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Effect of AM fungi and some organic fertilizers on growth and biochemical content of *Trigonella foenum graecum* L.

Bhosale K.S.[1*], Kale S.T.[1] and Shinde B.P.[2]
[1]P.G. Research Centre, Dept of Botany N. Wadia College, Pune, Maharashtra, India
[2]V.P. College of Arts, Science and Commerce, Baramati, Maharashtra, India
ksbhosale@gmail.com

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Abstract

A pot culture experiment was carried out to assess effect of Mycorrhizal fungi, Farm Yard Manure, Neem seed cake and combination of AM+FYM+ Neem seed cake on Trigonella foenum-graecum L. Plastic pots of 5 kg soil capacity were used for growing Trigonella seeds. Four sets were made for further experimentation. Each set included three control and three replicates. In the first set of an experiment three experimental pots were filled with mixture containing autoclave sterile garden soil and AM fungi in 9:1 ratio, control plants of all four sets filled only with garden soil. The second set included garden soil and FYM in 9:1 ratio. Garden soil and Neem seed cake was filled in third set with 9:1 ratio whereas forth set was filled with mixture of Garden soil with AM+FYM+Neem seed cake in 9:1 ratio. All plants further assessed for morphological parameters and biochemical contents.

Keywords: Trigonella, AM, FYM, Neem cake, biochemical content.

Introduction

Fenugreek (*Trigonella foenum-graecum* L.), the annual herbaceous legume crop belonging to family Fabacecae is usually known as Fenugreek and is considered as one of the oldest multipurpose medicinal herbs[1]. Plants of *Trigonella foenum-graecum* L. are bushy green, medium sized and pods are small and sickle shaped. It is a native of South-eastern Europe and Western Asia and widely cultivated in India which harbour great diversity of fenugreek. It is commonly found growing in the Mediterranean region of the world. In India; it is an important versatile *rabbi* season seed spice crop mainly grown in Rajasthan, Gujarat, Madhya Pradesh Maharashtra and Haryana. The AM fungi are major components of the soil microbial community, forming symbiotic association with roots of over 90% of the terrestrial plants. AM fungi have gained significance because of their role in growth and early establishment of plant species used in afforestation programmes, soil fertility, nutrient uptake and bio-control of plant diseases[2]. AM fungi are ecologically significant because they form relationships in the roots of a host plant in a symbiotic association. The host plant provides the fungus with soluble carbon sources and other nutrients, and the fungus provides the host plant with an increased capacity to absorb water and nutrients from the soil. Farm yard manure is an organic matter prepared from various kinds of animal excreta mixed with other organic materials such as crop residues, kitchen wastes, vegetable wastes, house sweepings etc. By using simple preservation techniques, the quality of FYM in terms of organic matter and plant nutrient content can be considerably improved and preserved for later use in crop production. Various tree leaves and seed cakes are rich source of nutrients to the crops as like neem. Neem cake (*Azadirachta indica* A. Juss) is the residue of seed kernel left after extraction of oil. Neem seeds are produced in huge amount. Neem cake most frequently used and worked as satisfactory nematode control, often comparable to that obtained with chemicals.

In present investigation the prominence has given to assess the usefulness of different organic fertilizers like AM fungi Farmyard manure, neem seed cake and mixture of all three on (*Trigonella foenum-graecum* L.), plants in pot experiment.

Materials and methods

A pot culture experiment was carried out at the Department of Botany, Nowrosjee Wadia College, Pune, in replicated randomized design with three replications for each treatment. The plants used for experiment was *Trigonella foenum-graecum* L. Inoculants used for the treatments were Mycorrhizal fungi (AM), Farm Yard Manure (FYM), Neem seed cake and combination of AM + FYM + Neem seed cake. *Trigonella* seeds were obtained from market. The plastic pots (20x20x30cm) of 5 kg soil capacity were used for growing the *Trigonella* seeds. Four sets were made for further experimentation. Each set included one control and three replicates. In the first set of an experiment three experimental pots were filled with mixture containing garden soil and AM fungi in 9:1 ratio. Control plants were filled only with garden soil. Similarly, the second set of all experimental pots were filled with mixture containing garden soil and FYM in 9:1 ratio. Garden soil and Neem seed cake was filled in third set with 9:1 ratio whereas forth set was filled with

mixture of Garden soil with AM + FYM + Neem seed cake in 9:1 ratio.

