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EFFECTS OF MYCORRHIZAE AS A SUBSTITUTE FOR INORGANIC FERTILIZER ON GROWTH AND YIELD OF TOMATO (*LYCOPERSICON ESCULENTUM* L.) AND SOY-BEAN (*GLYCINE MAX* L.), AND SOIL MICROBIAL ACTIVITY

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ABSTRACT

A greenhouse experiment was conducted to determine the influence of mycorrhizae as a substitute for inorganic fertilizer on growth and yield of Tomato (*Lycopersicon esculentum*) and Soybean (*Glycine max*) and soil microbial activity. Four doses of inorganic fertilizers viz recommended dose [Tomato- Urea:TSP:MOP=65:325:65 kg/ha and Soybean Urea:TSP:MOP=35:130:35 kg/ha), 1/2 and 1/4 of recommended dose and 0 dose] were applied to soil with standard dose of mycorrhizae (2 g/5L water). The above four treatments were tested in a Completely Randomized Design (CRD) with five replicates.

Results indicated that there was no significant difference in all parameters tested on plants treated with recommended dose and 1/2 of recommended dose of inorganic fertilizer with standard dose of mycorrhizae. The highest fruit wet weight (556 g/plant - 69% higher than the lowest) in tomato plants and the highest pod wet weight (18 g/ plant) in soybean was observed with $\frac{1}{2}$ of the recommended dose of inorganic fertilizer and lowest was observed in plants treated with standard dose of mycorrhizae without inorganic fertilizer (tomato -329 g/plant and soybean 4 g/plant). However the highest pod dry weight (8 g/plant) and seed dry weight (7 g/plant) were observed in 1/2 of recommended dose of inorganic fertilizer with standard dose of mycorrhizae in Soybean. The highest soil microbial activity was recorded in soil treated with standard dose of inorganic fertilizer) and the lowest was observed in soil treated with recommended dose of inorganic fertilizer (287 CO₂ mg/kg of soil). The activities of soil microorganisms were lower in soils treated with inorganic fertilizers considering the growth parameters and soil microbial activity it can be concluded that 1/2 of the recommended dose of inorganic fertilizer with standard dose of mycorrhizae is the best fertilizer mixture for crops, tomato and soybean. Addition of mycorrhizae to the field soil increases the soil microbial activity significantly.

Key words: Soybean, Mycorrhizae, Inorganic fertilizer, Tomato

INTRODUCTION

Inorganic fertilizer application enhances plant growth and yield because it absorbs quickly to soil and plants. Therefore, farmers apply maximum amount of inorganic fertilizers to their crops to achieve a higher yield. As a result of the excessive applications of inorganic fertilizer leaches to the ground causes water pollution. To minimize this situation, a combination of inorganic fertilizer with biological ingredients can be a better alternative for crop cultivation (Urban Creeks Council, 2001). Mycorrhiza (family Endogone) is a symbiotic association between mycorrhizal fungi and higher plants roots an act as an organic fertilizer/bio fertilizer. Mycorrhizae improve crop yield and increase the use of inorganic fertilizer by forming a bridge between the roots and the soil (University of Washington, 2006). It indirectly enhances the structure of the soil and improves air and water infiltration.

Tomato (*Lycopersicon esculentum*: Solanaceae) is the second most important vegetable crop next to potato. Present world production of tomato is about 100 million tons from 3.7 million hectares of cultivated land. Tomato is rich in many nutrients and is a primary ingredient in many Sri Lankan curries and sometimes paired with fish, prawns, okra *etc*.

Soybean (*Glycine max*: Fabaceae) is a mycotrophic (mycorrhizal) plant native to East Asia

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which is much depended on mycorrhizal symbiosis. Soybean is a profitable crop that is grown commercially for human consumption because it is rich in protein and other nutrients. At present soybean is one of the five major grain legumes cultivated in Sri Lanka. It is recognized as a potential food crop that can bridge the gap between the national needs and the availability of protein, as well as edible oil requirement in Sri Lanka (Arulandy, 1995).

The present study was conducted to find the growth and yield responses of tomato and soybean with mycorrhizae as a substitute for inorganic fertilizers. The main objectives of the study were to develop the best fertilizer combination (Inorganic fertilizer with Mycorrizae) for the optimum growth and yield of tomato and soybean and to compare the soil microbial activity of field and treated soils.

