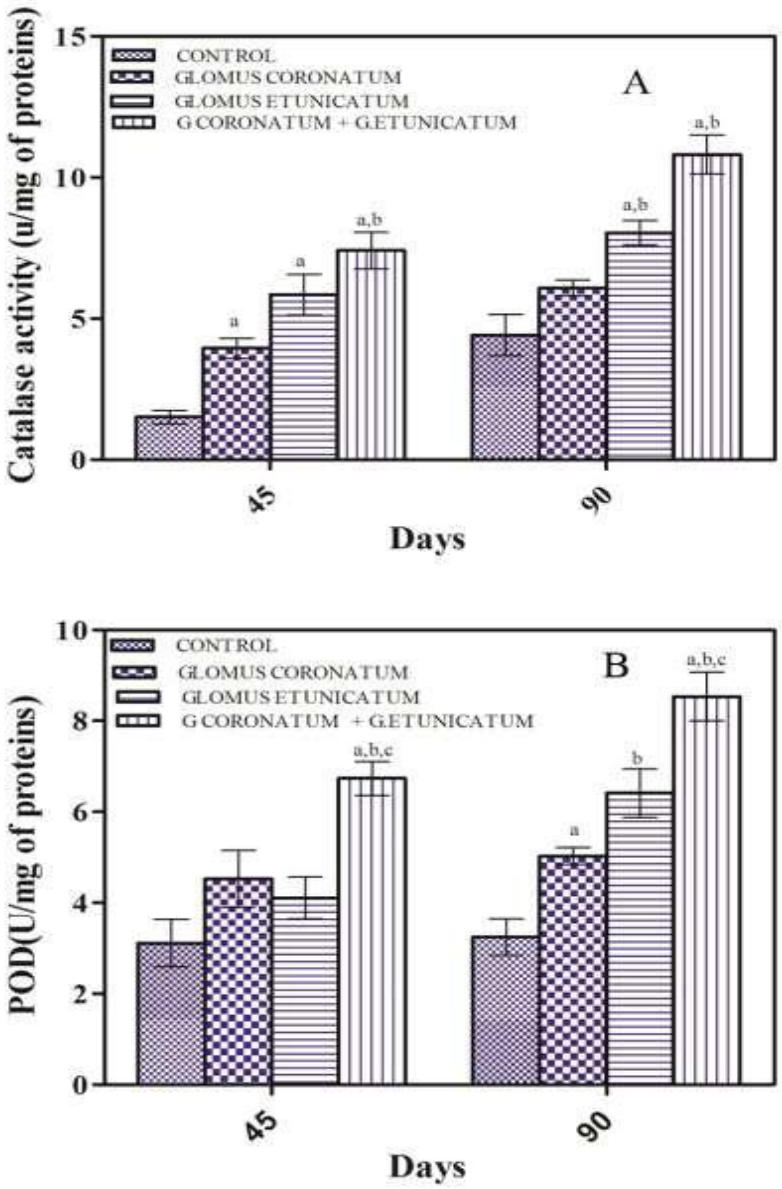

Figure 4.2: Effects of *G. coronatum, G. etunicatum, G. coronatum*+ *G. etunicatum* Catalase activity (µ/mg)(A), Total Peroxidase content(µ/mg) (B), of *V. minor.* Values are mean±SE; N=3 ap<0.05 Vs control, bp<0.05 Vs 10% *G. coronatum*cp<0.05 Vs 10%*G etunicatum*, dp<0.05 Vs *G. coronatum*+*G. etunicatum*; analysed by Two-way ANOVA followed by Bonferroni's multiple comparison test.

4.3 Non-Enzymatic Antioxidants

4.3.1 Phenols

Total phenol content of *V. minor* leaves were measured after 45 and 90 days ofsowing and the results obtained are given in (Fig. 4.3(A)). AMF concentrations increased the total phenol content. All AM Fungi applied (*G.coronatum, G. etunicatum* and *G. coronatum*+*G. etunicatum*) affected the total phenol content significantly. AM Fungi (*G. coronatum, G.*

etunicatum and *G. coronatum + G. etunicatum* showed an increase in total phenol content in appropriate manner. After 45 days, it increased by 53% in 10% *G. coronatum*; by 58% in 10% *G. etunicatum*; by 64% in 10% *G. coronatum + G. etunicatum*. After 90 days of AM Fungi treatment again it increased by 44% in 10% *G. coronatum*; by 55% in 10% *G. etunicatum*; by 68% in 10% *G. coronatum+G. etunicatum*. After 45 days of incubation the degree of total phenol content was minimum in 10% *G. coronatum* (0.53 mg/gFW) and maximum in 10% *G. coronatum+ G. etunicatum* (0.63 mg/gFW) as against 0.34mg/gFW total phenol content in control. After 90 days of incubation the degree of increases in total phenol content was minimum in 10% *G. coronatum* (0.63mg/gFW) and maximum in 10% *G. coronatum+ G. etunicatum* (0.76mg/gFW) as against 0.51mg/gFW in control. Data analysis was done by Two-way analysis of variance (ANOVA) and Bonferroni multiple comparison post test showed significant differences in total phenol content after 45 and 90 days between treated and untreated. It is concluded that AMF inoculation treatment had significant impact in the total phenol content of plant.