The set up for experiment started with autoclaving of garden soil at 115 lbs pressure for 30 minutes. The autoclave sterile soil then poured in sterilized pots by weighing 4.5 kg accurately. Then well-imbibed 10 to 15 seeds grown in each control and experimental pots. The plants were grown in normal conditions for 14 days and then the fertilizers dose was given in each pot. The dose of fertilizers was given after 15 days to avoid further complications due to dose of fertilizers and get acclimatized with edaphic and environmental conditions.

About 200 seeds of uniform size from *Pusa* cultivar were selected for the experiments. The seeds were surface sterilized with 0.1% $HgCl_2$, washed thoroughly 4 times in sterilized distilled water and then soaked in distilled water for 4 hours. 10 to 15 well-imbibed seeds were sown in each plastic pot. The set of experiment was placed in shed net under observation for studies with respect to morphological parameters like root length, shoot length, leaf number, surface area of leaves, number of branches, etc. Each set was further analysed for biochemical contents like chlorophyll, protein, vitamin C and reducing sugars.

The length of roots was measured with the help of thread and then the thread was used scale to measure the length or roots in cms. The shoot length and number of branches similarly measured and counted with the help of thread and recorded. The number of leaves counted and recorded simply by counting the leaves of control and experimental plants. The surface area of both control and experimental plants was measured by plotting of outline of leaves on graph paper and measured in square cms. An average fresh weight of the *Trigonella* plants was measured in weighing balance in grams. The dry weight of control and experimental plants was measured by drying the leaves in hot air oven at 50ºC. for 5 days and then measured in grams.

Analysis of Biochemical contents was carried according to following methods.

Chlorophyll Content: An amount of Chlorophylls a, b and total chlorophylls was carried out by Arnon's[3] method. An extract of chlorophyll was made using fresh green leaves of *Trigonella* (1g). The grinding of leaves done with the help of a mortar and pestle along with 10ml ice cold 80% acetone. The mixture of both then used to centrifuge at 3000rpm for 2 minutes. The supernatant solution used for reading absorbance and remaining pellet again re extracted for two times with 5 ml of 80% acetone. All the supernatants then used for taking further readings.

The supernatant later used to record an absorbance at 663 nm, 645 nm. The concentration of chlorophyll a, b and total chlorophyll calculated with the equations of Arnon as follows.

Total Chl: = (20.2×A 645-8.02×A 663) × 100/mg leaf weight

Chl-a = (12.7 × A663-2.69 × A645) × 10 /mg leaf weight

Chl-b = (22.9 × A645-4.61 × A663) × 10 /mg leaf weight

Protein content: Estimation as well as quantification of proteins were done by Lowry *et al*.[4] method. Isolation of protein was done using fresh leaves of *Trigonella*. The plants used for protein estimation were from control and experimental pots. The leaves sliced into small bits and from that weighed 0.5g plant material accurately and used to extract using 5ml of 0.1M phosphate buffer having pH 7.0. The plant extract then used to centrifuge for 15min at 10,000rpm. The supernatant discarded and the remaining pellet then used to dissolve in 2ml of 1.0N NaOH solution. This solution then used as a sample and 0.2 ml solution taken for protein estimation. The working standard dilutions of Bovine Serum Albumin (BSA) and extract of plants filled in a sequence of test tubes and final volume was accustomed to 1mL in every test tube. Later 5mL of reagent C was added in the series of tubes and the mixture was incubated for 10min. After incubation 0.5mL of folin ciocalteau was added and the mixture again incubated for 30min at dark. After incubation blue colour developed in the reaction mixture. This mixture then used read absorbance at 660nm on UV-visible spectrophotometer. BSA fraction V was used at the concentration of 50mg to dissolve in distilled water and used as standard protein. From this standard graph drawn for calculations.