MATERIALS AND METHODS

This study was conducted at the Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya. The study site is located in the low country wet zone (WL₂) with the annual rainfall of 1900 mm, the mean monthly temperature of 27.5 $^{\circ}$ C, and relative humidity of around 72 %.

Tomato (variety Bhathiya) and Soybean (variety PM 25) seeds were obtained from the Department of Agriculture. Nursery trays were filled using 1:1 ratio of compost and sand. Seeds were covered by thin soil layer and watered daily. After twenty one days of nursery period, healthy equal size (5 cm) seedlings were transplanted to pots to maintain three plants per pot. After 10-12 days weaker plants were thinned out and to leave two plants per pot.

Potting medium was prepared using 1:1:1:1 ratio of top soil, coir dust, sand and compost. Pots were filled with potting media and sterilized using Topsin (Methyl 70 %) fungicide (6 g/10 L of water). Then the pots were kept wet for seven days. Four doses of inorganic fertilizer; recommended level (Soybean NPK- 35:130:35, Tomato NPK- 30:150:40) without mycorrhizae (T1), 1/2 of recommended dose of Inorganic fertilizer with standard dose of mycorrhizae (2g mycorrhizae/5L water) (T2), 1/4 of recommended dose of Inorganic fertilizer with standard dose of mycorrhizae (T3) and Standard dose of mycorrhizae without Inorganic fertilizer and (T4) (2 g mycorrhizae/5L water) were used. Each treatment was replicated five times. All management practices were conducted according to the recommendations of the Department of Agriculture from seed germination to harvesting.

Tomato and soybean were used to investigate the effect of soil mixture on the number of leaves/plant (after 25th day- beginning of the vegetative stage and 45th day- end of the vegetative stage), number of flowers/plant, plant shoot height (cm), plant root height (cm), number of pods/ fruits per plant, number of nodules/plant (soybean), yield (g) /plant and microbial activity of the different soil mixtures separately.

Determination of soil microbial activity

Soil microbial activity was measured according to the CO₂ evolution method. Soil sample (10 g) from the tomato and soybean cultivated pot was taken in to a jam bottle and mixed with 3.5 ml distilled water. The controller set was filled with 3.5 ml of distilled water. Ignition tube was taken and filled with 3 ml of 2N-NaOH and then placed on the jam bottle so that the emitted CO_2 from the soil will be absorbed in to the NaOH solution. Soil samples were kept in dark room for one week. Then contents in ignition tubes were washed in to 250 ml conical flasks separately and mixed with 7.5 ml of 2N BaCl₂. Few drops of phenolphthalein were added and mixtures were titrated by 0.5N-HCl. Burette reading was taken at the point of which burette color changes from pink to colorless.

Experiment was carried out according to complete randomized block design with four replicates and 8 plants per replicate. Data were analyzed using SAS statistical software (version 9.1.3).

RESULTS AND DISCUSSION

Results revealed that the highest mean number of leaves at the day 25 (beginning of vegetative stage) was recorded in treatment with inorganic fertilizer recommended dose without mycorrhizae (T1) both in tomato (13 leaves/plant) and soybean (14 leaves/plant). But, it was not significantly different from 1/2 of the recommended dose of inorganic fertilizer with mycorrhizae standard dose (T2) (12 leaves/tomato plant, 14 leaves/soybean plant). The lowest number of leaves at the 25^{th} day was recorded in the treatment mycorrhizae standard dose without inorganic fertilizer (T4) (9 leaves/tomato plant, 11 eaves/soybean plant). T2 was not significantly different from ¹/₄ of the recommended dose of inorganic fertilizer with mycorrhizae standard dose (T3). Both T1 and T2 treatments were significantly different from T4. The number of leaves at the 25th day in T1 was increased 44% in tomato and 34% in soybean compared to T4. The rate of releasing of nutrients are much faster in the inorganic fertilizers thus they providing major elements at the early growth stage of plant (Lucus, 2001) and this could be the reason for this observation.

