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Studies on influence of AM fungi and some organic fertilizers on growth and biochemical content in *Allium cepa* L

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Abstract
Onion (*Allium cepa* L.) is an annual herb belongs to family Liliaceae. It is the most widely cultivated species of the genus *Allium*. In present investigation onion plants are evaluated for different growth and biochemical parameters like Chlorophyll content, Protein content, N, P and K content were evaluated. Evaluation of these parameters done using Onion plants with application of Arbuscular mycorrhizal (AM) fungi, Farm Yard Manure (FYM), Neem seed cake and combination of AM, FYM and neem seed cake etc. The research was conducted at the department of Botany, Nowrosjee Wadia College, Pune. The experimentation was done with three replicated randomized design for each treatment. Three control and three replicates were used for each set of experiment. The plastic pots (20 x20x 30 cm) of about 5 kg soil capacity were used for growing the *Allium cepa* L. seeds.

Keywords: onion, AM fungi, FYM, neem seed cake, growth, chlorophyll, protein

1. Introduction

The geographic origin of the onion is uncertain because the wild onion is extinct and ancient records of using onions span western and eastern Asia, Cumo, 2015 [1]. Food uses of onions date back thousands of years in China, Egypt and Persia. Onions were taken by the first European settlers to North America, where the Native Americans were already eating wild onions raw or cooked in various foods, Cumo, 2015 [1]. According to diaries kept by the colonists, bulb onions were one of the first crops planted by the Pilgrim Fathers. Onion has its own distinctive flavour and is used in soups, meat dishes, salads, and Sandwiches, and is cooked alone as a vegetable. Its pungency is due to the presence of a volatile oil (allyl propyl disulphide); Malik, 1994 [2]. It is a high value vegetable crop for its popularity in many spicy dishes use as matured bulbs or as green vegetables, when harvested earlier, Barzegar *et al*., 2008 [3], Mahantesh *et al*., 2008[4] and has medicinal uses; Corzo *et al*., 2007 [5]. The crop is cultivated in the semi-arid northern region of the country, where the soil is characterized with poor nutrients concentration and low organic matter, Amans *et al*., 1996 [6]. Onions have antibiotic, antiseptic, antimicrobial and carminative properties to help you stay away from infections. Onions are rich in sulphur, fibres, potassium, calcium, vitamin B, vitamin C and they are low in fat, cholesterol and sodium. It is an immediate cure for fever, common cold, cough, sore throat, allergies, etc. A mixture of onion juice and honey can cure these problems easily. A small piece of onion can work against side effects of fever if it is kept on the forehead. A small piece of onion, when inhaled, can stop or slow down the bleeding through the nose.

The term Mycorrhiza was coined from two Greek words "Myco" meaning fungus and "Rhiza" meaning root. Initially Frank, 1885 [7] gave the name "Mycorhiza" to the peculiar association between tree roots and ectomycorrhizal fungi. In this name second 'r' is introduced by Kelly, 1931 [8] and thus the name became Mycorrhiza. Mycorrhizal (AM) fungi form symbiotic relationships with plants roots. The fungus grows into the root and out into the soil. The plant responds by altering its physiology in a number of ways. The fungus in the soil absorbs water and nutrients from the soil and conducts them back to the root. The mycorrhizal plants are often more resistant to diseases, especially those diseases caused by pathogens in the soil. Mycorrhizal fungi are important because they frequently increase plant nutrient uptake, uptake of water, phosphorus and nitrogen to help plant growth. Root colonization with arbuscular mycorrhizal fungi (AMF) have enhanced the uptake of nutrients, especially, P, N, and other nutrients and improve plant growth, Smith and Read, 1997 [9], Gerdemann, 1963 [10]. It also reduced an amount of fertilizer required by plant Miyasaka *et al*., 2003 [11], Robson *et al*., 1981 [12], Jubort and Archer, 2000 [13] and reclaim degraded soil. The interactions of onion with AMF under field conditions were well documented by Haymann and Mossae, 1971 [14], Mossae and Haymann, 1971 [15], Mossae 1973 [16]. The fungi form a symbiotic association with host plant thereby improving the plants growth through acquisition of soil nutrients via their extramatrical hyphae. Other benefits of AMF for sustainable crop production are, resistance to environmental stress and biological control of root pathogens, Gianinazzi and Vosátka, 2004 [17], Vosátka and Albrechtov, 2008 [18]. Furthermore, complementary effect of AM fungi as an alternative for reducing fertilizer need of major crop species were reported by Mossae, 1981 [19], Lidermann and Davis, 2004 [20]. Farmyard manure is decomposed mixture of dung and urine of farm animals along with litter and left-over material from roughages or fodder fed to the cattle. On an average well decomposed farmyard manure contains 0.5 % N, 0.2 % $P_2O_5$and .0.5 % K_2O. They supply plant nutrients including micronutrients. It improves physical properties of soil like structure, water holding capacity etc., they increase the availability of nutrients e.g. Carbon dioxide released during decomposition acts as a CO_2 fertilizer. FYM provides all the nutrients that are required by plants but in