Vitamin C Content: Vitamin C content of from the leaves of *Trigonella* was estimated following the method of Birch *et al*.[5]. Initially working standard and stock standard solutions was prepared. The five ml of working standard was taken into a conical flask; in this 10ml of 4% oxalic acid was added. This solution was titrated against dye (42g Na_2CO_3 dissolved in 52 mg of 2-6-dichloro phenol indophenols and final volume raised up to 200mL with distilled water). The amount of dye consumed equivalent to amount of ascorbic acid. The sample was extracted from five g sugarcane stem in 4% oxalic acid and final volume raised to 100mL. Then the 5mL of the final mixture was added with 10mL of 4% oxalic acid and titrated against the dye and the amount of vitamin C was calculated by using following formula.

$$\text{Amount of Vitamin C mg/100 g sample} = \frac{0.5\text{ mg}}{\text{V1 mL}} \text{ x } \frac{\text{V2}}{100\text{ mL}} \text{ x } \frac{100\text{ mL}}{\text{Wt.of the sample}} \text{ x } 100$$

Estimation of reducing sugars: The reducing sugars were estimated by using Dinitrosalicylic acid (DNSA) reagent[6]. Weighed 1g leaves of *Trigonella* and extracted the sugars with hot 80% ethanol twice (5ml each time). Collected the supernatant and evaporated it by keeping it on a water bath at 80ºC. This extract was condensed on hot water bath to approximately 1.0ml and centrifuged at 10,000rpm for 10 minutes. The final volume of supernatant was adjusted to 10ml with distilled water and dissolved the sugars. Pipette out 0.2ml of the extract in test tubes and adjusted the final volume up to 3 ml with distilled water in all the tubes. Then added 3 ml of DNSA reagent and the contents were heated in a boiling water

bath for 5 minutes. One ml of 40% Rochelle salt solution was added in the warm contents. After cooling the contents, the intensity of dark red colour was recorded at 510 nm on UV-visible spectrophotometer. D-glucose at the concentration of 100 µg per ml was used to make standard graph. The calculation of amounts of reducing sugars done using standard graph.

Results and discussion

Root length in experimental plants was more as compared to controlled plants. Root length was 4.7cms in Mycorrhizal plants. It was highest i.e. 5.2cms in plants treated with AM+FYM+Neem seed cake. Whereas it was 4.9cms for the experimental plants treated with FYM and Neem seed cake. This was due to more availability of nutrients to the roots of plants. It was maximum in plants treated with combination of AM+FYM+Neem seed cake because Mycorrhizal fungi mobilized the nutrients and made them easily available for plants roots (Figure-1).

In all experimental plants shoot length was more than control plants. There was highest increase in shoot length (1.4cms) recorded for plants treated with AM+FYM+Neem seed cake. Mycorrhizal fungi increased absorption area of the roots so that more nutrients absorbed by the plant roots and hence resulted well as compared to control plants.

Similar mechanism of action took place with respect to surface area of the plants treated with AM+FYM+Neem seed cake. Similar results were obtained[7,8].

The number of branches in experimental plants were significantly different than controlled plants. More mobilized nutrients resulted in more absorption by the roots of the plants.