4.3.2 Total Carotenoids

Total carotenoids of *V. minor* leaves were measured after 45 and 90 days of sowing and the results obtained are given in (Fig. 4.3 (B)). AMF concentrations increased the total phenol content. All AM Fungi applied (*G. coronatum, G. etunicatum* and *G. coronatum+G. etunicatum*) affected the total carotenoids significantly. AM Fungi (*G. coronatum, G. etunicatum* and *G. coronatum + G. etunicatum* showed an increase in total carotenoids content in appropriate manner. After 45 days, it increased by 38% in 10% *G. coronatum*; by 50% in 10% *G. etunicatum*; by 62% in 10% *G. coronatum +G. etunicatum*. After 90 days of AM Fungi treatment again it increased by 34% in 10% *G.coronatum*; by 44% in 10% *G. etunicatum*; by 60% in 10% *G. coronatum + G. etunicatum*. After 45 days of incubation the degree of total carotenoids were minimum in 10% *G. coronatum* (4.50 mgg-1FW) and maximum in 10% *G. coronatum+ G. etunicatum* (5.92mgg-1FW) as against 2.30 mg/gFW total carotenoids in control. After 90 days of incubation of AMF the degree of increase in total carotenoids were minimum in 10% *G. coronatum* (3.82mgg-1FW) and maximum in 10% *G. coronatum+ G. etunicatum* (6.23mgg-1FW) as against 2.31mgg-1FW in control. Data analysis was done by Two-way analysis of variance (ANOVA) and Bonferroni multiple comparison post test showed significant differences in total carotenoids content after 45 and 90 days between treated and untreated. It is concluded that AMF inoculation treatment had significant impact in the total carotenoids of plant.

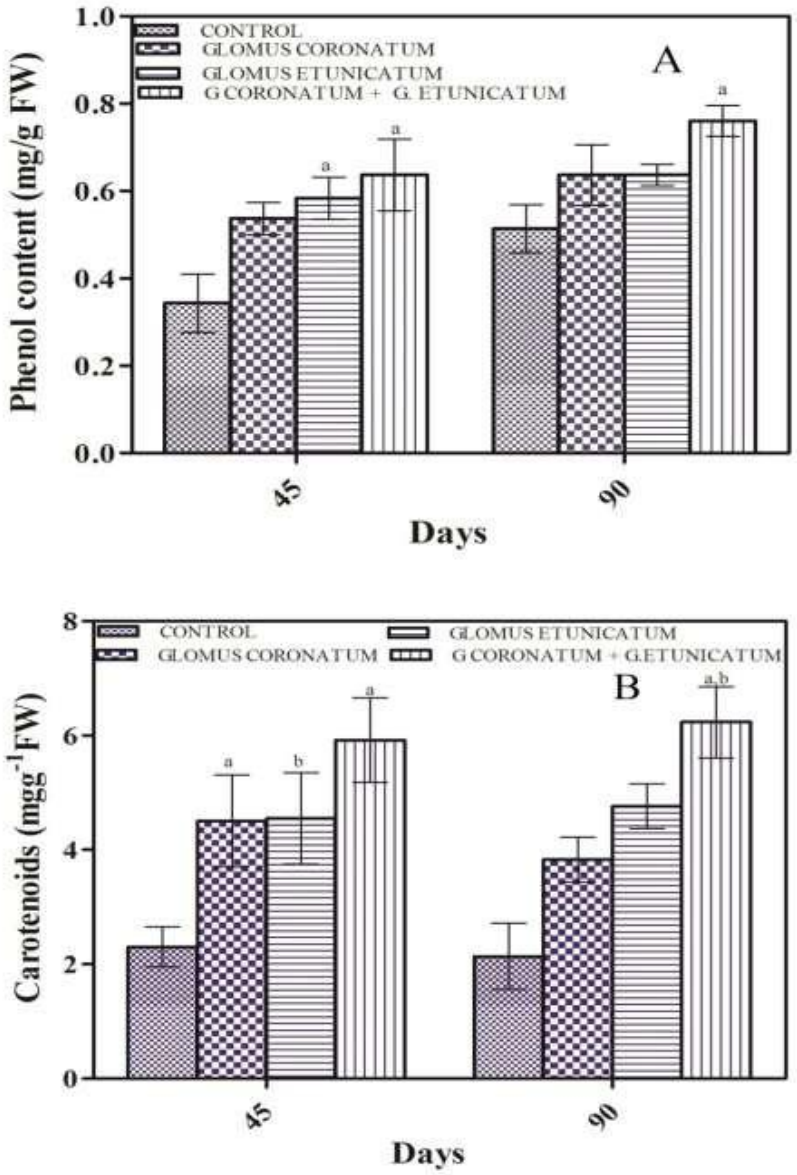

Figure 4.3: Effects of *G. coronatum, G. etunicatum, G. coronatum+ G. etunicatum* Phenol content (mg/gFW) (A), Carotenoids (mgg-1FW) (B), of *Vinca minor.* Values are mean±SE; N=3ap<0.05 Vs control, bp<0.05 Vs 10% *G. coronatum*cp<0.05 Vs 10% *G. etunicatum*, dp<0.05 Vs *G. coronatum+G. etunicatum*; analysed by Two-way ANOVA followed by Bonferroni' multiple comparison test.

4.3.3 Total Flavonoids

Total Flavonoids of *V. minor* leaves were measured after 45 and 90 days of sowing and the results obtained are given in (Fig. 4.4(A)). AMF concentrations increased the total Flavonoids. All AM Fungi applied (*G. coronatum, G. etunicatum* and *G. coronatum+G. etunicatum*) affected the total flavanoids significantly. AMFungi (*G. coronatum, G.*

