EFFECT OF INDIVIDUAL AND COMBINED APPLICATION OF BIOFERTILIZERS ON MORPHOLOGICAL AND BIOCHEMICAL CONSTITUENTS OF *ARACHIS HYPOGAEA* L.

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***ABSTRACT*:** A pot experiment entitled "Effect of individual and combined application of biofertilizers on morphological and biochemical constituents of (*Arachis hypogeae* L.)'' was carried out at College of Vivekandha College of Arts and Sciences for Women (Autonomous), Tiruchengode. The experiment comprising six treatments of nutrient management *viz*., T_1: Control, T_2: FYM, T3: Vermicompost, T4: *Azospirillum,* T5: *Rhizobium,* T6: FYM + Vermicompost + *Azospirillum* + *Rhizobium,* were evaluated in randomized block design with three replications. The experimental soil was clayey in texture, low in N, medium in available P, K and S. The groundnut seeds were tested in the experiment. The experimental results revealed that significantly higher values of growth parameters *viz.*, germination studies, shoot and root length, fresh weight, dry weight, total leaf area and also biochemical analyses such as chlorophyll, protein, amino acid, reducing sugar and non-reducing sugar was observed in combined application of soil amendments (FYM + Vermi + *Azospirillum* + *Rhizobium*). Biofertilizers usage in crops not only saves the costly chemical fertilizers application to certain extent, but also help in obtaining quality produce through secretion of plant growth promoting hormones like IAA, GA and cytokinins.

Keywords: Groundnut, Vermicompost, FYM, Biochemicals, *Rhizobium*

I. INTRODUCTION

Groundnut (*Arachis hypogeae* L.) is a major commercial oil seeded crop in India, China, Brazil, Nigeria and USA. Among the oilseed crop grown in India, groundnut occupies pre-dominant position. In recent years, crop cultivation requires the use of chemical fertilizer, but it is expensive for people who have not capacity to buy fertilizer. Therefore, the current trend is to explore the possibilities of supplementing organic fertilizer like FYM, castor cake, vermicompost *etc.* with biofertilizers. Biofertilizers have shown positive interaction with organic manure in legume crops. Organic manure in conjunction with biofertilizers will sustain and maintain the productivity of soil. Therefore, it is needed to compare various organic manure and biofertilizers in order to find out most effective combination. Keeping this objective in view, the present investigation was conducted to study the effect of organic manure and biofertilizers on growth of groundnut.

Sustainable agriculture aims at long term maintenance of natural resources and agricultural productivity with minimal adverse impact on the environment. It emphasizes optimal crop production with minimal external inputs, reducing dependence on commercial inputs and substituting them with internal resources [1]. At present, there is a need for developing an efficient nutrient management system with the use of organic manures, inorganic fertilizers and biofertilizers to maintain soil fertility and for better crop production [2]. The high content of both micro and macro nutrients in organic manures along with the slow release of phosphorus could reduce the nutrient deficiency problems and lower the magnitude of phosphorus fixation.

Now-a-days there is a need to devise alternate ways to collect, process, compost, utilize organic manure as well as biofertlizers like *Azotobacter*, *Azospirillum*, *Acetobacter*, *Rhizobium, Azolla*, Blue green algae and Phosphate solubilizing bacteria enrich fertility status of the soil [3]. The chemical fertilizers like N, P and K have played significant role on increasing the yield and quality in plants during early seventies. But in recent years the usage of chemical fertilizers indiscriminately in an unbalanced manner has been shown to result in several problems like loss of fertility, soil health and multiple nutrient deficiencies and loss of microbial activities etc, which ultimately resulting in reduced crop productivity and quality [4].

Vermicomposting is a process by which epigeic earthworm species are used for the conversion of organic wastes into vermicompost, excellent organic manure or it is the degradation of organic waste by earthwormic consumption. Vermicomposting is a solid waste as resources [5]. Composting have agricultural and other biowastes is the most widely adapted process for their recycling into the soil for replenishing with the scavenged nutrients, particularly the organic matter and micronutrients. The trace elements like Zn, Cu, Cr, Mn, Fe *etc* are essential for plants; they may become injurious for health of plants, animals and human beings. Therefore the concentration of trace elements in compost should not exceed the prescribed limits [6]. The main objective of this study was to investigate the effect of organic fertilizers and biofertilizers on morphological and biochemical constituents of groundnut.

II. MATERIALS AND METHODS

The pot culture was conducted to study the growth and biochemicals of groundnut (*Arachis hypogaea* L). In this regard, the materials used and the methods followed were given bellow.

2.1. Experimental site

The experimental site was located at Tiruchengode, namakkal Disrtict, Tamil Nadu, India. The experiment outline was entirely randomized block design, with three repetitions.

2.2. Pot culture experiment

Pot culture experiments were conducted with groundnut to known the effect of FYM, Vermicompost, biofertilizers (*Rhizobium* + *Azospirillum*) on morphological and biochemical parameters of ground nut (*Arachis hypogaea*).

