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Comparative studies on effect of am fungi, some organic fertilizers and chelated micronutrients on growth of *Coriandrum sativum* L.

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Abstract
Coriander (*Coriandrum sativum* L.), is annual herbaceous crop belonging to family Apiaceae is usually known as Coriander and is highly used as one of the oldest multipurpose medicinal and spice herbs. Plants of Coriandrum sativum are bushy green, medium sized and plants are small and sickle shaped. It is widely cultivated in India. Most of the farmers grow Coriandrum by using chemical fertilizers; which can have adverse effect on health of consumers of the same. Its use in daily diet is outstanding, almost every kitchen has its importance to spice up the food. Chemically grown Coriandrum can have adverse effect on health of human being. Hence it was necessary to study its growth parameters with respect to some organic fertilizers a pot culture experiment was carried out. In this experimentation the assessment was carried out with respect to effect of Mycorrhizal fungi, Vermicompost, Green Manure and Chelate on *Coriandrum sativum*. for growing *Coriandrum* 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 autoclave sterile garden soil and AM fungi in 9:1 ratio, control plants of all four sets filled only with only garden soil., the second set of all experimental pots were filled with mixture containing garden soil, AM fungi and GM in 8:1:1 ratio. The pots of the third set were filled Garden soil and Vermicompost and AM with 8:1:1 ratio. The fourth set filled with 3 kg garden soil and 6 grams of chelated micronutrients. All plants further assessed for morphological parameters and biochemical contents.

Keywords: AM fungi, vermicompost, green manure, chelate, growth

Introduction
It is a native of South eastern Europe and Western Asia and widely cultivated in India. It is commonly found growing in the Mediterranean region of the world. In India; it is an important versatile *rabbi* season spice crop mainly grown in Rajasthan, Gujarat, Madhya Pradesh Maharashtra and Haryana. Although it is a *rabbi* crop it is grown during all seasons all over India. From earliest times, Greeks and the Romans used it as a medicine, spice and cattle fodder and so it was and still known as Greek hay. It is also used to treat a variety of health problems in Egypt, Greece, Italy, and South Asia Acharya *et al.,* (2008) [1]. Seeds of Coriander seeds are used as a yellow dye, in cosmetics and for medicinal commitments.

Arbuscular mycorrhizal (AM) fungi have an extraordinary importance as they increase nutrient acquisition by the plant as well as resistance to biotic and abiotic stress, Barea and Jeffries (1995) [2] Barea *et al*., (2002a), [3] Barea *et al*., (2002b), [4], Barea *et al*., (2002c), [5] In fact, the symbiosis with AM fungi has been proposed as one of the mechanisms of heavy metal plant tolerance Hildebrandt et al., (2007) [6] and water stress avoidance Augé (2004) [7], Ruíz-Lozano and Azcón, (1996) [8], Ruíz-Lozano *et al.*,(1995) [9]. Nonetheless, the mycorrhizal component may disappear or, at least, be severely depleted in degraded soils, so it may be necessary to reinforce or replace it by appropriate inoculation Requena *et al.*, (1996) [10].

Use of vermicompost as an organic fertilizer and substitute for chemical fertilizer is advised by pioneers of organic farming. Earthworm processed organic waste often referred to as vermicompost are finely divided peat like material with high porosity, aeration, drain ability and water holding capacity. Vermicompost contains nutrients in the readily available form to the plants such as nitrate, exchangeable P, K, Ca and Mg Edwards, and S. Burrows (1988) [11]. It also contains biologically active substance such as plant growth regulators Tomatic *et al.,* (1987) [12]. The application of vermicompost not only add plant nutrients and growth regulators to the soil but also add to physical, chemical and biological properties of the soil. Its other effects are reduced soil erosion, deodourification of obnoxious smell, destruction of pathogens and detoxification of soil pollutant. It has also been reported to promote nodulation in legumes resulting in to higher production Tandon (1991) [13].