limited quantities. It helps in maintaining C:N ratio in the soil and also increases the fertility and productivity of the soil, Kirshnan, 2005 [21]. It improves the physical, chemical and biological properties of the soil. It improves both the structure and texture of the soils. It increases the water holding capacity of the soil. Due to increase in the biological activity, the nutrients that are in the lower depths are made available to the plants. It acts as much, thereby minimizing the evaporation losses of moisture from the soil. Neem, *Azadirachta indica* L. is native to the arid regions of the Indian sub-continent, where it grows to 12-24 m high at altitudes between 50 and 100 m with 130 mm of sufficient rainfall per annum for its normal growth. In India, neem is known for its use and is more utilized in rice cultivation. Fresh fruit yield per neem tree ranges between 37 and 50 kg per year. Forty kg fruit yields nearly 24 kg of dry fruit (60%), which in turn gives 11.52 kg of pulp (48%), 1.1 kg of seed coat (4.5%), 1 kg of husk (25%) and 5.5 kg of kernel (23%). The kernel gives about 2.5 kg of neem oil (45%) and 3.0 kg of neem cake (55%). There has been an evident shift all over the world from synthetic pesticides to non-synthetic ones; this is largely because of the wide spread awareness of the side effects of these synthetic pesticides not only on plants and soil but also on other living organisms, Subbalakshmi, 2012, [22]. Neem seed cake is used as manure, fertilizer, soil conditioner, coating agent, fumigant, pesticide and insecticide, Subbalakshmi, 2012, [22]. Neem seed cake performs the dual function of both fertilizer and pesticide, acts as a soil enricher, reduces the growth of soil pest and bacteria, provides macro nutrients essential for all plant growth, helps to increase the yield of plants in the long run, bio degradable and Eco friendly and excellent soil conditioner.

In present investigation emphasis is given to study the effect of organic fertilizers on growth of Onion. An effect of AM Fungi, Farm Yard Manure, Neem seed cake and combination of all these on growth and biochemical parameters.

2. Materials and Methods

The research was conducted at the department of Botany, Nowrosjee Wadia College, Pune. The experimentation was done with three replicated randomized design for each treatment. The plants used for experiment was Allium cepa L. fertilizers were used for the treatments included Mycorrhizal fungi (AM), Farmyard Manure (FYM), Neem seed cake and combination of AM+FYM+ Neem seed cake. *Allium cepa* L. seeds were obtained from seed vendor. The plastic pots (20x20x 30 cm) of about 5 kg soil capacity were used for growing the Onion seeds. Four sets were used for the experimentation. Each set of four included three control and three replicates. In the first set of three experimental pots were filled with mixture containing garden soil and AM fungi in 9:1 ratio and control without AM fungi. The soil with AM fungi included Propagules of *Acaulospora appendiculata*, *A. gerdemanni*, *Glomus convolutum*, *G. fasciculatum* and *Scutellospora calospora*. In all sets of experimentation only experimental pots were filled with respected organic fertilizers while control pots were filled only with garden soil. The second set of 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, fourth set was filled with mixture of garden soil with AM + FYM + Neem seed cake in 9:1 ratio.

At the beginning, garden soil used for experimentation was autoclaved at 115 lbs pressure for 30 minutes. The autoclave sterile soil then added in sterilized pots by weighing 4.5 kg accurately. The onion seeds were already grown in Petri plate were sprouted forming a radical. These sprouted two 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.