2.3. Preparation of pot

Pot culture experiment was conducted for a period of 75 days. Red soil and garden soil free from pebbles and stones were filled in plots separately. The 10 kg of soil and sand in the ratio of 3:1 were filled before sowing.The seeds of groundnut seeds (*Arachis hypogaea* (L.) were obtained from Regional Research Institute, TNAU, Periyar Nagar at Virudhachalam taluk, Cuddalore district, Tamil Nadu, India. Sowing of groundnut crop was done at spacing of 30cm x 10cm. The field was irrigated immediately after sowing for assured seed germination.

T_1 : Control

T_2 : FYM

T3: Vermicompost

T4: *Azospirillum*

T5: *Rhizobium*

T6: FYM+ Vermicompost+ *Azospirillum*+ *Rhizobium*

Pre-sowing irrigation was given to ensure uniform germination. Irrigation was given at 7 DAS with due care to avoid excess flooding of water. Uniform irrigation was given for 4 times in a month. Five plant samples were randomly collected at regular intervals (seedling and flowering) and they were used for observations of morphological parameters like root length, shoot length, fresh weight and dry weight of root and shoot.

2.4. Germination studies

Germination study was conducted with the groundnut (*Arachis hypogaea*). The seeds were surface sterilized with 0.2 per cent of $HgCl_2$ for two minutes and thoroughly washed with tap water. The seeds were equispacially arranged in petriplates lined with filter paper and moistened with respective concentrations (T1, T2, T3, T4, and T5). The control set was maintained by using tap water. On the seventh day, the germination percentage was observed.

$$\text{Germination (\%)} = \frac{\text{No of seeds germinated}}{\text{No. of seeds placed for germination test}} \times 100$$

2.5. Morphological parameters

Five plants were taken from each treatment and their shoot and root length was measured by using a scale and these values were recorded. Five plant samples were collected from each treatment. They are washed thoroughly with tap water and then distilled water. After, the fresh weight of plant materials was taken in an electrical single pan balance after drying in air. Then, the samples were kept in hot air oven at 80°C for 24 hours. They were taken and kept in desiccators for some time. Then, the dry weight of plant samples were taken by using electrical single pan balance.

2.6. Biochemical analyses

Chlorophyll content (mg g-1 fresh weight of leaves) was determined through organic solvent (80% acetone) extraction method as described by [7]. Estimation of total soluble proteins was done by the method [8]. Total free amino acids were extracted from plant tissues and determined according to method [9]. Reducing and non-reducing sugar determined by the method [10] following standard procedures. The significance of treatments was analyses using one way ANOVA. Significant differences between treatments were determined using Turkey's multiple range tests ($P<0.05$).

III. RESULTS

The application of various types of soil amendments (T1-FYM, T2- Vermicompost, T3-*Azospirillum*, T4-*Rhizobium* and T5-FYM+Vermi+Azo+Rhizo) on Groundnut (*Arachis hypogaea* L.) on its germination percentage, morphological, biochemical, yield parameters and soil analysis were analyses for the present study.

Soil sample was taken after harvesting from each treatments of the experimental site and soil chemical properties were determined. The soil samples were analyzed for selected chemical properties mainly for soil pH, total nitrogen, available phosphorus and organic carbon using the appropriate laboratory procedures. The T5 (FYM + Vermi + *Azospirillum* + *Rhizobium*) analysis results showed that the organic C and organic matter is high, implying that this organic fertilizer can be a good source of plant nutrients. Therefore, application of inorganic NPS fertilizers along with well decomposed T5 (FYM + Vermi + *Azospirillum* + *Rhizobium*) with very high nutrient content is justified to produce good yield of potato at the study site. The change in total N, P, S after harvest (appendix table 1) relative that incorporation of T5 (FYM + Vermi + *Azospirillum* + *Rhizobium*) and mineral N, P, K fertilizers could improve the fertility status of the soil.

3.1. Germination percentage of *Arachis hypogaea* L.

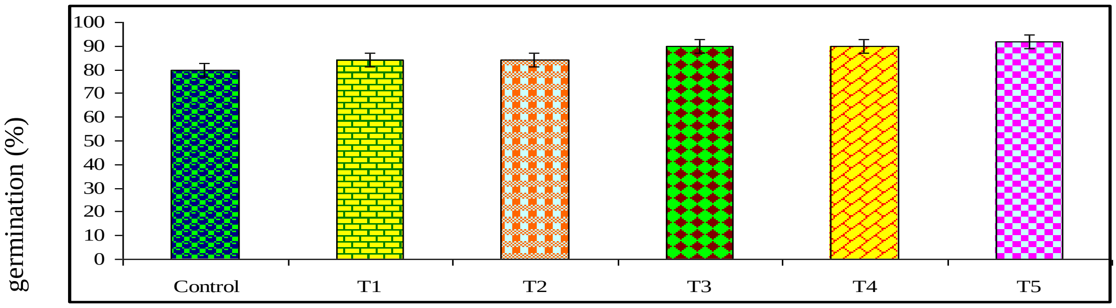

Fig.1 Effect of various soil amendments on germination percentage of Groundnut (*Arachis hypogaea* L.)

