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Arbuscular Mycorrhizal Fungi and Organic Amendments as alternatives for Inorganic Fertilizer on growth, yield and Vitamin C content of Chili (*Capsicum annum* L.)

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Abstract

This study was investigated to compare Biofertilizer (AMF), Biochar, Compost and Inorganic Fertilizer on Vegetative, Reproductive Properties and Vitamin C content of Chili (*Capsicum annum* L.) The pot experiment was conducted at the Faculty of Applied Sciences, Rajarata University of Sri Lanka. Control (without soil amendments) was consisted of 3000g of soil in pot with one chili plant. The treatments were arranged in a randomized complete block design (RCBD) with four replicates. Vitamin C content of chili fruits were tested after harvesting. Treatments were field soil only (T_0), field soil and 3% rock phosphate (T_1), field soil and arbuscular mycorrhizae (T_2), field soil and arbuscular mycorrhizae and 3% rock phosphate (T_3) field soil and 5% biochar (T_4), field soil, 5% biochar and 3% rock phosphate (T_5), field soil and 5% compost (T_6) field soil, 5% compost and 3% rock phosphate (T_7), field soil and inorganic fertilizer (T_8), field soil, inorganic fertilizer and 3% rock phosphate (T_9). According to Tukey's Pairwise Comparison test, the application of mycorrhizae and compost with or without rock phosphate significantly ($p<0.05$) improved the relative growth rate of chili compared to T_0 T_4, T_5 and T_8. However, number of fruits were significantly high ($p<0.05$) with mycorrhizae, biochar and compost with rock phosphate. Vitamin C content was significantly ($p<0.05$) high in all the treatments except T_0,T_1 and T_5. Organic and biofertilizer (AMF) with rock phosphate could be used as effective substitutes for inorganic fertilizers for chili cultivation in Sri Lanka.

Keywords: *Arbuscular mycorrhizae, Organic fertilizers, Inorganic fertilizers, Capsicum annuum L.*

Introduction

The present- day agricultural crop production is highly dependent on inorganic fertilizer and some other agro-chemicals. However, the application of inorganic fertilizers to soil are known to affect adversely on the soil microorganisms and therefore, the soil fertility and crop productivity will be affected in the long run (Seneviratne, 2009). Furthermore, it is also found that inorganic fertilizer contaminate soil with several toxic metals which could be accumulated in living systems and human bodies through the food chains, and cause health problems (Chandrajith *et al.*, 2010). Therefore, use of inorganic fertilizer cannot be continued as a sustainable practice of crop production. As a result, there is a renewed interest on the reduction of inorganic fertilizer applications by alternatives (Jayawardhane and Yapa, 2018; Kulasooriya *et al*., 2018). Traditionally, natural organic manure such as animal droppings, green manure, and compost, etc. used as alternatives for inorganic fertilizer. Also, beneficial microorganisms, biochar, and rock phosphate (RP) found to be currently trending in organic agriculture (Pathirana and Yapa, 2020).
Chili pepper (*Capsicum annum* L.: solanaceae) is popular throughout the world due to its nutritional, medicinal as well as the economic value (Rahmanet *et al*., 2013). Chili is rich in vitamins (A, B_5, C, and E), minerals (K, Mg, Fe, Ca, and P), antimicrobial, and anticancer compounds (Saleh *et al*., 2018). Arbuscular mycorrhizal fungi (AMF) are important soil microorganisms which form a symbiotic association with most of the higher plant roots. Through these mutualistic associations, AMF provide several benefits to crop plants including nutrient and water supply (Siddiqui and Pichtel, 2008), disease resistance (Siddiqui and Pichtel, 2008), Heavy metal tolerance (Davies *et al.*, 2002), and improve soil properties (Gaur and Adholeya, 2004). Biochar is a type of soil amendment prepared by a variety of biomass feedstocks, including wood, crop, manures, and yard wastes via the pyrolysis under limited oxygen and below 700 ^{0}C temperature. Biochar can enhance soil moisture and nutrient availability (Lehmann and Joseph, 2009), remediate contaminated lands (Woolf *et al.*, 2010), and provide habitat for soil microorganisms (Baldock and Smernik, 2002). Rock phosphate is a good

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alternative phosphorous source for synthetic Triple super phosphate (TSP) and is recommended for organic farming due to its less solubility (Wanninayake *et al*., 2021).
Therefore, the present study was conducted to assess the effects of selected biofertilizer and organic amendments, which are the potential substitutions for synthetic fertilizer on the growth, yield and vitamin C content of green chili.