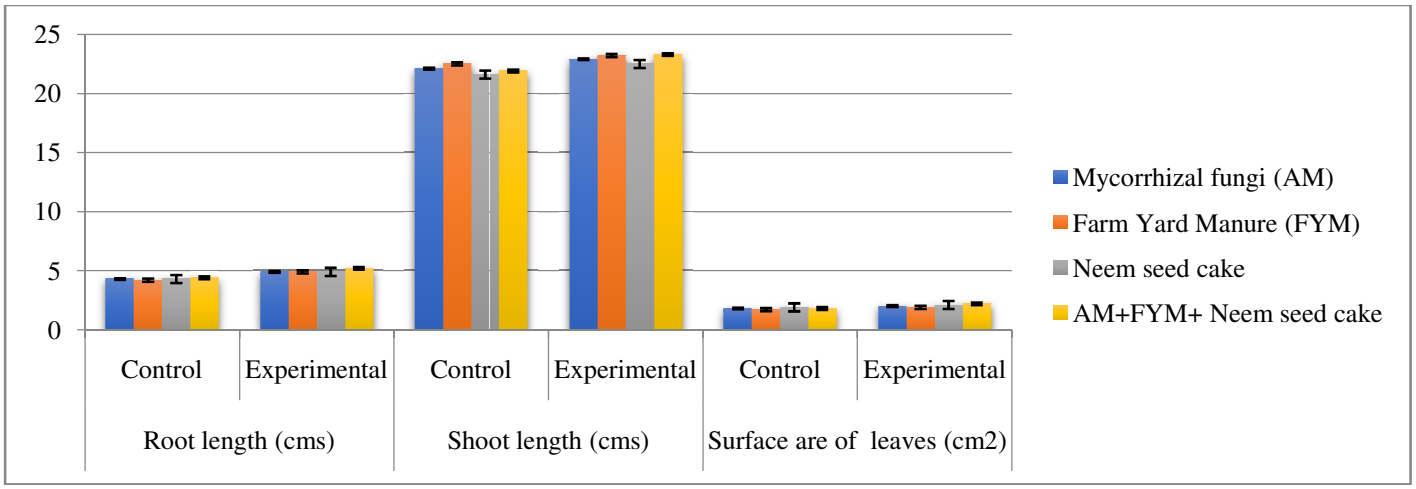

Figure-1: Effect of Mycorrhizal fungi (AM), Farm Yard Manure (FYM), Neem seed cake and combination of AM+FYM+ Neem seed cake on root length and shoot length and surface area of *Trigonella* leaves.