Fig.1 shows that the effect of soil amendments (T1-FYM, T2-, Vermicompost, T3-*Azospirillum*, T4-*Rhizobium* and T5-FYM + Vermi + Azo + Rhizo) on germination percentage of Groundnut (*Arachis hypogaea* L.) when compared to control. The highest germination (90, 90%) was found in pots which were applied with T5-FYM+Vermi+Azo+Rhizo amendments followed by T4-vermicompost (84%), T3- *Azospirillum*, T2- Vermicompost and T1- FYM whereas, lowest germination percentage was observed in control.

3.2. Morphological parameters

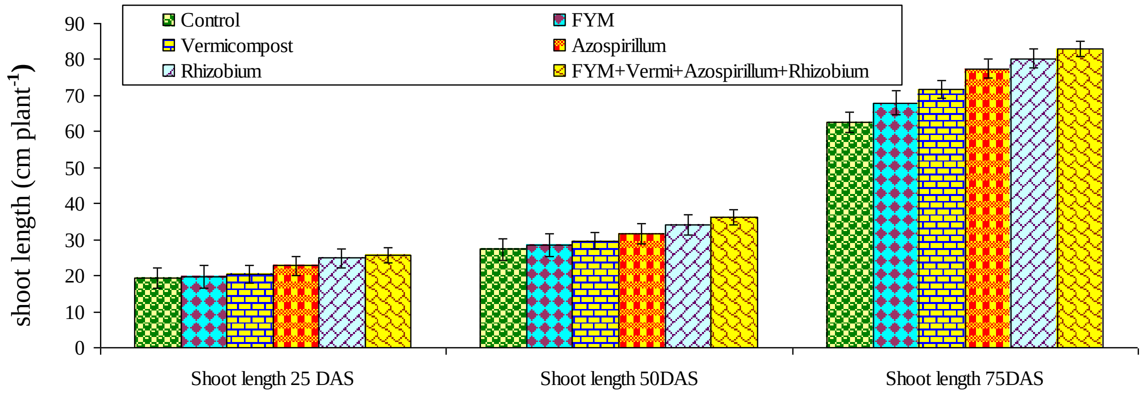

Fig.2 Effect of different soil amendments on shoot length

Fig. 2 clearly revealed that different soil amendments (Farm Yard Manure, Vermicompost, *Azospirillum*, *Rhizobium* and FYM + Vermi + *Azospirillum* + *Rhizobium*) on shoot length of Groundnut (*Arachis hypogaea* L.) in 25, 50, 75 DAS with respect to control. The lowest shoot length was observed in control soil while, the shoot length was significantly increased in all the soil amendments in both days. The highest shoot length was observed in combined application of soil amendments (FYM + Vermi + *Azospirillum* + *Rhizobium*). This resulted due to the improved growth status of plants because of the presence of organic amendments in the soil.

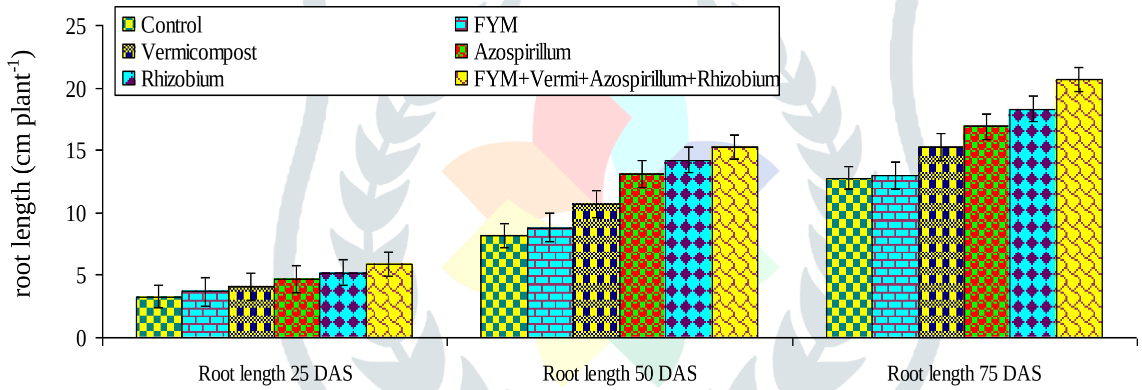

Fig.3 Effect of different soil amendments on root length

Fig. 3 clearly revealed that different soil amendments (Farm Yard Manure, Vermicompost, *Azospirillum*, *Rhizobium* and FYM + Vermi+ *Azospirillum* + *Rhizobium*) on root length of Groundnut (*Arachis hypogaea* L.) in 25, 50, 75 DAS with respect to control. The lowest root length was observed in control soil while, the root length was significantly increased in all the soil amendments in both days. The highest shoot length was observed in combined application of soil amendments (FYM + Vermi + *Azospirillum* + *Rhizobium*). This resulted due to the improved growth status of plants because of the presence of organic amendments in the soil.