etunicatum and *G. coronatum+G. etunicatum* showed an increase in total flavonoids in appropriate manner. After 45 days, it increased by 35% in 10% *G. coronatum*; by 40% in 10% *G. etunicatum*; by 50% in 10% *G. coronatum+G. etunicatum*. After 90 days of AM Fungi treatment again it increased by 42% in 10% *G. coronatum*; by 53% in 10% *G. etunicatum*; by 63% in 10% *G. coronatum+G. etunicatum*. After 45 days of incubation, the degree of total flavonoids were minimum in 10% *G. coronatum* (0.35 $mgg^{-1}FW$) and maximum in 10% *G. coronatum+ G.etunicatum* (0.48 $mgg^{-1}FW$) as against 0.17 mg/gFW total flavanoids in control. After 90 days of incubation, the degree of increases in total flavanoids were minimum in 10% *G. coronatum* (0.39 $mgg^{-1}FW$) and maximum in 10% *G. coronatum+ G. etunicatum* (0.54 $mgg^{-1}FW$) as against 0.23 mgg-1FW in control. Data analysis was done by Two-way analysis of variance (ANOVA) and Bonferroni multiple comparison post test showed significant differences in total flavonoids after 45 and 90 days between treated and untreated. It is concluded that AMF inoculation treatment had significant impact in the total flavonoids of plant. in 10% *G. coronatum* (0.35 mgg-1FW) and maximum in 10% *G. coronatum+ G.etunicatum* (0.48 $mgg^{-1}FW$) as against 0.17 mg/gFW total flavanoids in control. After 90 days of incubation, the degree of increases in total flavanoids were minimum in 10% *G. coronatum* (0.39 mgg-1FW) and maximum in 10% *G. coronatum+ G. etunicatum* (0.54 $mgg^{-1}FW$) as against 0.23 mgg-1FW in control. Data analysis was done by Two-way analysis of variance (ANOVA) and Bonferroni multiple comparison post test showed significant differences in total flavonoids after 45 and 90 days between treated and untreated. It is concluded that AMF inoculation treatment had significant impact in the total flavonoids of plant.

4.3.4 Tocopherol

Tocopherol content of *V. minor* leaves was measured after 45 and 90 days of sowing and the results obtained are given in (Fig. 4.4(B)). AMF concentrations increased the tocopherol content. All AM Fungi applied (*G. coronatum*, *G. etunicatum* and *G. coronatum* + *G. etunicatum*) affected the tocopherol content significantly. AM Fungi (*G. coronatum*, *G. etunicatum* and *G. coronatum* + *G. etunicatum* showed an increase in tocopherol content in appropriate manner. After 45 days, it increased by 23% in 10% *G. coronatum*; by 36% in 10% *G. etunicatum*; by 49% in 10% *G. coronatum* + *G. etunicatum*. After 90 days of AM Fungi treatment again it increased by 40% in 10% *G. coronatum*; by 63% in 10% *G. etunicatum*; by 72% in 10% *G. coronatum* + *G. etunicatum*. After 45 days of incubation the degree of total carotenoids was minimum in 10% *G. coronatum* (0.61 μgmg^{-1}) and maximum

in 10% *G. coronatum*+ *G. etunicatum* (1.50µgmg^{-1}) as against 0.35µgmg-1 tocopherol content in control. After 90 days of incubation the degree of increases in tocopherol content was minimum in 10% *G. coronatum* (0.96µgmg^{-1}) and maximum in 10% *G. coronatum*+ *G. etunicatum* (1.65µgmg^{-1}) as against 0.67µgmg^{-1}in control. Data analysis was done by Two-way analysis of variance (ANOVA) and Bonferroni multiple comparison post test showed significant differences in tocopherol content after 45 and 90 days between treated and untreated. It is concluded that AMF inoculation treatment had significant impact in tocopherol content of plant.

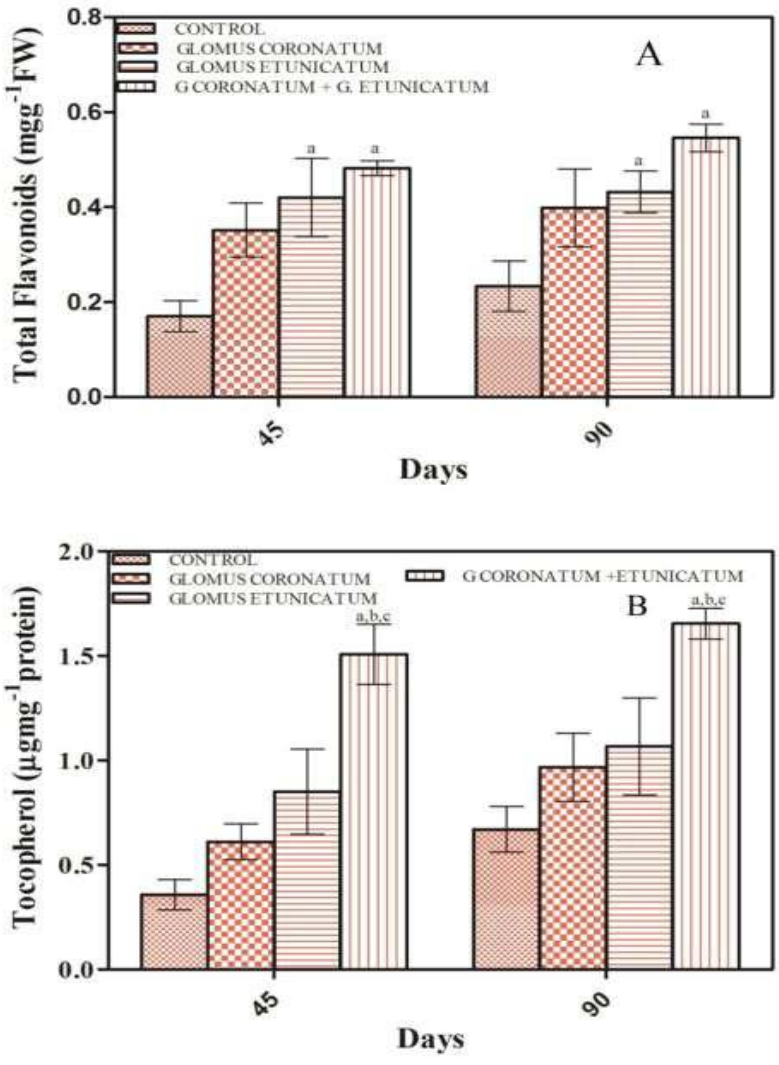

Figure 4.4: Effects of *G. coronatum, G. etunicatum, G. coronatum*+ *G. etunicatum* Total Flavonoids content (mgg-1FW) (A), Tocopherol (µgmg-1FW) (B), of *Vinca minor.* Values are mean±SE; N=3ap<0.05 Vs control, bp<0.05 Vs 10% *G.coronatum*cp<0.05 Vs 10% *G. etunicatum*, dp<0.05 Vs *G. coronatum*+*G. etunicatum*; analysed by Two-way ANOVA followed by Bonferroni's multiple comparison test.