Green 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. Hence, by applying adequate FYM, especially in the soil of drought prone areas, water holding capacity of agricultural soil and soil fertility status can be increased and the productivity can be greatly sustained. Chelate deficiencies are major problem in the crop production in the present-day agricultural programs Mishra (2014) [14]. Micronutrient fertilizers are gaining importance day by day and would play a major role in bringing stability and sustainability in the production of food, spices crop, oil seeds in the coming decade. A Chelate described a kind of organic complexes, inorganic sources such as sulphates of Cu, Mn, Fe and Zn are the most

common metallic salts used in the fertilizer industry because of their readily availability to plants and water solubility. A chelate refers to a ring system those results when a metal ion combines with two or more electron donor groups of a single molecule. This is the characteristic feature of that makes this compound useful in agriculture. The plant availability of certain micronutrients fertilizers reduced by transformation of added micronutrient into forms that plants unable to absorb. Chelating agents play a key role increase productivity and yield of agricultural crops and also increase micronutrient deficiency Sekhon (2000) [15].

In present investigation the importance has given to evaluate the efficacy of different organic manures like AM fungi, Vermicompost, green manure and chelated micronutrients with respect to growth and biochemical parameters on (Coriandrum sativum L.), plants in pot culture 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 *Coriandrum sativum* L. Inoculants used for the treatments includes Mycorrhizal fungi (AM). Apart from Am fungi for other sets of experiments Green Manure, Vermicompost and Chelate respectively added. Coriandrum seeds were obtained from market. The plastic pots (20 x 20 x 30 cm) of 3 kg soil capacity were used for growing the Coriandrum 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, AM fungi and GM in 8:1:1 ratio. Garden soil and Vermicompost and AM was filled in third set with 8:1:1 ratio whereas forth set was filled with mixture of Garden soil with AM + GM + VC + Chelate in 7:1:1:1 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 3 kg accurately. Then well-imbibed 10 to 15 seeds grown in each control and experimental pots. The plants were grown in normal conditions for 20 days and then the fertilizers dose was given in each pot. The dose of fertilizers was given after 20 days to avoid further worries due to dose of fertilizers and get acclimatized with edaphic and environmental conditions.

About 200, healthy seeds were selected for the experiments. The seeds were surface sterilized with 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 grown 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 A, Vitamin C and Phenols.

Morphological parameters measured by following procedure. 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 number of leaves counted and recorded simply by counting the leaves of control and experimental plants. An average fresh weight of the *Coriandum* 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 50 ºC for 5 days and then measured in grams.

Biochemical contents analysed by following procedure.

Chlorophyll Content: Amount of Chlorophyll a; chlorophyll b and total chlorophyll were determined by Arnon (1949) [16]. Chlorophyll extract was prepared from fresh leaves of Coriandrum (1g) by grinding in a mortar and pestle, together with 10 ml of ice cold 80% acetone. The homogenate was centrifuged at 3000 rpm for 2 minutes. The supernatant was saved and pellet was re extracted twice with 5 ml of 80% acetone. All the supernatants were pooled and saved. The absorbance of the extract was recorded at 663 nm, 645 nm and the concentration of chlorophyll a, chlorophyll b and total chlorophyll was calculated using Arnon's equations as follows.

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

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

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

Proteins: Estimation and quantification of proteins were done by Lowry *et al.*, (1951) [17] method. The fresh leaves of Coriandrum from control and experimental plants were cut into small pieces separately and 0.5 g plant material was extracted with 5 ml of 0.1 M phosphate buffer (pH 7.0). The extract was centrifuged at 10,000 rpm for 15 min. The supernatant was discarded and the pellet was dissolved in 2 ml of 1.0 N NaOH solution. This was used as a sample and 0.2 ml was taken for the estimation of proteins. The working standard of BSA and plant extract was taken in a series of test tubes and final volume was adjusted to 1 mL in each tube. Then 5 mL of reagent C was added in all the tubes and incubated the mixture for 10 min. This was followed by addition of 0.5 mL of folinciocalteau and incubated at dark for 30 min. The blue colour developed in the reaction mixture was read at 660 nm on UV-visible spectrophotometer. Bovine serum albumin fraction V (BSA) was used at the concentration of 50 mg and dissolved in distilled water and used as a standard protein to prepare the standard graph. The amount of protein was calculated with the help of standard graph.