Seeds of Bhima super cultivar were selected for the experiments. The seeds were surface sterilized with 0.1% $HgCl_2$, washed thoroughly with sterilized distilled water and then soaked in distilled water for 3 hours. Two 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, leaf diameter, number of scale leaves, etc. Each set was further analysed for biochemical contents like chlorophyll and protein

Morphological Parameters were assessed by following procedure. Root length: 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. Shoot length: The shoot length similarly measured and counted with the help of thread and recorded. Number of leaves: The number of leaves counted and recorded simply by counting the leaves of control and experimental plants. Number of scale leaves: The number of scale leaves counted and recorded simply by counting the leaves of control and experimental plants. Diameter of leaves: The diameter of both control and experimental plants was measured with the help of thread and recorded. Diameter of bulbs: The diameter of bulbs of both control and experimental plants was measured with the help of thread and recorded. Fresh weight: An average fresh weight of the experimental and control plants was measured by using weighing balance in grams. Dry weight: The dry weight of control and experimental plants was measured by drying the leaves in hot air oven at 50º C. 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, [23]. 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, [24] 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.

Analysis of N, P and K content: In dried samples of onion bulb tissues N, P, K elements were determined according the methods described by Pregl, 1945, [25], Traugh and Mayer 1939 [26] and Brown and Lilleland 1946, [27] respectively. This analysis was carried out in Zuari agrochemical laboratory Pune at the end of season of onion plants i.e. on 105th day. Analysis of these major nutrients was done from bulbs.

3. Results and Discussion

By using the pot experimental design results of various fertilizers treatments on Allium cepa L. revealed different notable effect on yield and other parameters like root length, shoot length, number of leaves, number of scale leaves, diameter of leaves, diameter of bulb, fresh weight and dry weight. Among the different experimental sets with treatment of Mycorrhizal fungi (AM), Farm Yard Manure (FYM), Neem seed cake and combination of AM+FYM+ Neem seed cake were found to be better and efficient in escalating growth parameters over control and other treatments respectively. Table 1.

Table 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 Onion leaves.

Sr. No.	Treatment	Root length (cms)		Shoot length (cms)	
		Control	Experimental	Control	Experimental
1	Mycorrhizal fungi (AM)	2.7	3.1	26.3	31.3
2	Farm Yard Manure (FYM)	2.5	3.1	28.1	32.2
3	Neem seed cake	2.3	2.9	27.6	32.6
4	AM+FYM+ Neem seed cake	2.4	3.2	26.2	34.3

Root length was ranging from 2.3 cms to 2.7 cms in control plants and 2.9 cms to 3.2 cms in experimental plants. It was maximum (3.2cms) in the plants added with AM+FYM+ Neem seed cake. There was more root length of all experimental plants than control plants. Similar trend observed with respect to shoot length. It was ranging from 26.2 cms to 28.1 cms in control plants. On the other hand, in experimental plants it was ranging from 31.3 cms to 34.3 cms. It was highest in the plants added with AM+FYM+ Neem seed cake (Table 1). These elevated results were due to availability of essential nutrients in FM, Neem seed cake and combination of AM+FYM+ Neem seed cake. In first and fourth set of experiment there is presence of AM fungi which have mobilised soil nutrients and made it available for the plants roots so that this trend was observed. Similar result was found also by Rather *et al*., 2003[28], Sharma *et al*., 2003 [29], Kumar and Chillar, 2001 [30], Dixit, 1997 [31], Mallanagouda, 1995 [32].

Number of leaves observed was 7 and 8 in all control plants whereas, in experimental plants it was 8 to 9. It was 7 and 9 respectively in control and experimental plants treated with AM fungi as well as AM+ FYM + Neem seed cake (Table No.2). The storage leaves are nothing but scales leaves of onion. In control plants they were ranging between 11 and 14 and 12 to 16 in experimental plants. The number of scale leaves were more in all experimental plants than control plants. Maximum difference as seen in plants treated with combination of AM+FYM+ Neem seed cake. Organic manures activate many species of living organisms which release phytohormones and may stimulate the plant growth and absorption of nutrients Arisha *et al*., 2003 [33] such organisms need nitrogen for multiplication. Similar result was also reported by Sharma *et al*., 2003 [29] and Rumpel, 1998 [34]. They found that animal manure applications increased onion yield (Table 2).

Table 2: Effect of Mycorrhizal fungi (AM), Farm Yard Manure (FYM), Neem seed cake and combination of AM+FYM+ Neem seed cake on number of leaves and number of scale leaves of Onion.