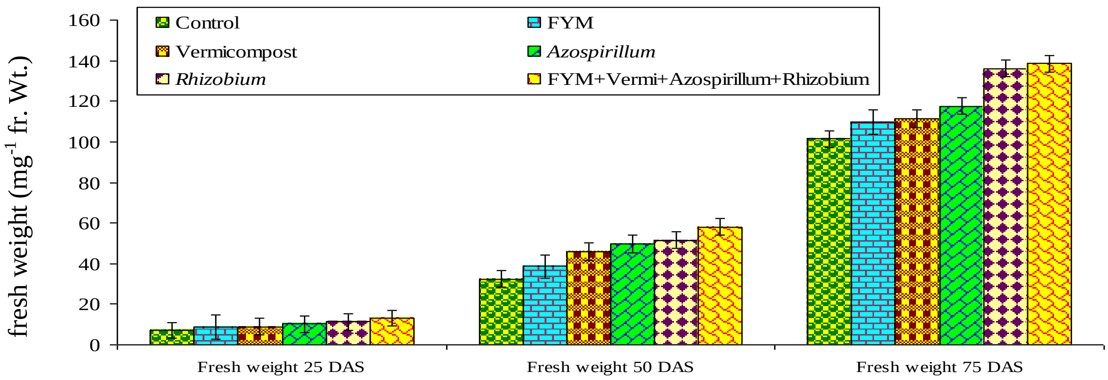

Fig.4 Effect of different soil amendments on fresh weight

Fig. 4 clearly revealed that different soil amendments (Farm Yard Manure, Vermicompost, *Azospirillum*, *Rhizobium* and FYM + Vermi+ *Azospirillum* + *Rhizobium*) on fresh weight of Groundnut (*Arachis hypogaea* L.) in 25, 50, 75 DAS with respect to control. The lowest fresh weight was observed in control soil while, the fresh weight was significantly increased in all the soil amendments in both days. The highest fresh weight was observed in combined application of soil amendments (FYM + Vermi + *Azospirillum* + *Rhizobium*). This resulted due to the improved growth status of plants because of the presence of organic amendments in the soil.

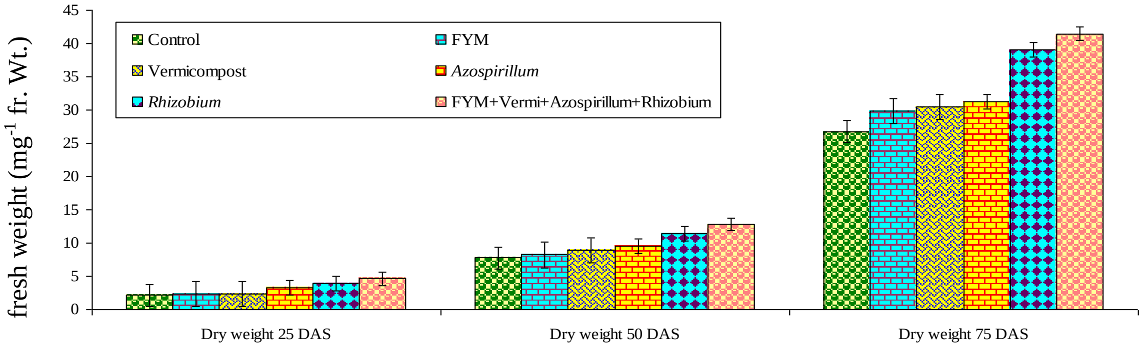

Fig.5 Effect of different soil amendments on dry weight

Fig. 5 clearly revealed that different soil amendments (Farm Yard Manure, Vermicompost, *Azospirillum*, *Rhizobium* and FYM + Vermi+ *Azospirillum* + *Rhizobium*) on dry weight of Groundnut (*Arachis hypogaea* L.) in 25, 50, 75 DAS with respect to control. The lowest dry weight was observed in control soil while, the dry weight was significantly increased in all the soil amendments in both days. The highest dry weight was observed in combined application of soil amendments (FYM + Vermi + *Azospirillum* + *Rhizobium*). This resulted due to the improved growth status of plants because of the presence of organic amendments in the soil.

3.3. Biochemical parameters

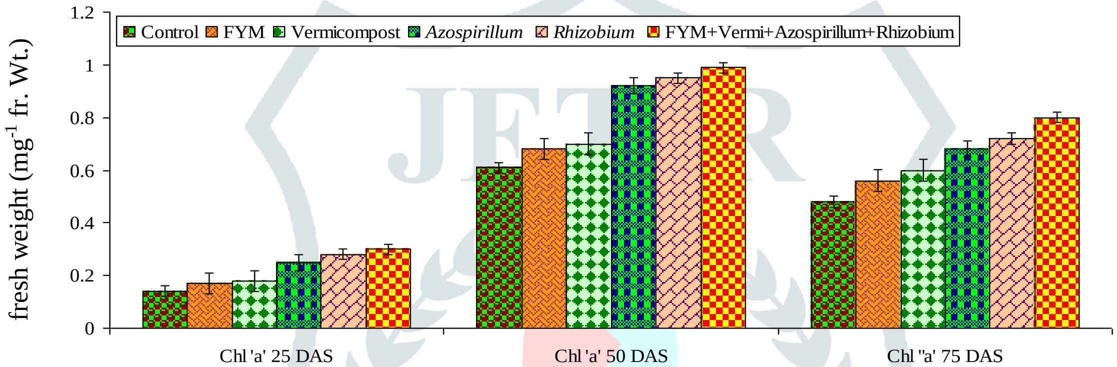

Fig.6 Effect of different soil amendments on chlorophyll

Fig. 6 shows that different soil amendments (Farm Yard Manure, Vermicompost, *Azospirillum*, *Rhizobium* and FYM + Vermi+ *Azospirillum* + *Rhizobium*) on chlo 'a' of Groundnut (*Arachis hypogaea* L.) in 25, 50, 75 DAS with respect to control. The lowest chl 'a' was observed in control soil while, the chl 'a' was significantly increased in all the soil amendments in both days. The highest chl 'a' was found in pots which were applied with combined application of soil amendments (FYM + Vermi+ *Azospirillum* + *Rhizobium*) in 50 DAS. This resulted due to the improved photosynthetic pigments of plants because of the presence of organic amendments in the soil.