Vitamin A & C: Vitamin C content of from the leaves of Coriandrum was estimated following the method of Birch *et al.*, (1933) [18]. Initially working standard and stock standard solutions was prepared. The five ml of working standard was taken into a conical flask; in this 10 ml of 4% oxalic acid was added. This solution was titrated against dye (42 g Na_2 CO_3 dissolved in 52 mg of 2-6-dichloro phenol indophenols and final volume raised up to 200 mL 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 100 mL. Then the 5 mL of the final mixture was added with 10 mL of 4% oxalic acid and titrated against the dye and the amount of vitamin A and C was determined by presence or absence of Reddish-brown colour.

Total phenolics: The total phenolics were estimated by using Dinitrosalicylic acid (DNSA) reagent Miller (1972) [19]. Weighed 1 g leaves of *Coriandrum* and extracted the sugars with hot 80% ethanol twice (5 ml 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 1.0 ml and centrifuged at 10,000 rpm for 10 minutes. The final volume of supernatant was adjusted to 10 ml with distilled water and dissolved the sugars. Pipette out 0.2 ml 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 this Red rose colour observed for studying presence or absence of Phenolic compounds.

Results and Discussion

The pot experimental results of different treatments in *Coriandrum sativum* L. revealed different significant responses of yield attributes *viz.* shoot length, number of leaves, fresh weight and dry weight. Among the different experimental sets with treatment of Mycorrhizal fungi (AM), Green manure (GM), Vermicompost and combination of AM+GM+VC+Chelate were found to be superior and effective in increasing growth parameters over control and other treatments respectively. Root length in experimental plants was more as compared to controlled plants. Root length was 3.5cms in Mycorrhizal plants. It was highest i.e. 4.2 cms in plants treated with GM + AM. Whereas it was 3.8 cms for the experimental plants treated with Green manure & Vermicompost. There was 1.6cms increase in root length in plants treated with AM. The trend showed that plants treated with AM+GM, AM+VC, AM+Chelate as increased root length in all experimental plants. In all experimental plants shoot length was more than control plants. It was 19.5 cms in Mycorrhizal plants, 20.8 cms GM, 20.9 cms in 21.2 and 22.5 cms in AM + Green manure + Vermicompost treated plants. There was highest increase in shoot length (1.4cms) recorded for plants treated with AM+GM, AM+VC, AM+Chelate. 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. Table 1.

Table 1: Effect of Mycorrhizal Fungi (AM), AM +Green Manure (GM), Vermicompost + AM and Combination of AM + GM + VC + Chelate on Root Length and Shoot Length and Surface Area of *Coriander* Leaves.

Sr. No.	Treatment	Root length (cms)		Shoot length (cms)	
		Control	Experimental	Control	Experimental
1.	Mycorrhizal fungi (AM)	3.5	4.2	19.5	20.6
2.	Green manure + AM	3.8	4.0	20.8	21.4
3.	Vermicompost + AM	4.4	4.9	20.9	22.3
4.	AM+GM+VC+Chelate	4.5	5.2	21.2	22.5

The number of branches in experimental plants were significantly different than controlled plants. It was 6.8 in Mycorrhizal plants, 6.3 in AM+GM, 6.2 in AM+VC and 7.2 in treated plant AM + GM + VC + Chelate plants. There was highest increased number of branches (7.8) recorded in plants treated with AM + GM + VC + Chelate. The number of leaves was 70.5 and 73.8 in Mycorrhizal controlled and experimental plants respectively, it was 71.2 and 78.2 in GM. 71.4 and 76.3 cms in Vermicompost and 72.5 and 80.4 in treated plants. There was AM + GM + VC + Chelate highest difference in number of leaves recorded (11.1) for plants treated with AM + GM + VC + Chelate. The number of branches in experimental plants were significantly different than controlled plants. It was 6.8in Mycorrhizal plants, 6.3 in AM+GM, 6.2 in AM+VC and 7.2 in treated plant AM + GM + VC + Chelate plants. There was highest increased number of branches (7.8) recorded in plants treated with AM + GM + VC + Chelate.