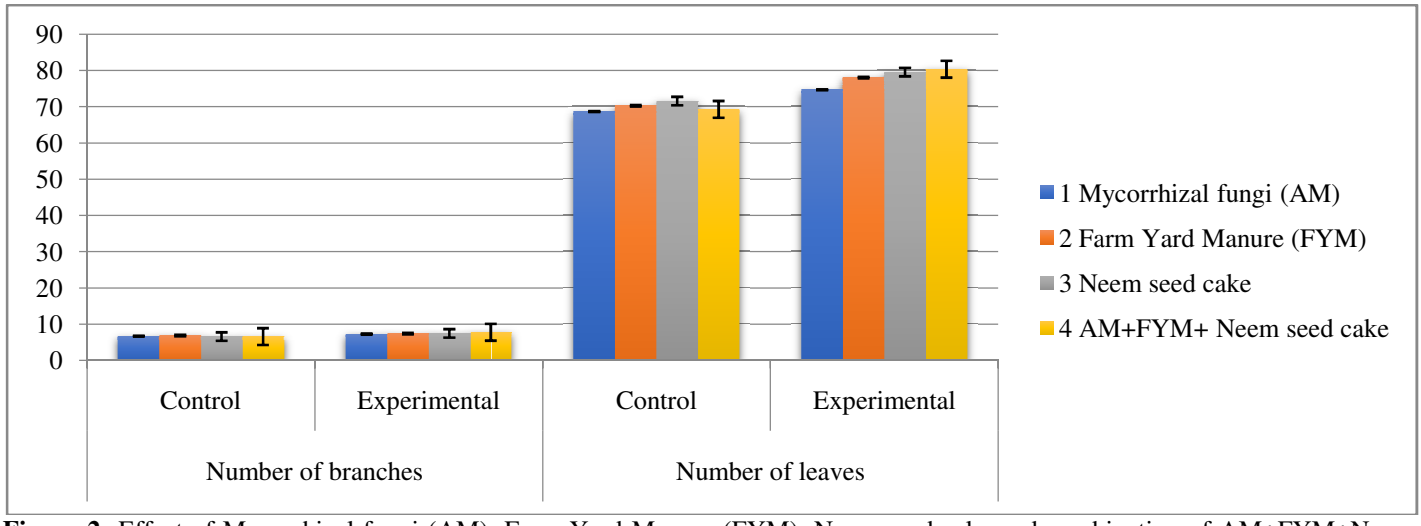

Figure-2: Effect of Mycorrhizal fungi (AM), Farm Yard Manure (FYM), Neem seed cake and combination of AM+FYM+Neem seed cake on number of branches, number of leaves of *Trigonella* leaves.

More absorption increased the roots and shoot length and surface area of leaves which resulted in to increase in absorption and photosynthetic efficiency. This leads to increased number of branches, number of leaves. Our results corroborate with Purbey and Sen[9] and manures by Khiriya and Singh[10] and Aishwath *et al.*[11].

The fresh weight in all controlled plants was comparatively less than in experimental plants. Increased photosynthetic efficiency resulted in more formation of sugars. This resulted in increased storage and thus there was increased fresh weight of experimental plants. The fresh weight had an impact on the dry weight of the all experimental plants. Our results harmonize with the results with Ashif Al. *et al.*[12] and Sunitha. *et al.*[13].

Amount of Chlorophyll a, b and total chlorophylls was recorded maximum experimental plants treated with AM+FYM+ Neem seed cake. This is due to the beneficial effect of AM+FYM+ Neem seed cake on these parameters might be due to its contribution in supplying additional plant nutrients and increasing availability of native soil nutrients with increased microbial activity. These results are in close agreement with that of Bhosale and Shinde[14], Khiriya *et al.*[15] and Jat *et al.*[16].