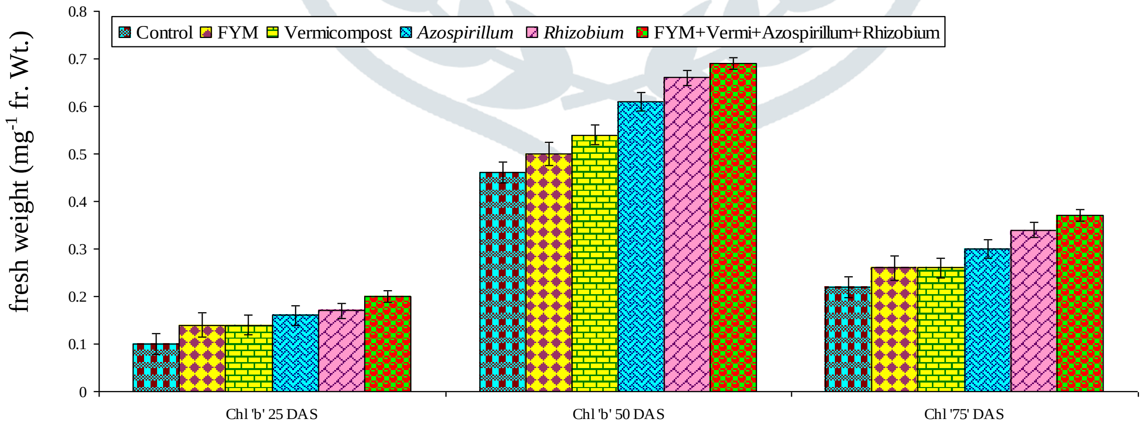

Fig.7 Effect of different soil amendments on chlorophyll

Fig. 7 shows that different soil amendments (Farm Yard Manure, Vermicompost, *Azospirillum*, *Rhizobium* and FYM + Vermi+ *Azospirillum* + *Rhizobium*) on chl 'b' of Groundnut (*Arachis hypogaea* L.) in 25, 50, 75 DAS with respect to control. The lowest chl 'b' was observed in control soil while, the chl 'a' was significantly increased in all the soil amendments in both days. The highest chl 'b' was found in pots which were applied with combined application of soil amendments (FYM + Vermi+ *Azospirillum* + *Rhizobium*) in 50 DAS. This resulted due to the improved photosynthetic pigments of plants because of the presence of organic amendments in the soil.

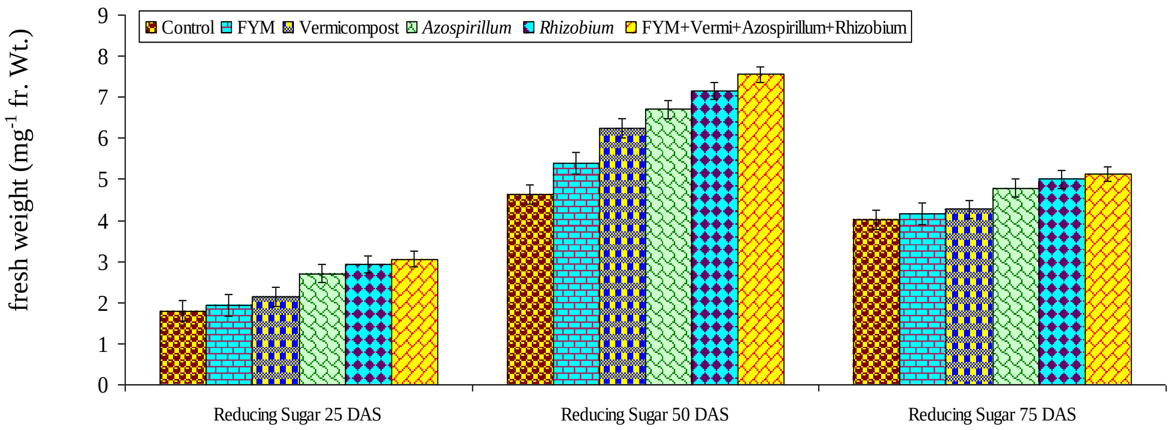

Fig.8 Effect of different soil amendments on reducing

Fig. 8 shows that different soil amendments (Farm Yard Manure, Vermicompost, *Azospirillum*, *Rhizobium* and FYM + Vermi+ *Azospirillum* + *Rhizobium*) on reducing sugar of Groundnut (*Arachis hypogaea* L.) in 25, 50, 75 DAS with respect to control. The lowest reducing sugar was observed in control soil while, the reducing sugar was significantly increased in all the soil amendments in both days. The highest reducing sugar was found in pots which were applied with combined application of soil amendments (FYM + Vermi+ *Azospirillum* + *Rhizobium*) in 50 DAS. This resulted due to the improved biochemical parameters of plants because of the presence of organic amendments in the soil.