The number of leaves was 70.5 and 73.8 in Mycorrhizal controlled and experimental plants respectively, it was 71.2 and 78.2 in GM. 71.4 and 76.3 cms in Vermicompost and 72.5 and 80.4 in treated plants. There was AM + GM + VC + Chelate highest difference in number of leaves recorded (11.1) for plants treated AM + GM + VC + Chelate. 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. More absorption increased the roots and shoot length and surface area of leaves which resulted in to increase in absortion and photosynthetic efficiency. Thes leads to increased number of branches, number of leaves. Our results corroborate with Purbey and Sen (2007) [20], Khiriya and Singh (2003) [21], Aishwath *et al.*, (2011) [22]. Table 2.

Table 2: Effect of Mycorrhizal Fungi (AM), AM +Green Manure (GM), Vermicompost + AM and Combination of AM + GM + VC + Chelate on Number of Branches, and Leaves of *Coriandrum*.

Sr. No.	Treatment	Number of branches (AVG)		Number of leaves (AVG)	
		Control	Experimental	Control	Experimental
1.	Mycorrhizal fungi (AM)	6.8	7.1	70.5	73.8
2.	AM+ Green Manure	6.3	7.3	71.2	78.2
3.	AM+Vermicompost	6.2	7.6	71.4	76.3
4.	AM+GM+VC+ Chelate	7.2	8.1	72.5	80.5

Fresh weight in all controlled plants was comparatively less than in experimental plants. It was 13.9 and 14.4 g for Mycorrhizal controlled and experimental plants respectively. Whereas, it was 14.3 g and 16.1 g in GM, 14.1g and 17.6 g for GM, 16.8 g and 17.9 g for AM + GM + VC + Chelate. Fresh weight and dry weight showed same trend in plants treated with AM + GM + VC + Chelate. Fresh and dry weight was more in all experimental plants than controlled plants. Fresh weight in all controlled plants was comparatively less than in experimental plants. It was

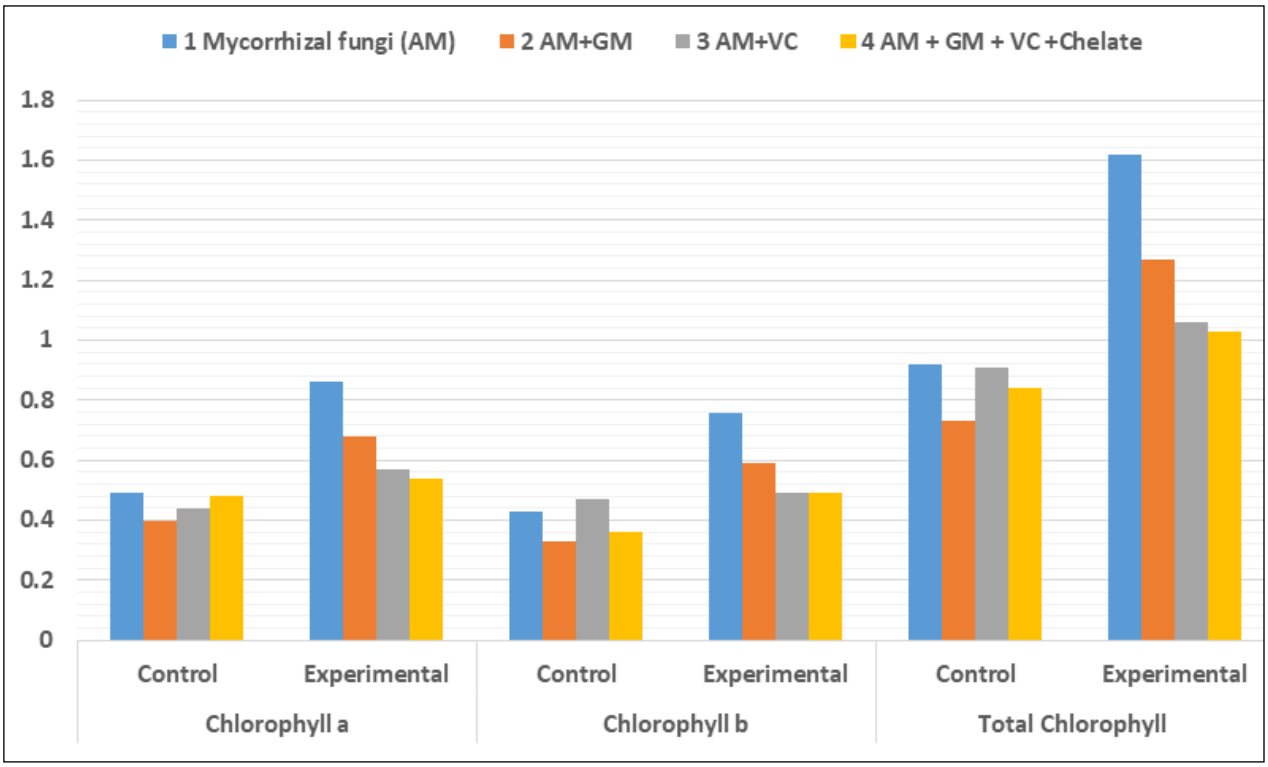
13.9 and 14.4 g for Mycorrhizal controlled and experimental plants respectively. Whereas, it was 14.3 g and 16.1 g in GM, 14.1g and 17.6 g for GM, 16.8 g and 17.9 g for AM + GM + VC + Chelate. Fresh weight and dry weight showed same trend in plants treated with AM + GM + VC + Chelate. Fresh and dry weight was more in all experimental plants than controlled plants. The fresh weight in all controlled plants was comparatively less than inexperimental 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 *et al.*, (2009) [23], Sunitha *et al.*, (2010) [24]. Table 3.