4.3.5 Lipid Peroxidation

Estimation of lipid peroxidation was performed in terms of total malonoaldehyde (MDA) content in the leaves of *V. minor* after 45 and 90 days of sowing the results obtained are given in (Fig. 4. 5(A)). Total MDA content AMF concentrations decreased the total MDA content. All AM Fungi applied (*G. coronatum, G. etunicatum* and *G. coronatum + G. etunicatum*) affected total MDA content significantly. AM Fungi (*G. coronatum, G. etunicatum* and *G. coronatum + G. etunicatum* showed a decrease in total MDA content in appropriate manner. After 45 days, it decreased by 63% in 10% *G. coronatum*; by 65% in 10% *G. etunicatum*; by 71% in 10% *G. coronatum + G. etunicatum*. After 90 days of AM Fungi treatment again it decreased by 52% in 10% *G.coronatum*; by 56% in 10% *G. etunicatum*; by 67% in 10% *G. coronatum + G. etunicatum*. After 45 days of incubation the degree of total MDA content was minimum in 10% *G. coronatum* (1.02nmolg-1FW) and maximum in 10% *G. coronatum+ G. etunicatum* (1.23nmolg^{-1}FW) as against 1.06nmolg^{-1}FW total MDA content in control. After 90 days of incubation the degree of increases in total MDA content was minimum in 10% *G. coronatum* (1.03nmolg- 1FW) and maximum in 10% *G. coronatum + G. etunicatum* (1.24nmolg^{-1}FW) as against 1.97 nmolg^{-1}FW in control. Data analysis was done by Two-way analysis of variance (ANOVA) and Bonferroni multiple comparison post test showed significant differences in total MDA content after 45 and 90 days between treated and untreated. It is concluded that AMF inoculation treatment had significant impact in total MDA content of plant.

4.3.6 Total Chlorophyll

Total chlorophyll content in *V. minor* leaves was also analysed after 45 and 90 days of sowing and the results obtained are given in (Fig. 4.5(B)). AMF concentrations increased the total chlorophyll. All AM Fungi applied (*G. coronatum, G. etunicatum* and *G. coronatum + G. etunicatum*) affected the total chlorophyll significantly. AM Fungi (*G. coronatum, G. etunicatum* and *G.coronatum + G.etunicatum* showed an increase intocopherol content in appropriate manner.After 45 days, it increased by 37% in 10% *G. coronatum*; by 48% in 10% *G.etunicatum*; by 66% in 10% *G. coronatum + G. etunicatum*. After 90 days of AM Fungi treatment again it increased by 32% in 10% *G. coronatum*; by 54% in 10% *G. etunicatum*; by 65% in 10% *G. coronatum + G. etunicatum*. After 45 days of incubation the degree of total chlorophyll was minimum in 10% *G. coronatum* (0.027 mgg^{-1}FW) and maximum in 10% *G. coronatum+ G. etunicatum* (0.048mgg^{-1}FW) as against 0.018mgg-1FW total chlorophyll in control. After 90 days of incubation the degree of increases in total chlorophyll was minimum

in 10% *G. coronatum* (0.026mgg-1FW) and maximum in 10% *G. coronatum*+ *G. etunicatum* (0.053mgg^{-1}FW) as against 0.017mgg^{-1}FW in control. Data analysis was done by Two-way analysis of variance (ANOVA) and Bonferroni multiple comparison post test showed significant differences in total chlorophyll after 45 and 90 days between treated and untreated. It is concluded that AMF inoculation treatment had significant impact in total chlorophyll of plant.