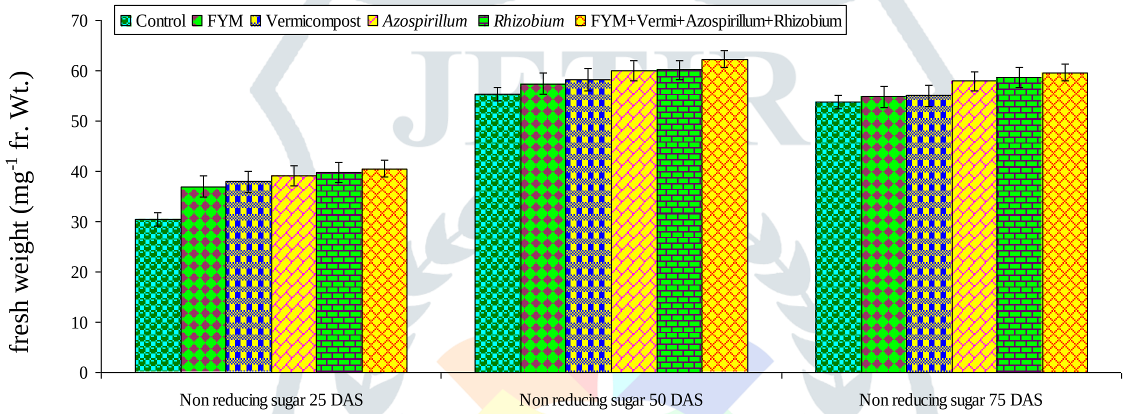

Fig.9 Effect of different soil amendments on non-reducing sugar

Fig. 9 shows that different soil amendments (Farm Yard Manure, Vermicompost, *Azospirillum*, *Rhizobium* and FYM + Vermi+ *Azospirillum* + *Rhizobium*) on non-reducing sugar of Groundnut (*Arachis hypogaea* L.) in 25, 50, 75 DAS with respect to control. The lowest non-reducing sugar was observed in control soil while, the non-reducing sugar was significantly increased in all the soil amendments in both days. The highest non-reducing sugar was found in pots which were applied with combined application of soil amendments (FYM + Vermi+ *Azospirillum* + *Rhizobium*) in 50 DAS. This resulted due to the improved biochemical parameters of plants because of the presence of organic amendments in the soil.

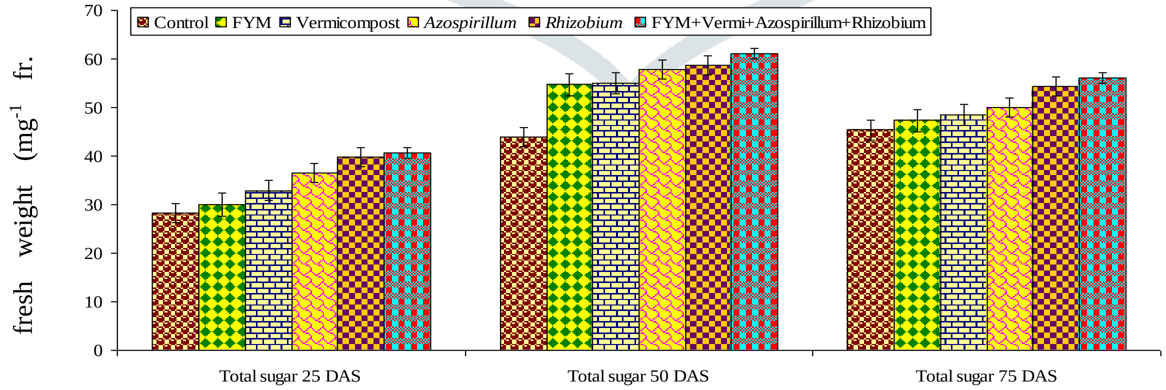

Fig.10 Effect of different soil amendments on total sugar

Fig. 10 shows that different soil amendments (Farm Yard Manure, Vermicompost, *Azospirillum*, *Rhizobium* and FYM + Vermi+ *Azospirillum* + *Rhizobium*) on total sugar of Groundnut (*Arachis hypogaea* L.) in 25, 50, 75 DAS with respect to control. The lowest total sugar was observed in control soil while, the total sugar was significantly increased in all the soil amendments in both days. The highest total sugar was found in pots which were applied with combined application of soil amendments (FYM + Vermi+ *Azospirillum* + *Rhizobium*) in 50 DAS. This resulted due to the improved biochemical parameters of plants because of the presence of organic amendments in the soil.