Table 3: Effect of Mycorrhizal Fungi (AM), AM +Green Manure (GM), Vermicompost + AM and Combination of AM + GM + VC + Chelate on Fresh Weight and Dry weight of *Coriandrum.*

Sr. No.	Treatment	Fresh weight (gm)		Dry weight (gm)	
		Control	Experimental	Control	Experimental
1.	Mycorrhizal fungi (AM)	13.9	14.4	1.1	1.2
2.	AM+ Green manure	14.3	16.1	1.2	1.3
3.	AM+ Vermicompost	14.1	17.6	1.2	1.4
4.	AM+GM+VC+ Chelate	16.8	17.9	1.3	1.6

Chlorophyll a was recorded lowest in 0.49 mg/g in controlled plants of Green manure while it was highest 0.86 mg/g in plants treated with AM + GM + VC + Chelate. On the other hand, chlorophyll b was recorded least 0.47 mg/g and maximum in control and experimental plants treated with Total chlorophyll was highest 0.91 mg/g in plants treated with plants treated with AM + GM + VC + Chelate. Amount of Chlorophyll a, b and total chlorophylls was recorded maximum experimental plants treated with AM+GM. This is due to the beneficial effect of AM+Chelate 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 Khiriya *et al.*, (2001) [25], Jat *et al.*, (2006) [26]. Fig. 1.

Fig 1: Effect of Mycorrhizal fungi (AM), AM +Green Manure (GM), Vermicompost + AM and combination of AM + GM + VC + Chelate on Chlorophyll content in *Coriandum.*



The protein content in all experimental plants was considerably more than control plants. It was lowest 0.32g in control neem Vermicompost plants and highest 0.44 mg/g in experimental plants in Chelate. Amount of protein in leaves was found improved under AM+FYM+ Neem seed cake treatment when compared to control plants, Protein content. Similar results were recorded by Gianinazzi-Pearson and Gianinazzi (1995) [27], Arines *et al.*, (1993) [28]. Fig. 2.