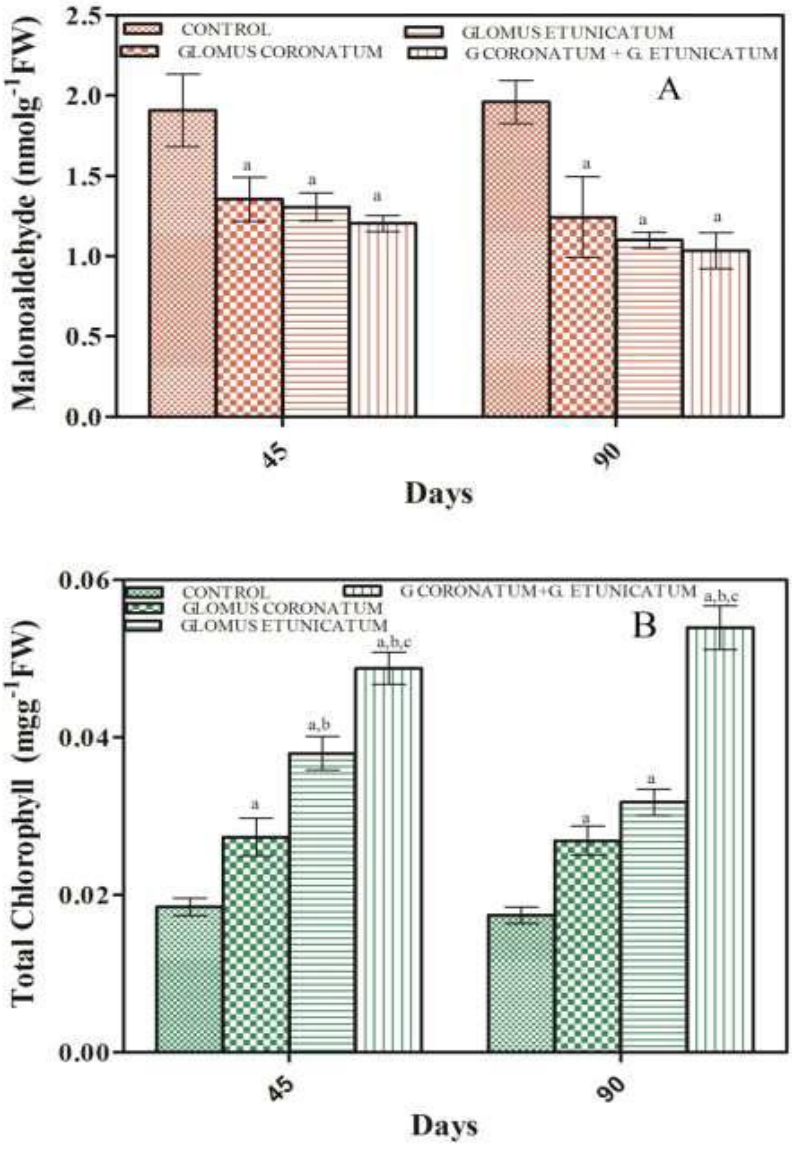

Figure 4.5: Effects of *G.coronatum, G.etunicatum, G. coronatum*+ *G.etunicatum* Malanoaldehyde (nmolg-1FW) (A), Total Chlorophyll (mgg-1FW) (B), of *Vinca minor.* Values are mean±SE; N=3 ap<0.05 Vs control, bp<0.05 Vs 10% *G. coronatum*cp<0.05 Vs 10% *G. etunicatum*, dp<0.05 Vs *G coronatum*+*G. etunicatum*; analysed by Two-way ANOVA followed by Bonferroni's multiple comparison test.

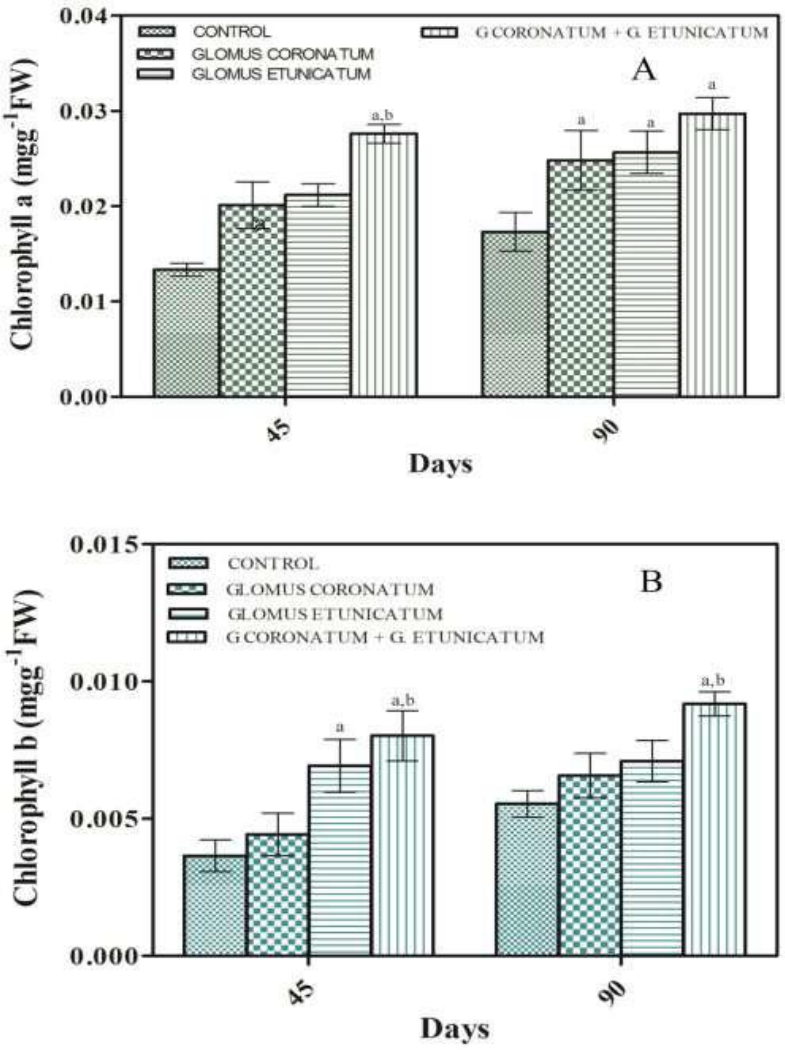

Figure 4.6: Effects of *G. coronatum, G. etunicatum, G. coronatum+ G. etunicatum* Chlorophyll a (mgg-1FW) (A), Chlorophyll b (mgg-1FW) (B), of *Vinca minor.* Values are mean±SE; N=3 ap<0.05 Vs control, bp<0.05 Vs 10% *G. coronatum*cp<0.05 Vs 10%*G etunicatum*, dp<0.05 Vs *G. coronatum+G. etunicatum*; analysed by Two-way ANOVA followed by Bonferroni's multiple comparison test.