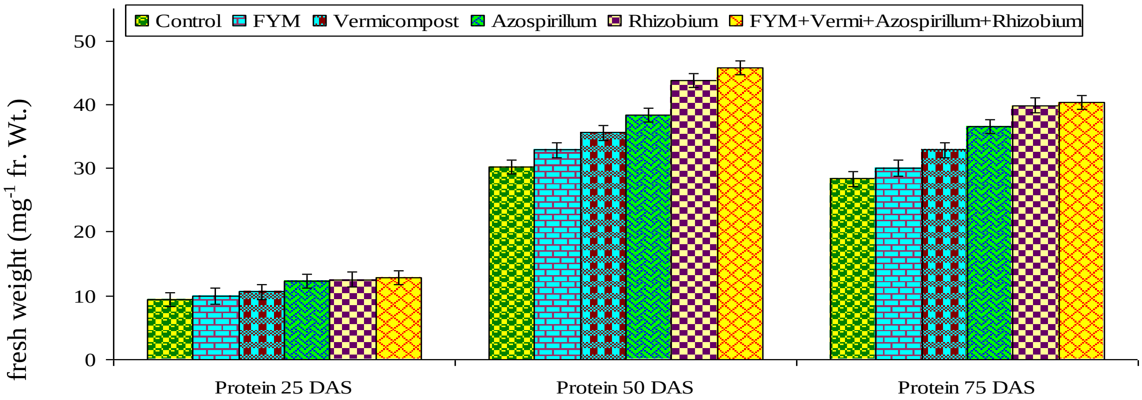

Fig.11 Effect of different soil amendments on protein

Fig. 11 shows that different soil amendments (Farm Yard Manure, Vermicompost, *Azospirillum*, *Rhizobium* and FYM + Vermi+ *Azospirillum* + *Rhizobium*) on protein content of Groundnut (*Arachis hypogaea* L.) in 25, 50, 75 DAS with respect to control. The lowest protein content was observed in control soil while, the protein content was significantly increased in all the soil amendments in both days. The highest protein content was found in pots which were applied with combined application of soil amendments (FYM + Vermi+ *Azospirillum* + *Rhizobium*) in 50 DAS. This resulted due to the improved biochemical parameters of plants because of the presence of organic amendments in the soil.

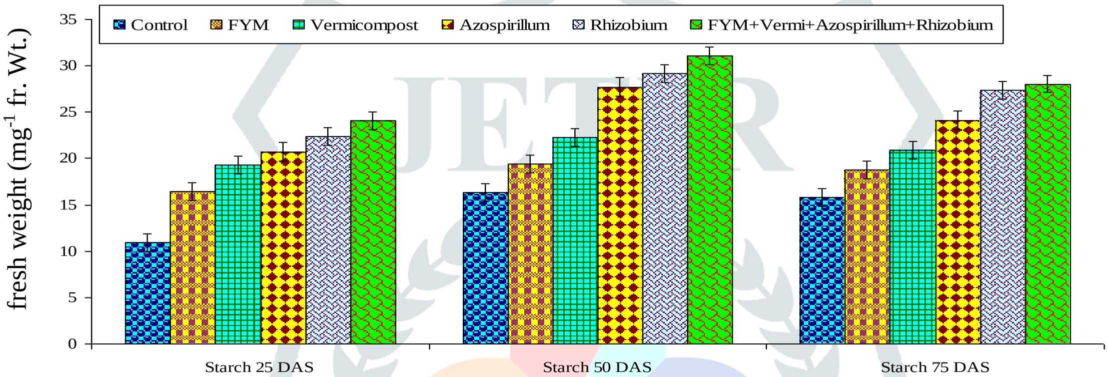

Fig.12 Effect of different soil amendments on starch

Fig. 12 shows that different soil amendments (Farm Yard Manure, Vermicompost, *Azospirillum*, *Rhizobium* and FYM + Vermi+ *Azospirillum* + *Rhizobium*) on starch content of groundnut (*Arachis hypogaea* L.) in 25, 50, 75 DAS with respect to control. The lowest starch content was observed in control soil while, the starch content was significantly increased in all the soil amendments in both days. The highest starch content was found in pots which were applied with combined application of soil amendments (FYM + Vermi+ *Azospirillum* + *Rhizobium*) in 50 DAS. This resulted due to the improved biochemical parameters of plants because of the presence of organic amendments in the soil.

IV. DISCUSSION

In the course of presenting the results of the experiment entitled "Effect of fertilizer on Soil Fertility, yield and quality of ground nut (*Arachis hypogaea* (L.)" significant variation in the criteria used for treatment evaluation were noted. In this chapter, the significant results or those assuming a definite pattern are discussed to establish the cause and effect relationship in the light of available evidence and literature. In accordance with the findings of [11] observed that bio-fertilizers significantly increased plant height, root length, root growth, alkaloid content and N, P, K, Ca and Mg uptake in *Catharanthus roseus* in comparison to the uninoculated control. Similar reports were documented in different organic amendments on fresh and dry weight of various crop plants, sweet marjoram [12]; *Gymnema sylvestre* [13]. The amount of vermicompost had a significant effect on not only growth and flowering of the Marigold plants, but also on the plant shoot and root biomass, plant height and diameter of the flowers [14].