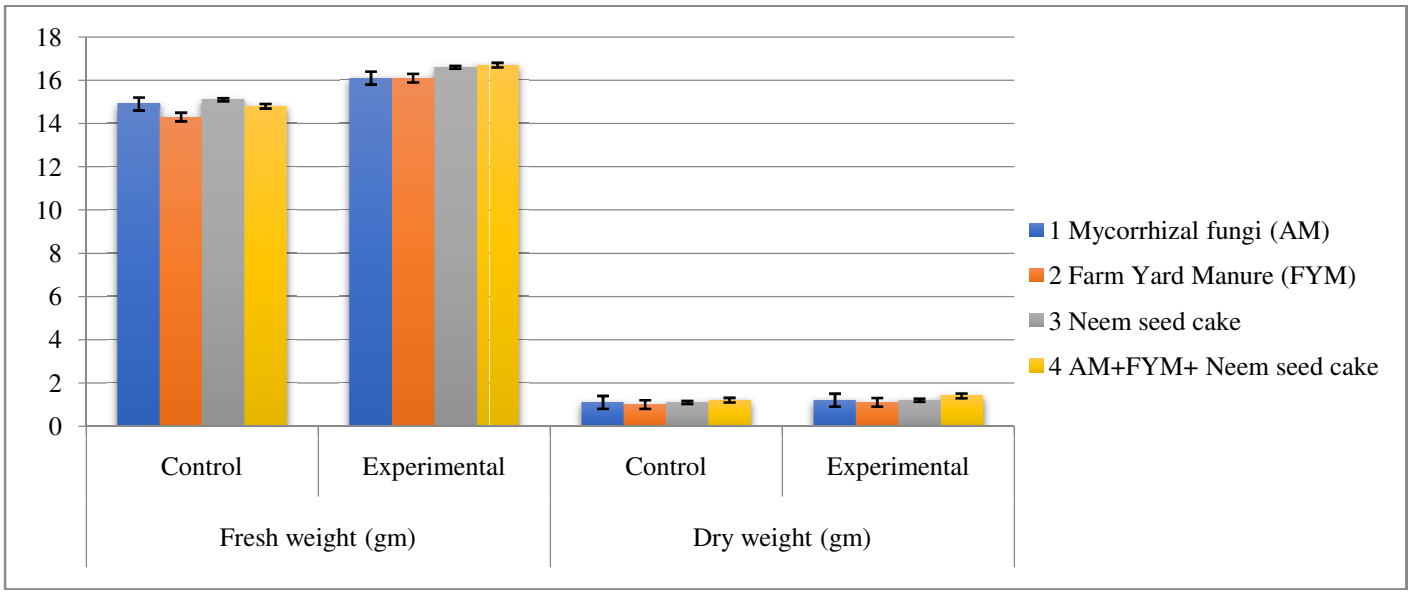

Figure-3: Effect of Mycorrhizal fungi (AM), Farm Yard Manure (FYM), Neem seed cake and combination of AM+FYM+ Neem seed cake on fresh weight and dry weight of *Trigonella.*

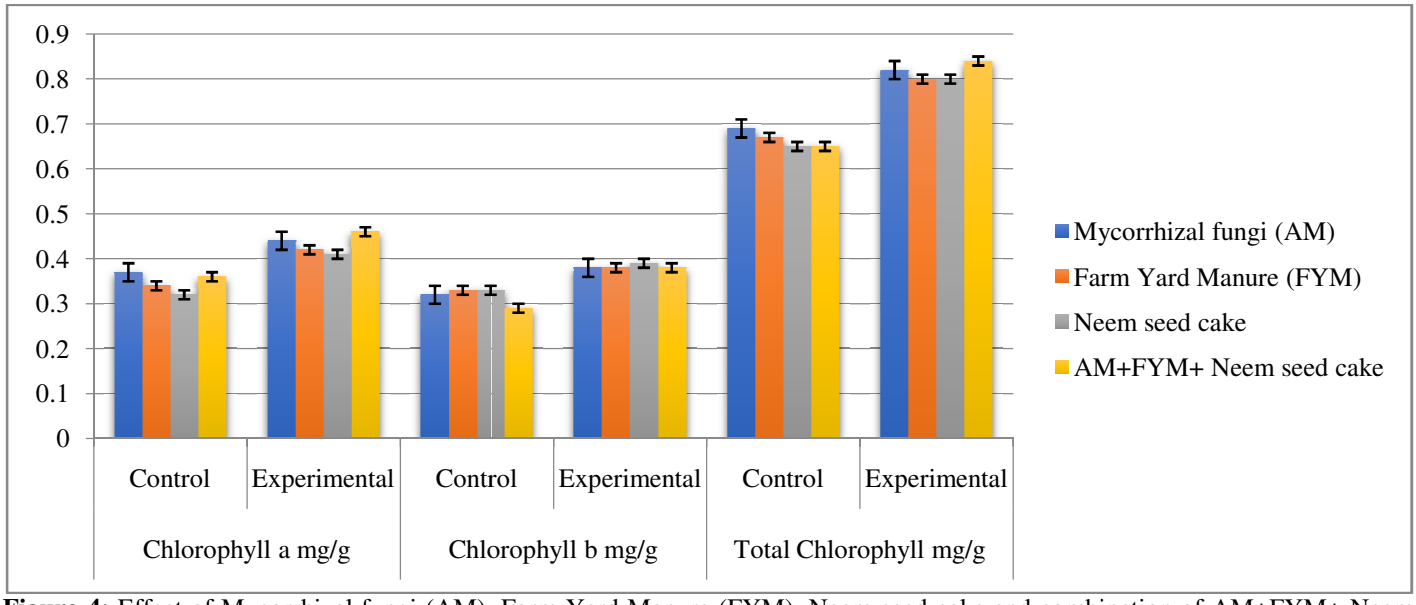

Figure-4: Effect of Mycorrhizal fungi (AM), Farm Yard Manure (FYM), Neem seed cake and combination of AM+FYM+ Neem seed cake on Chlorophyll content in *Trigonella.*

The average content of protein and reducing sugars in leaves was found improved under AM+FYM+Neem seed cake treatment when compared to control plants, Protein and reducing sugar content. Similar results were recorded by Gianinazzi-Pearson and Gianinazzi[17], Arines *et al.*[18].