The highest number of leaves at the 45th day (end of the vegetative stage) was recorded in

out fertilizer (T4) (37 leaves/ tomato plant, 19 leaves/ soybean plant). T1 was not significantly different from 1/2 of the recommended dose of inorganic fertilizer with mycorrhizae standard dose (T2) (43 leaves/tomato plant, 30 leaves/soybean plant). ¹/₄ of the recommended dose of inorganic fertilizer with mycorrhizae standard dose (T3) (39 leaves/ tomato plant, 24 leaves/soybean plant) and T4 were not significantly different from each other. According to the results, number of leaves at 45 days in T2 increased 23% in tomato and 57% soybean respectively compare to T4. In mycorrhizae, nutrient content is relatively low and the nutrients are not readily available for plant uptakes (Ramanie et al 2008) compared with inorganic fertilizer.

The highest shoot height was observed in T1 (96 cm tomato and 56 cm soybean) and the lowest was recorded in T4 (79 cm tomato and 33 cm soybean) (Table 1 and Table 2a) shoot height was not significantly different from T1 and T2 (92 cm tomato; 52 cm soybean) however both treatments were significantly different from T3 (82 cm tomato, 39 cm soybean) and T4 (79 cm tomato, 33 cm soybean) (Table 1, Table 2 and Fig. 2). Plant height in T2 increased 21% in tomato and 68% soybean compared to T4. There were no significant (P>0.05) differences in plant root length



Fig. 1 Effects of different concentrations of inorganic fertilizer and mycorrhizae on number of fruits per plant of Tomato from 1st harvest

inorganic fertilizer recommended dose without mycorrhizae (T1) (48 leaves/ tomato plant and 28 leaves/ soybean plant) and lowest was recorded in mycorrhizae standard dose withamong all the treatments (Table 1 and Table 2a). However, highest root length was recorded in T4 (26 cm tomato, 21 cm soybean) and lowest recorded in T1 (22 cm tomato, 17

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cm soybean). The reason is that via mycorrhizae, plant increases root surface area as an adaptation to adverse soil conditions (Seran *et al.* 2010). Plant roots in T4 treatment had larger nodules in soybean while there were no nodules observed in other treatments (Fig. 3). soybean plant). The lowest mean number of fruits was recorded in T4 (18 fruits/plant, 4 pods/soybean plant). Number of fruits in T2 was increased by 47% in tomato and 283 % in soybean compared to T4.

However the highest wet weight of fruits/

Table 1: Growth parameters of Tomato with inorganic fertilizer and mycorrhizae, Four doses of inorganic fertilizer; recommended level without mycorrhizae (T1), 1/2 of recommended dose of inorganic fertilizer with standard dose of mycorrhizae (2g mycorrhizae/5L water) (T2), 1/4 of recommended dose of inorganic fertilizer with standard dose of mycorrhizae (T3) and standard dose of mycorrhizae without inorganic fertilizer and (T4) (2 g mycorrhizae/5L water) were used.

Treatment	No of leaves at 25 days	No.of leaves at 45 days	Plant shoot height (cm)	Plant root length (cm)	No.of flow- ers/plant	No of fruits/plant	Wet weight of fruits/ plant (g)	Soil micro- bial activity (CO ₂ mg/kg soil)
T1	13 ^a	48 ^a	96 ^a	22 ^a	32 ^a	27 ^a	556 ^a	287d
T2	12 ^{ab}	43 ^{ab}	92 ^a	22 ^a	30 ^{ab}	25ª	548 ^a	349c
T3	11 ^{bc}	39 ^b	82 ^b	24 ^a	26 ^{bc}	21 ^{ab}	379 ^b	373c
T4	09 ^c	39 ^b	79 ^b	26 ^a	23°	18 ^b	329 ^b	464a
T5 Field soil	-	-	-	-	-	-	-	419b

Column values followed by the same letter are not significantly different as determined by Duncan's multiple range test(P=0.05)

Table 2a: Vegetative growth parameters of Soybean with inorganic fertilizer and mycorrhizae.