Sr. No.	Treatment	Number of leaves		Number of scale leaves	
		Control	Experimental	Control	Experimental
1	Mycorrhizal fungi (AM)	7	9	11	12
2	Farm Yard Manure (FYM)	7	8	12	14
3	Neem seed cake	8	9	14	15
4	AM+FYM+ Neem seed cake	7	9	14	16

Diameter of leaves and bulb recorded more in experimental plants than control pants. In experimental plants diameter of leaf and bulb was (2.1 and 4.8 cms respectively) highest in experimental plants treated with combination of AM+FYM+ Neem seed cake for AM fungi, FYM and neem seed cake also the effect was positive in experimental plants than control plants. It is assumed that AM fungi have the potential to reduce the high application rate of fertilizer needed to produce high onion yield [10]. Moreover, onion plant benefits positively to AM symbiosis DeMello, 2003[35], Stribley, 1990[36], Plenchette *et al*., 1983[37] (Table 3).

Table 3: Effect of Mycorrhizal fungi (AM), Farm Yard Manure (FYM), Neem seed cake and combination of AM+FYM+ Neem seed cake on diameter of leaves and diameter of bulb of Onion.

Sr. No.	Treatment	Diameter of leaf		Diameter of bulb	
		Control	Experimental	Control	Experimental
1	Mycorrhizal fungi (AM)	1.6	2	4.3	4.6
2	Farm Yard Manure (FYM)	1.7	2	4.2	4.4
3	Neem seed cake	1.6	1.9	4.2	4.5
4	AM+FYM+ Neem seed cake	1.8	2.1	4.3	4.8

The fresh weight of onion bulbs was 56 g in control and 63 g in experimental showing 7 g more weight than control plant in AM treated plants. In plants treated with FYM showed 5 g more weight. Similar trend was observed in case of plants treated with neem seed cake showing 8 g more fresh weight of bulb.

Table 4: 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 Onion.

Sr. No.	Treatment	Fresh weight		Dry weight	
		Control	Experimental	Control	Experimental
1	Mycorrhizal fungi (AM)	56	63	4.3	4.4
2	Farm Yard Manure (FYM)	64	69	4.4	4.6
3	Neem seed cake	63	71	4.4	4.6
4	AM+FYM+ Neem seed cake	61	73	4.2	4.7

In experimental plants treated with combination of AM+FYM+ Neem seed cake sowed 12 g more fresh weight than control plants. Overall, there was increased fresh weight of onion bulbs in experimental plants as compared with control plants this might be due to availability of more nutrients due to presence of AM fungi. Actually, AM fungi mobilized nutrients in the rhizosphere of Onion and made it available for roots. Similar findings with those obtained by Olalla *et al*., 2004 [38] (Table 4).

Chlorophyll a, b and total chlorophylls were noticeably more than that of control plants. Chlorophyll a was ranging between. 36 mg/g to 42 mg/g. in all control plants. On the other hand, it was 0.43 to 0.46 mg/g in experimental plants (Figure 1). Similar drift was recorded with respect to chlorophyll b and total chlorophylls. Healthy plants generally contain more chlorophyll as they are without any disease and full with nutrient supply due to AM fungi (Graph 1). Arbuscular mycorrhizal symbiosis increased the rate of photosynthesis, and so as to increase the rates of photosynthetic storage and export at the same time, Auge, 2001 [39]. It has been proved that the amount of chlorophyll in mycorrhizal plants was higher than non-mycorrhizal plants, Gemma *et al*., 1997 [40], Davies *et al*., [41], Mathur and Vyas, 1995 [42] and higher concentration of chlorophyll is associated with higher photosynthesis rate, Davies *et al*., [41]. Different VAM fungi have different effects on photosynthesis in the condition of drought stress, Dixon *et al* 1994 [43], Bhosale and Shinde, 2011 [44] obtained similar results.