Increased Vitamin C content in all experimental plants because of readily available mobilized nutrients in the rhizosphere soil of the plants. AM fungi made all nutrients available. FYM and Neem seed cake contains all major, minor and micro nutrients essential for plant growth. Due to their presence all these essential nutrients were available for the plat growth and this has resulted in increased vitamin C content in experimental plants as compared with control plants. Our results are match up with the result of Meena *et al.*[19]. Therefore, it can be concluded on the basis of present investigation that, AM fungi mobilize the soil nutrients and made them available to the plants roots. Ultimately it has positive effects over the increase in morphological parameters as well as biochemical contents.

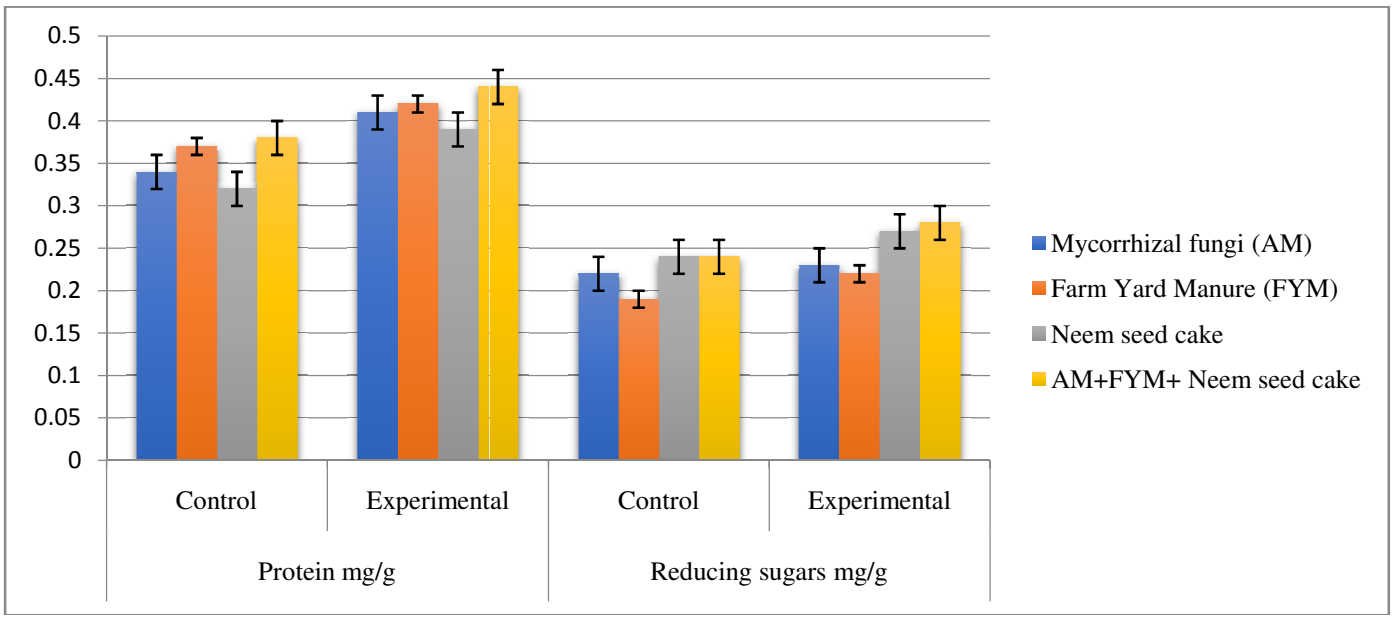

Figure-5: Effect of Mycorrhizal fungi (AM), Farm Yard Manure (FYM), Neem seed cake and combination of AM+FYM+ Neem seed cake on Protein and reducing sugar content in *Trigonella.*