Treatment	No of leaves at 25 days	No of leaves at 45 days	Leaf width (cm)	Leaf length (cm)	Plant shoot height (cm)	Plant root length (cm)	Soil microbial activity (CO ₂ mg/kg soil)
T1	14 ^a	28^{a}	6 ^a	8 ^a	56 ^a	17 ^a	250d
T2	14^{a}	30 ^a	6^{ab}	8 ^a	52 ^{ab}	17 ^a	350c
Т3	11 ^b	24 ^{ab}	4 ^{bc}	6^{ab}	39 ^{ab}	19 ^a	370c
T4	11 ^b	19 ^b	4 ^c	6 ^b	33 ^b	21 ^a	460a
T5 Field soil	-	-	-	-	-	-	411b

The highest number of flowers per plant observed in T1 (32 flowers/ tomato plant, 55 flowers/ tomato plant) and the lowest recorded in T4 (23 flowers/tomato plant, 21 flowers/soybean plant) (Table 1 and Table 2b). The number of flowers was not significantly difference between T1 and T2 (30 flowers/tomato plant, 53 flowers/soybean plant). Number of flowers in T2 was 38% higher in tomato and 160% higher in soybean compared to T4.

As indicated in table 1, Fig.1 and Table 2b, the highest number of fruits was recorded in T1 (27 fruits/tomato plant, 18 pods/soybean plant). However not significantly different from T2 (25 fruits/tomato plant, 16 pods/

plant was recorded in T1 (556 g in tomato and 16g in soybean) but not significantly different from T2 (548 g/tomato plant, 16 g/soybean plant). Both of the values were significantly different from T3 and T4. Lowest wet weight of fruits/pods per plant was recorded in T4

 Table 2b: Reproductive growth parameters of Soybean with inorganic fertilizer and mycorrhizae.

Treat- ment	No of flow- ers per plant	No of pods per plant	Wet weight of pods (g)/plant	Dry weight of pods (g)/ plant	Dry weight of seeds(g)/ plant
T1	55 ^a	46 ^a	18 ^a	8^{a}	7 ^a
T2	53 ^a	42 ^a	16 ^a	8 ^a	7 ^a
T3	31 ^b	22 ^b	07 ^b	4 ^b	3 ^b
T4	21 ^b	12 ^b	04 ^b	3 ^b	2 ^b

Column values followed by the same letter are not significantly different as determined by Duncan's multiple range test (P=0.05).



Fig. 2 Effects of different concentrations of inorganic fertilizer and mycorrhizae on plant height of Soybean.

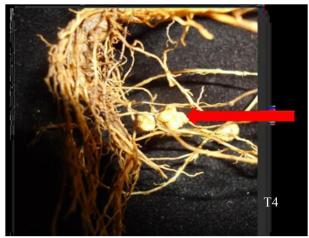


Fig. 3 Root nodules in Soybean in the T4 treatment

(329 g/tomato plant, 4 g/soybean plant) (Table 2b). Wet weight of pods in T2 was higher than 69% tomato and 300% soybean compared to T4. However highest pod dry weight (8 g/plant) and seed dry weight (7 g/ plant) were observed in 1/2 of recommended dose of inorganic fertilizer with standard dose of mycorrhizae in Soybean.

But the highest seed wet weight of pods was observed in T1. Reason for that may be there were more succulent tissues in pods of T1 due to inorganic fertilizer. As a result of that, plants in T2 may more resistant to pest and diseases than plants in T1.

The highest microbial activity (emitted CO_2 mg/kg of soil) was observed in T4 for both crops 464 and 460 CO₂ mg/kg soil for tomato and soybean respectively and was significantly different from all the other treatments. Field soil recorded the next highest microbial activity (419 - 411 CO2 mg/kg of soil) because it was not treated by topsin but all the other soils in T1,T2,T3 and T4 were sterilized before planting tomato. T1 recorded the lowest soil microbial activity (287 - 250 CO₂ mg/ kg of soil) and the same results were observed by Seran et al. (2010) where soil micro and macro organisms are reduced with the presence of inorganic fertilizers as compared to organic fertilizers. Increment of soil microbial activity of T4 was 61.08 % compared to the T1. There was no significant difference between soils T2 $(349 - 350 \text{ CO}_2 \text{ mg/kg of soil})$ and T3 (373 - 370 CO₂ mg/kg of soil) for soil microbial activity. But microbial activity of both T2 and T3 were significantly different from field soil, T1 and T4 treatments.

CONCLUSION

Half recommended dose of inorganic fertilizer with standard dose of mycorrhizae is the best



Fig. 4 Effects of different concentrations of inorganic fertilizer and mycorrhizae on number of pods per Soybean plant

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fertilizer mixture for tomato and soybean based on plant growth parameters and soil microbial activity. Addition of mycorrhizae increases the soil microbial activity significantly compared to field soil

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