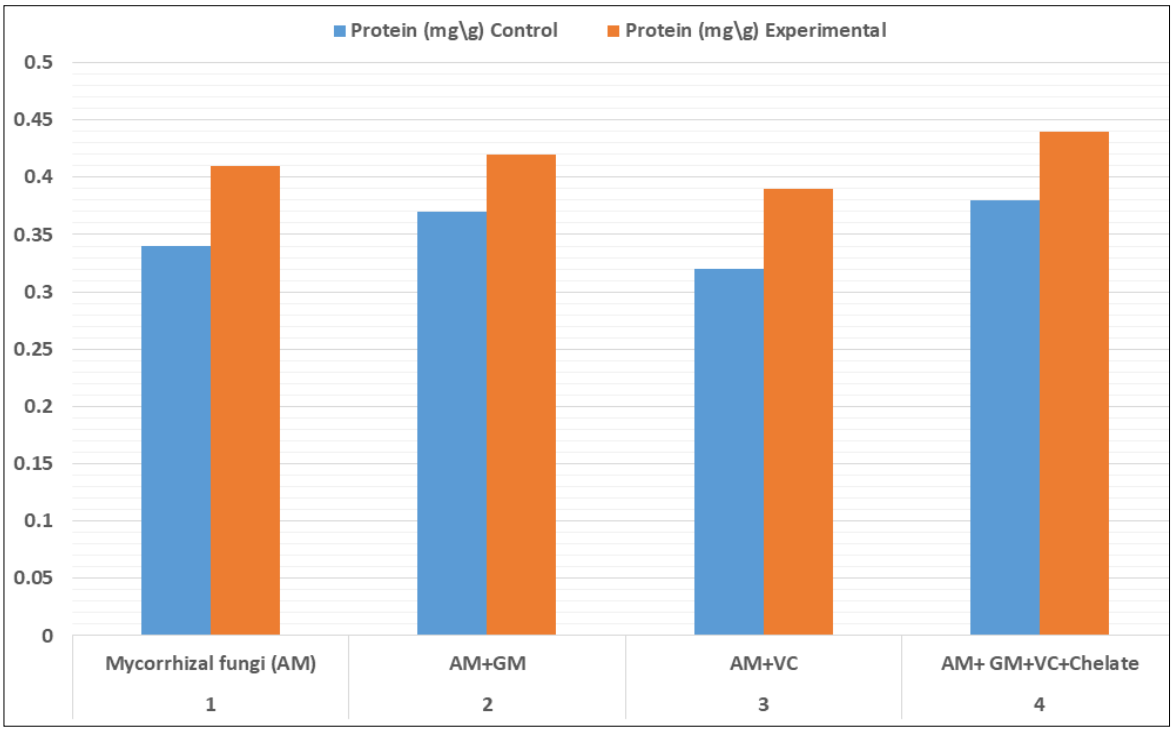


Fig 2: Effect of Mycorrhizal fungi (AM), AM +Green Manure (GM), Vermicompost + AM and combination of AM + GM + VC + Chelate on Protein content in Coriandum

Qualitative tests for vitamin A and C was carried out for all control and experimental plants. Both vitamins A and C were present in both controlled and experimental plants. The results are presented in Table 4. Increased Vitamin A and C content in all experimental plants because of readily available mobilised nutrients in the rhizosphere soil of the plants. AM fungi made all nutrients available. GM and Vermicompost 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 & A content in experimental plants as compared with control plants.

Table 4: Effect of Mycorrhizal fungi (AM), AM +Green Manure (GM), Vermicompost + AM and combination of AM + GM + VC + Chelate on Vitamin A and C content in *Coriandrum.*

Sr. No.	Treatment	Vitamin A (mg/g)		Vitamin C (mg\g)	
		Control	Experimental	Control	Experimental
1.	Mycorrhizal fungi (AM)	++	++	++	++
2.	Green Manure (GM) + AM	++	++	++	++
3.	Vermicompost + AM	++	++	++	+ +
4.	AM + GM + VC + Chelate	++	++	++	++

Conclusions

Effect of Mycorrhizal fungi (AM), AM +Green Manure (GM), Vermicompost + AM and combination of AM + GM + VC + Chelate has positive effect on root length, shoot length, number of branches and number of leaves of Coriandum. Fresh weight and dry weight of *Coriandum* plants complemented with Mycorrhizal fungi (AM), AM +Green Manure (GM), Vermicompost + AM and combination of AM + GM + VC + Chelate showed better results. Biochemical content like Chlorophyll, proteins, vitamin A and C were improved by AM + GM + VC + Chelate. Among organic fertilizers combination of AM fungi and green manure has highest effect overall growth and biochemical parameters. AM fungi and vermicompost nearly has equal effects over growth parameters. AM fungi mobilized the soil nutrients and made them available to the plants roots. Vermicompost and green manure has high nutrient content so there was increased yield. AM alone showed little less efficient results as these fungi are nutrient mobilizers. Effect of organic fertilizers is best in presence of AM fungi only so it must be used in combination with other organic fertilizers. It is suggested to the farmers to use AM fungi along with other organic fertilizers to escalate the production and yield

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