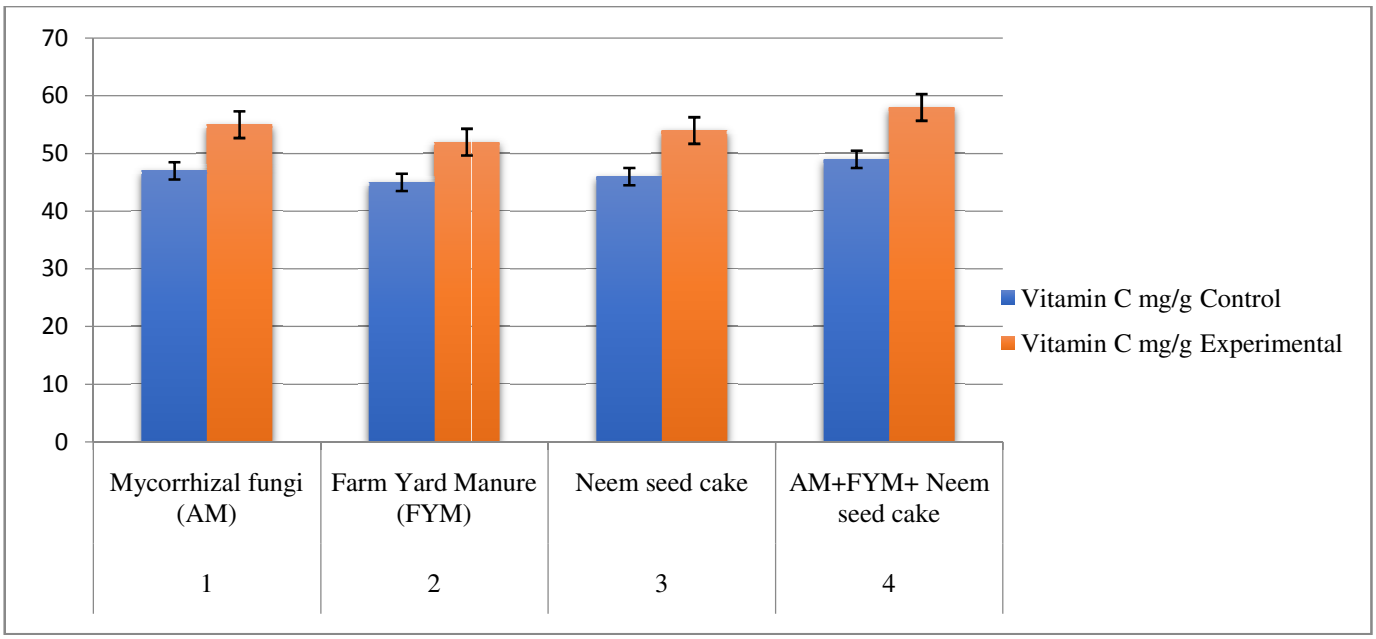

Figure-6: Effect of Mycorrhizal fungi (AM), Farm Yard Manure (FYM), Neem seed cake and combination of AM+FYM+Neem seed cake on Vitamin C content in *Trigonella.*

Conclusion

Root length, shoot length, surface area of leaves, number of branches and number of leaves in experimental plants was more as compared to controlled plants. Plants treated with combination of FYM+AM fungi+Neem seed cake showed best result as compared with other set of experiments. Fresh weight and dry weight was recorded highest in plants treated with combination of FYM+AM fungi+ Neem seed cake followed by neem seed cake only. Chlorophyll, protein, reducing sugars and Vitamin C content were more in all experimental plants than control plants. AM fungi, FYM and Neem seed cake also showed worthy results but the results were outstanding when applied in combination.

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