4.3.7 Chlorophyll a

Chlorophyll a content in *V. minor* leaves was also analysed after 45 and 90 days of sowing and the results obtained are given in (Fig. 4.6(A)). AMF concentrations increased the Chlorophyll a. All AM Fungi applied (*G. coronatum, G. etunicatum* and *G. coronatum + G. etunicatum*) affected the significantly. AMFungi (*G. coronatum, G. etunicatum* and *G. coronatum+G.etunicatum* showed an increase in Chlorophyll a in appropriate manner. After 45 days it increased by 48% in 10% *G. coronatum*; by 60% in 10% *G. etunicatum*; by 69% in 10% *G. coronatum + G. etunicatum*. After 90 days of AM Fungi treatment again it increased

by 58% in 10% *G. coronatum*; by 68% in 10% *G. etunicatum*; by 72% in 10% *G. coronatum+G. etunicatum*. After 45 days of incubation the degree of total chlorophyll was minimum in 10% *G. coronatum*(0.020 mgg^{-1}FW) and maximum in 10% *G. coronatum+ G. etunicatum* (0.028mg-$^{-1}$FW) as against (0.018mgg^{-1}FW) Chlorophyll a in control. After 90 days ofincubation the degree of increases in Chlorophyll a was minimum in 10% *G. coronatum* (0.024mgg^{-1}FW) and maximum in 10% *G. coronatum+ G. etunicatum* (0.029mgg^{-1}FW) as against 0.017mgg^{-1}FW in control. Data analysis was done by Two-way analysis of variance (ANOVA) and Bonferroni multiple comparison post test showed significant differences in Chlorophyll a after 45 and 90 days between treated and untreated. It is concluded that AMF inoculation treatment had significant impact in Chlorophyll a of plant.

4.3.8 Chlorophyll b

Chlorophyll b content in *V. minor* leaves were also analysed after 45 and 90 days of sowing and the results obtained are given in Fig. 4. 6(B). AMF concentrations increased the Chlorophyll b. All AM Fungi applied (*G. coronatum*, *G. etunicatum* and *G. coronatum + G. etunicatum*) affected the significantly. AMFungi (*G.coronatum*, *G. etunicatum* and *G. coronatum + G. etunicatum* showed an increase in Chlorophyll b in appropriate manner. After 45 days It increased by 37% in 10% *G. coronatum*; by 50% in 10% *G.etunicatum*; by 70% in 10% *G. coronatum + G. etunicatum*. After 90 days of AM Fungi treatment again it increased by 55% in 10% *G. coronatum*; by 61% in 10% *G. etunicatum*; by 79% in 10% *G. coronatum + G. etunicatum*. After 45 days of incubation the degree of chlorophyll b was minimum in 10% *G. coronatum* (0.004 mgg-1FW) and maximum in 10% *G. coronatum+ G. etunicatum* (0.008mgg^{-1}FW) asagainst 0.003mgg^{-1}FW Chlorophyll b in control. After 90 days of incubation the degree of increases in Chlorophyll b was minimum in 10% *G. coronatum* (0.006mgg^{-1}FW) and maximum in 10% *G. coronatum+ G. etunicatum* (0.009mgg^{-1}FW) as against 0.005mgg^{-1}FW in control. Data analysis was done by Two-way analysis of variance (ANOVA) and Bonferroni multiple comparison post test showed significant differences in Chlorophyll b after 45 and 90 days between treated and untreated. It is concluded that AMF inoculation treatment had significant impact in Chlorophyll b of plant.

DISCUSSION

Nutrient analysis (Na, K+) after 45 and 90 days of AMF inoculation (*Glomus coronatum, Glomus etunicatum, Glomus coronatum + Glomus etunicatum*) in *V. minor* plants were increased in appropriate manner. AMF inoculation has been reported to increase the P content

in plants through the enhanced uptake by the hyphae (Lozano *et al*., 2000). This suggests that AMF increased P uptake may reduce the negative effect of NaCl in plants. Similar, AMF inoculated plants have been proclaimed to enhance K+ ions under NaCl stress Kadian *et al*. (2013), increasing plants ability to cope with ROS and to regulate water balance. K ions plays a key role in plant metabolism; under excess soil salinity, Na ions compete with K for binding sites essential for various cellular functions but cannot replace the function Giri *et al*. (2007) and Na-induced K ions deficiency has been implicated in growth and yield reductions of various crops, including tomato (Lopez *et al*., 1996). Thus, in accordance with our results, numerous studies on a wide variety of crops have shown that K ion concentration in plant tissue declines as soil salinity increases. However, previous studies frequently reported enhanced K uptake by mycorrhizal symbionts (Evelin *et al*., 2009).

In AMF inoculation (*Glomus coronatum, Glomus etunicatum* and *Glomus coronatum* + *Glomus etunicatum*) after 45 and 90 days antioxidant activity (CAT, POD) were increased in plants treated with AMF in combination than control or untreated plants of *Vinca minor*. Maintenance of low ROS or reduced oxidative damage in AM plants is also due to the enhanced activity of some of the antioxidant enzymes where, they convert the toxic molecules (ROS) into non–toxic or less toxic molecules. The level of antioxidant enzymes such as superoxide dismutase (SOD), catalase and peroxidise was found to be significantly high in mycorrhizal plants than non–mycorrhizal plants when grown under stressful environment (Porcell and Ruiz–Lozano, 2004; Wu *et al*.,2006 b; Khalafallah and Abo-Ghalia, 2008). Inoculation with *Glomus mosseae* in strawberry under drought stress increased the activity of antioxidant enzymes like catalase (CAT), ascorbate peroxidase (APX) accumulation of free proline and soluble protein, and decreased the MDA content and plasma membrane conductivity (Baozhong *et al*., 2010). The mycorrhizal association helps the plants to overcome the oxidative stress damage and hence, the plants could continue to grow and produce without much of yield penalty under stress conditions. In general, AMF can affect the overall plant biomass through its role in P acquisition (Auge, 2001). AMF inoculation induced enhancement in activities of antioxidant enzymes demonstrated in the present study support the findings of Abdel Latef and Chaoxing (2011). Mycorrhizal fungi promote antioxidant activity by utilizing various appliances such as: (a) enhancing nutrient uptake (b) increasing the efficiency of the host plants by increasing their growth (c) producing phytochemicals such as flavonoids. Some researchers in the recent years showed that mycorrhizal inoculation can increase in antioxidant activity and phenolic compounds (Huang