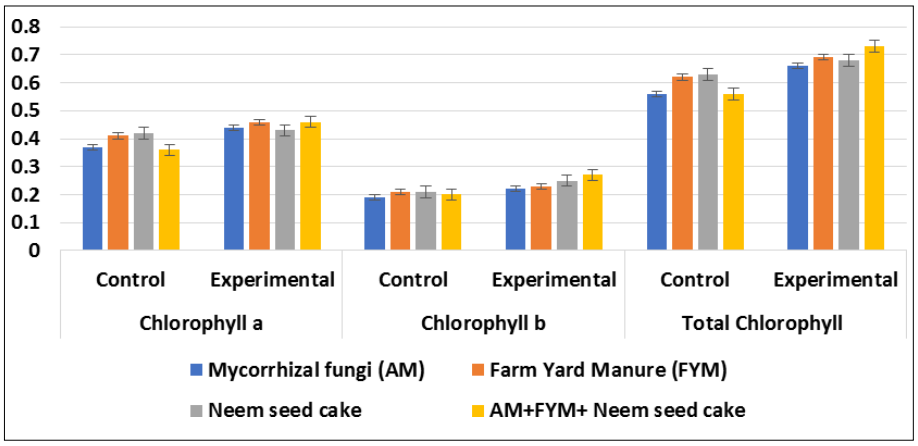

Fig 1: Effect of Mycorrhizal fungi (AM), Farm Yard Manure (FYM), Neem seed cake and combination of AM+FYM+ Neem seed cake on Chlorophyll content in Onion.

Protein content in experimental plants were less as compared with control plants in plants treated with Am fungi and those treated with AM+FYM+ Neem seed cake. This might be due to presence of AM fungi protein might have been utilised and hence recorded less as compared with control plants. On the other hand, plants treated with FYM and neem seed cake showed more protein in experimental plants than control plants (Figure 2). This might be due to presence of AM fungi protein might have been utilised and hence recorded less as compared with control plants. On the other hand, plants treated with FYM and neem seed cake showed more protein in experimental plants than control plants. These findings were in agreement with those of Ruiz Lozano *et al*., 1996 [45], Subramanian and Charest 1995[46] and Wu *et al*., 2006 [47].

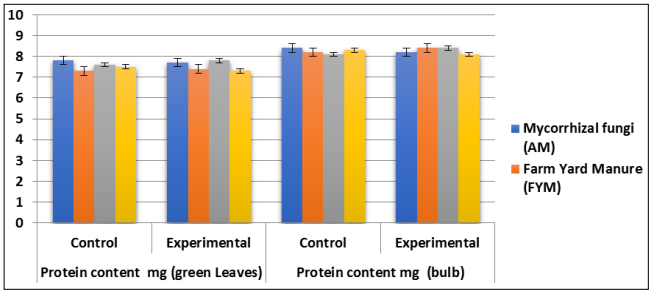

Fig 2: Effect of Mycorrhizal fungi (AM), Farm Yard Manure (FYM), Neem seed cake and combination of AM+FYM+ Neem seed cake on Protein content in bulb and leaves of Onion.

Major nutrients like N, P and K were recorded more in all experimental plants than control plants. The obtained results are in good accordance with that reported by, Thalooth *et al*., 2006 [48], Fatma and Shafeek 2000 [49] and Fawzy *et al*., 2010 [50].

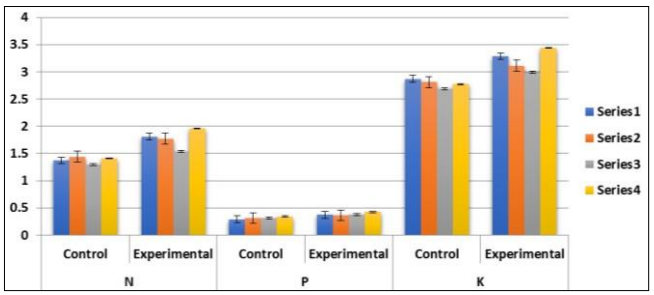

Fig 3: Effect of Mycorrhizal fungi (AM), Farm Yard Manure (FYM), Neem seed cake and combination of AM+FYM+ Neem seed cake on N, P and K content in Onion.

4. Conclusions

There was positive effect of AM fungi, Neem seed cake and Farm Yard Manure (FYM) has positive effect on root length, shoot length of Onion plants. Number of leaves and scale leaves were significantly more in experimental plants treated with fungi and Neem seed cake and FYM. Biochemical content like Chlorophyll, proteins, were enhanced by AM fungi, Neem seed cake and FYM. Effects of all organic fertilizers alone were positive on growth and biochemical content. Among all organic fertilizers FYM alone has best results over AM fungi and Neem seed cake. AM fungi can mobilize the soil nutrients and make them available to the plants roots hence AM treated plants were superior than non-treated plants. Neem seed cake and FYM has high nutrient content so there was increased yield. Effect of organic fertilizers is best in presence of AM fungi on N, P and K content in onion were higher than control plants. Thus, farmers can use AM fungi along with other organic fertilizers to increase the yield of onion crop.

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