[15] in a study on wheat, nitrogen stabilizer bacteria including *Azotobacter* and *Azospirillum* had a significant effect on the number of spikes in plant. [16] suggested that significantly higher plant height, number of leaves per plant, leaf area per plant and leaf area index over lower levels of vermicompost was recorded in response to application of vermicompost at the rate of 6 t ha^{-1}. Vermicompost is known to contain micronutrients apart from major nutrients. [17] observed on pepper plants found that, using biofertilizers Microbin and Biogen significantly increased the vegetative growth characters (plant length, number of leaves and stems per plant). The plant growth is enhanced by the application of PGPR through ACC-deaminase activity and increased nutrients uptake by the plant and root growth [18]. The plant growth is enhanced by the application of PGPR through ACC-deaminase activity and increased nutrients uptake by the plant and root growth [18].

Besides that, the use of these bio-fertilizers significantly improved growth parameters. However, the improvement in these characters were found limited when these bio-fertilizers were used alone. These findings are agreement with earlier worker of [19] & [20]. Thus applying vermicompost + PGPR increased the vegetative growth of onion plants to be similar to those resulted from the RMF were reported by [21], [22] and [23]. [24] concluded that Vermicompost recorded significant increase in growth parameters when compared to other treatments and the farmers could realize the importance of organic manure which serves environment friendly. Biofertilizers like PSB, VAM and *Azotobacter* can improve plant survival, vegetative growth, Leaf Area Index, Harvesting Index and tuber yield in potato through effective nitrogen utilization and release of plant growth promoting substance (PGPS) [25].

The beneficial effects of foliar treatments on tomato plants are materialized by increasing cellular chlorophyll synthesis. This increase is detected as a first sign in the cultivation, after foliar fertilizer application by converting the leaves colour in a dark green [26]. Similar observation was made by [27], reported the increase of chlorophyll content in rice leaf up to flowering stage due to inoculation of cyanobacteria. [28] reported that there was increased chlorophyll content in rice plant influenced by mixture of several bioinoculants.

The protein was increased in treated with combined inoculation of biofertilizers (T5) of ground nut treatments in pot experimenst. Similar result by [29] found that the leaf protein content increased up to 50th day and then declined which was influenced by combined inoculations. [30] observed that the immobilized cyanobacterial inoculation influences the increasing trend of leaf protein content up to 60th day and then declined. Similar trend was also observed in the present study in which the combined form of bioinoculant application both in field and pot experiments (T5) influenced the increasing content of protein up to 50th day. The protein was observed maximum up to 50th day in most of the early study by individual type of inoculation. In the present work the combined, dual and individual form of inoculation represent a different type of increasing trend up to 50th day.

Significant level of variation in the level of protein content in leaves of different varieties of mustard under the field trial of 2005-2006 reveals differential rate of nitrogen assimilation by crop plants. The progressive increase in the protein content up to T2 treatment of combined dose of biofertilizer and chemical fertilizer may be due to higher absorption of nitrogen and phosphorous from soil by crop plants due to their availability under the influence of application of biofertilizer [31].

The results amino acids of ground nut as influenced by different treatments in both field and pot experiment. In rice plant generally the amino acid content gradually increased up to 60th day and declined thereafter [32], [33]Sushil Pradhan, 2000). [30] also reported that the amino acid content gradually increases up to 60th day and then declined. The amino acid was increased in treated with combined inoculation of biofertilizers (T5) of ground nut treatments in pot experimenst. The similar result found out by[32], [33]. [30] also reported that the amino acid content gradually increases up to 75th day.

The starch was increased in treated with combined inoculation of biofertilizers (T5) of ground nut treatments in pot experimenst, [33] found that the starch content of leaf of rice seedling treated with cyanobacteria was found to increase significantly when compared to the control. In the present study also similar trend was noticed that both in field and pot experiments. The starch content increased gradually up to 50th day. It was also noticed that the T5 treatment both in pot and field (combined inoculation) showed better results when compared to dual and individual form of bioincoulant application.

Significant level of variation in total soluble sugar content in leaves among the different varieties may be attributed towards the variable rate of photosynthesis leading to production of variable amount of photosynthate among the different varieties. Higher biosynthesis of chlorophyll and photosynthesis of flag leaf of plants under the biofertilizer treated plots might have resulted towards higher level of total soluble sugar content of leaves. The results also reveal that dual inoculation of biofertilizers have pronounced influence on biosynthesis of carbohydrates in leaves [34]. Higher accumulation of sugar in leaves of mustard under cycocel treated plots might be due to higher rate of photosynthesis. Our findings were similar with the earlier findings on *Siderites montana* [35] and on Iris [36]. These carbohydrate changes are of particular importance because of their direct relationship with such physiological processes as photosynthesis, translocation and respiration.

V. CONCLUSION

The results of present study could be concluded that for higher productivity and profitability from groundnut cultivation, among the treatments the only combined applications (FYM + Vermi+ *Azospirillum* + *Rhizobium*) play a major role in improving growth and yield of different field crops, vegetables, flowers and fruits. Biofertilizers could be increase plant growth and yield also depends on the quantity of combined fertilizers applied in the plant medium. Use of fertilizers in horticulture at large scale could solve the management and disposal problem associated with macrophytes and also resolves the deficiency of organic matter in such soil in addition to nutrient depletion.

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