et al., 2011; Banuelos *et al*., 2014). Perner *et al*. (2008) communicated that inoculation of onion plants with arbuscular mycorrhizal fungi increased the antioxidant activity by increasing phenolic compounds as a result of plant defense mechanism. AMF can also alter plant product qualities. Phenolic production in plants can be affected by biotic and abiotic factors and AMF colonization may be one way to alter or enhance phenolic production or composition within the host plant (Ganz *et al*., 2002; Toussaint *et al*., 2007). Phenolic content of *V. minor* plant increases when inoculated with *Glomus coronatum, Glomus etunicatum and Glomus coronatum+ Glomus etunicatum* after 45 and 90 days. Increases in phenolic compounds in host plants as a result of arbuscular mycorrhizal (AM) fungus inoculation have been reported (Ling-Lee *et al*., 1977) (fig 4.3(A)). Inoculation of plants with AMF results in an overall increase in the production of some new phenolic compounds during the progression of the infection (Devi *et al*., 2002). Colonization by arbuscularmycorrhizal fungi (AMF) is known to improve plant nutrient up- take and use (Clark and Zeto, 2000) and stress tolerance (Smith and Read, 1997). AMF colonization can also alter or enhance phenolic production within the host plant (Toussaint *et al*., 2007; Ganz *et al*., 2002). Enhancement of secondary metabolite accumulation in plants is of great importance in production of culinary herbs with antioxidant activity. The relationship between AMF and phenolic content alteration, within various plants, has been reviewed by Yao *et al*. (2007) and Toussaint (2007) and both agree that numerous factors need clarification in order to better define the relationship between AMF and its host plant's secondary metabolite production. Plant phenolic compounds are dormant candidates as signals during mycorrhizal formation. Some reports show that flavonoids, together with the aforetime mentioned strigolactones, can exert a positive effect on hyphal growth during the establishment of the AMF symbiosis (Mandal *et al*., 2010).

Total carotenoids were increased by inoculation of AMF (*Glomus coronatum*, *Glomus etunicatum, Glomus coronatum + Glomus etunicatum*) after 45 and 90 days (fig. 4.3(B)). It is well-known that AMF can stimulate carotenoid metabolic pathways in plant roots (Walter *et al*., 2010; Strack *et al*., 2006; Walter *et al*., 2007). AM symbiosis activates the plastidial methylerythritole phosphate (MEP) pathway by increasing transcript levels of MEP and carotenoid biosynthetic and cleavage genes Strack *et al*. (2003), which stimulates the production of carotenoids and carotenoid cleavage products including C13 cyclohexenone derivatives (e.g., blumenol), C14 apocarotenoids (e.g., mycorradicin) and strigolactones. Increased quantity of lycopene and β-carotene in fruits of tomato inoculated with *Glomus* sp.

and those carried out by Mena-Violante *et al.* (2006), who reported enhanced carotenoid content in chile ancho (*Capsicum annuum* L. cv. San Luis) plants inoculated with *G. fasciculatum* and subjected to drought. It is known that chloroplasts of mycorrhizal plants can have higher and more numerous plastoglobuli Kainulainen *et al*. (2000), the subcellular compartment where carotenoids are mainly localized during the chloroplast to chromoplast transformation. Carotenoids are pigmented compounds that are uniquelysynthesized by higher plants, algae, fungi and bacteria. During photosynthesis, carotenoids present in the thylakoid membrane of chloroplasts harvest light and transfer the absorbed energy to the reaction center of photosystems. Carotenoids also protect Chl from photo-damage by dissipating the excess of energy and quenching reactive oxygenspecies (ROS) produced under excess light (Demming-Adams *et al*., 1996). Major dietary carotenoids include hydrocarbons, such as β-carotene, α- carotene and lycopene, as well as xanthophylls or oxygen-containing carotenoids, including β-cryptoxanthin, lutein and zeaxanthin. Most carotenoids have pro vitaminA activity.

As increase in Flavonoids were also observed in Inoculation of AMF (*Glomus coronatum, Glomus etunicatum and Glomus coronatum+ Glomus etunicatum*) as compared to untreated or control plants of *V. minor.* Anthocyanins belong to the large family of phenolic compounds collectively known as flavonoids. The consequences of AMF on the levels of anthocyanins in host plants has been studied in different crops, such as Cherry, tomato inoculated with *G. etunicatum, Glomus coronatum* (Poulton *et al*., 2001), different cultivars of the culinary herb commonly known as "basil" (Lee *et al*., 2009) and strawberry (Castellanos-Morales *et al*., 2010) associated with *G. intraradices.* In all these studies, results shows that mycorrhizal plants had increased concentrations of total anthocyanins in leaves, stems and/or fruits compared to their respective non-mycorrhizal controls. A number of flavonoids illustrate a luminous stimulatory effect on AMF hyphal growth and this effect seems to depend on the chemical structure of the compound (Becard *et al*., 1992; Scervino *et al*., 2006). During pre-symbiotic growth and when applied to plants inoculated with AMF, flavonoids exhibit an AMF fungal genus- and species specific effect (Scervino *et al*., 2005). Once plants were colonized by AMF the pattern of flavonoids is dramatically changed (Morandi, 1996; Vierheilig *et al*., 1998). Larose *et al*. (2002) and Harrison *et al*. (1993) linked the alterations of the flavonoid pattern in mycorrhizal roots to the developmental stage of the AM symbiosis. Whereas during root penetration and the early establishment of AMF intervening levels of a number of flavonoids are detected in roots, at a later stage of root

colonization characterized among others by abundant collapsed arbuscules, high levels of flavonoids such as the phytoalexin medicarpin have been reported (Larose *et al*., 2002). At a later stage ofthe AM association, when the AMF is well established, changes of the flavonoid pattern play a regulatory role (Larose *et al*., 2002; Catford *et al*., 2006).

After 45 and 90 days of AMF inoculation (*Glomus coronatum, Glomus etunicatum, Glomus coronatum+ Glomus etunicatum*) in *Vinca minor* plants tocopherol ratio increases as compared to control/untreated plants (fig.4.4(B). Tocopherols may play important roles in plants beyond their antioxidant function in photosynthetic membranes. Plants impaired in tocopherols show alterations in germination, export of photoassimilates, leaf senescence and responses to abiotic stresses (Falk *et al*., 2010). Supplementation of the human diet with vitamin E contained in fruits and vegetables is considered to provide health benefits against cardiovascular diseases through its antioxidant activity, the prevention of lipoprotein oxidation and the inhibition of platelet aggregation (Clarke *et al*., 2008). In general terms, mycorrhizal symbiosis induced the accumulation of tocopherol in leaves. The most relevant increases in theamount of tocopherols were observed in external leaves of any variety or cultivar of lettuce after applying a mixture of *G. mosseae* and *G. intraradices*. Inspite of all mycorrhizal plants, the highest levels of tocopherols were found in external leaves of the red leaf lettuce Maravillade Verano. Lizarazo *et al.* (2010) concluded that increased levels of tocopherols with leaf ageing represent a general trait in plants. Since AMF can shorten the life cycle of their host plants Garmendia *et al*. (2004) the possible different phenological stage of mycorrhizal and non-mycorrhizal lettuces could explain why the outer leaves of mycorrhizal lettuces had greater amounts of tocopherols than the external leaves of their non-mycorrhizal controls (Baslam *et al*., 2012).

Lipid peroxidation decreases after inoculation of AMF (*Glomus coronatum, Glomus etunicatum, Glomus coronatum+Glomus etunicatum*) after 45 and 90 days (fig. 4.5(A)). Mycorrhizal association has been shown to have significantly higher antioxidant activity in several systems. For instance in maize, Zhu *et al*. (2011b), have reported low melondialdehyde (MDA) content in mycorrhizal plants. In several systems, MDA has been shown to damage the membranes and disrupting the cell metabolism. Therefore, low level of MDA observed in maize plants associated with AMF is an indication of positive effect of AMF on reducing the ROS level in plants. Such reduced level of MDA has also been reported by others in different plants (Porcel and Ruiz–Lozano, 2004; Wu *et al*., 2006a). In

the current study, MDA formation was found to be higher in the non mycorrhizal roots than in the mycorrhizal ones in the presence of increasing CaCO3 concentrations. The reduction of MDA in arbuscular mycorrhizal roots in comparison with non-arbuscular mycorrhizal roots may demonstrate that AMF could alleviate the peroxidation of membrane lipids. The root protection against CaCO3 stress by mycorrhizal colonization is probably related to a reduction of ROS production by inducing ROS scavenging systems. Among the most common mechanism for detoxifying ROS synthesized during stress response, POD induction has been reported inseveral studies (Abdel Latef and He, 2011). Zhu *et al.* (2010), Latef and Chaoxing (2011), reported that low temperature stress increased MDA contents, and AM plants had lower MDA content as compared with the non-AM plants.

Total chlorophyll, Chlorophyll a, Chlorophyll b was increased when AMF inoculation (*Glomus coronatum, Glomus etunicatum, Glomus coronatum* + *Glomus etunicatum*) was done after 45 and 90 days. Numerous researchers Garmendia *et al*. (2004), Afek *et al*. (1990), Sohrabia *et al.* (2012), have found increased concentrations of total Chlorophyll in leaves of several crops (onion, strawberry, pepper and chickpea) inoculated with different AM species belonging to the genus *Glomus* (*G. mosseae*, *G. versiforme*, *G. intraradices*, *G. etunicatum* or *G. deserticola*, *G. coronatum*). Selvaraj *et al.* (2009) also observed increased levels of Chlorophyll in *Pogostemon patchouli*, an aromatic herb belonging to the mint family and cultivated for its essential oil used in cosmetics, when it was inoculated with isolates of seven indigenous AMF (*Acaulospora scrobiculata*, *Gigaspora margarita*, *G. aggregatum*, *G. geosporum*, *G. mosseae*, *Sclerocystis pakistanika* and *Scutellospora heterogama*); however, Chl enhancements varied with the AMF species inoculated. Baslam *et al.* (2011) reported that the symbiosis of greenhouse grown lettuce plants with AMF resulted in enhanced amounts of Chl in leaves. Enhanced chlorophyllase activity causes degradation of photosynthetic pigments and associated proteins of photosynthetic pigment complex (Ahanger *et al*., 2014; Abd-Allah *et al*., 2015a). AMF inoculation causes an increase in the uptake of minerals like magnesium resulting in maintained chlorophyll biosynthesis. Our observation of positive effects of AMF on the cholorphyll synthesis as well as the mitigation of salt induced deleterious changes support the findings of *Vicia faba* (Hashem *et al*., 2014) *Ephedra aphylla* (Alqarawi *et al*., 2014). The increased levels of Chlorophyll in mycorrhizal lettuce plants presumably would have assigned to achieve higher photosynthetic rates in the whole plant, thus benefiting both growth of host plants (fig 4.5(B)) and the development and functionality of the symbiosis.

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