Material and Methods

Treatment application

A pot experiment was conducted inside a planthouse under natural light conditions at Rajarata University of Sri Lanka in Mihinthale (80 21′ 0" North, 800 30′ 0" East), Sri Lanka, during 2017-2018. The day temperature in the planthouse during the trial was 31°C -35°C and night temperature was 30°C with the 68% relative humidity. Annual precipitation in the area is between 1000-1500 mm. The experiment was designed as complete randomized block design with four replicates. There were five different manure treatments including the control, and depending on the addition of rock phosphate (RP), there were total of ten treatments: field soil only (T_0), field soil and 3% rock phosphate (T_1), field soil and arbuscular mycorrhizae (T_2), field soil and arbuscular mycorrhizae and 3% rock phosphate (T_3) field soil and 5% biochar (T_4), field soil, 5% biochar and 3% rock phosphate (T_5), field soil and 5% compost (T_6) field soil, 5% compost and 3% rock phosphate (T_7), field soil and inorganic fertilizer (T_8), field soil, inorganic fertilizer and 3% rock phosphate (T_9). Before adding treatments, each pot was filled with a potting mixture containing sieved field soil, obtained at the depth of 0-10 cm, from the fields with previously no added synthetic fertilizer. Composite soil samples containing AMF spores and fine corn root fragments obtained from the upper layer of a corn field were used as AMF inoculum. Biochar was produced by following double barrel method (Agegnehu *et al.*, 2016) using wood chips, paddy husks and paddy straw (3:2:2 ratio). Commercially available Eppawala rock phosphate and compost were used for desired treatments. AMF inoculum, biochar and compost were applied at the rate of 250g per pot, and rock phosphate was added at 90g per pot. Recommended doses of NPK were added in inorganic fertilizer treatments. The final weight of soil mixture per pot was adjusted into 3kg by changing the weight of initial potting mixture (Table 1). Seeds of variety chili pepper (*Capsicum annuum* L.) was grown in a tray and one plant was transplanted to each pot.

Data Collection

The agronomic parameters such as height of the plant (cm), relative growth rate, number of leaves per plant, leaf area index, number of branches per plant as the growth parameters and number of flowers per plant, number of fruits per plant and weight of fruits (g) as the yield parameters were measured. Furthermore, photosynthesis rate and ascorbic acid content of chili were measured.

Table 1. Treatment combinations of the pot experiment

Treatment		Amount of soil (g)	Amount of amendment (g)	Rock phosphate (3%) (g)
T_0	field soil only	3000	-	-
T_1	field soil and 3% rock phosphate	2910	-	90
T_2	field soil and arbuscular mycorrhizae	2750	250	-
T_3	field soil and arbuscular mycorrhizae and 3% rock phosphate	2660	250	90
T_4	field soil and 5% biochar	2750	250	-
T_5	field soil, 5% biochar and 3% rock phosphate	2660	250	90
T_6	field soil and 5% compost	2750	250	-
T_7	field soil, 5% compost and 3% rock phosphate	2660	250	90
T_8	field soil and inorganic fertilizer	2995	5	-
T_9	field soil, inorganic fertilizer and 3% rock phosphate	2905	5	90

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Agronomic data

Height of the plant (cm) was measured at harvesting stage and relative growth rate was calculated. Number of leaves, branches, flowers, and fruits per plant were measured from the date of transplanting to harvesting stage periodically. Fresh weight of fully-matured fruit was recorded after harvesting. Leaf area was determined by using leaf area meter and photosynthetic rate was measured by portable photosynthesis system (LI-6400XT).

Ascorbic acid content of chili

The ascorbic acid content of harvested fruits was determined by adopting the procedure of Orobiyi *et al*., (2015). Chili pepper fruits (0.5g) were washed with water and homogenized with 5 ml of 4% oxalic acid using mortar and pestle. The homogenates were centrifuged at 5,000 rpm for 10 minutes and the supernatants were filtered. The residues were made up to 25 ml with 4% oxalic acid. The ascorbic acid content was estimated by spectrophotometry using 2, 4 dinitrophenylhydrazine reagent at 540 nm wave length and the absorbance was measured. The standard curve was used to measure the vitamin C content.

Results and Discussion

The application of mycorrhizae and compost with or without rock phosphate significantly ($p<0.05$) improved the relative growth rate of chili compared to control and other treatments (Figure 1). Chili plant height was increased from transplanting onwards, reaching its maximum height at 4th month. The maximum plant growth was observed in mycorrhizal applied chili plants. However, number of fruits were significantly high ($p<0.05$) with mycorrhizae, biochar and compost with rock phosphate (Figure 2). The growth and yield parameters of chilies were greatly influenced by phosphorus. The findings of the current study showed that rock phosphate applied plants showed the higher values for most of investigated growth parameters and yield parameters.

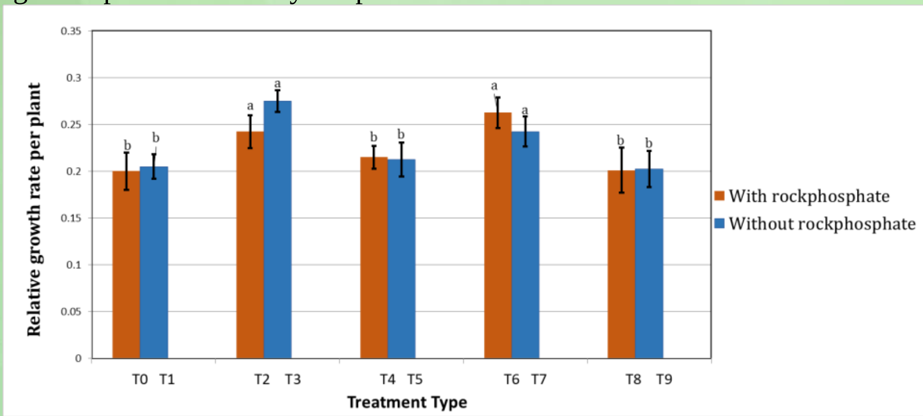

Figure 1. Effect of the different treatments for relative growth rate of chili plants. Means share a same letter denoted no significant difference at $p < 0.05$.

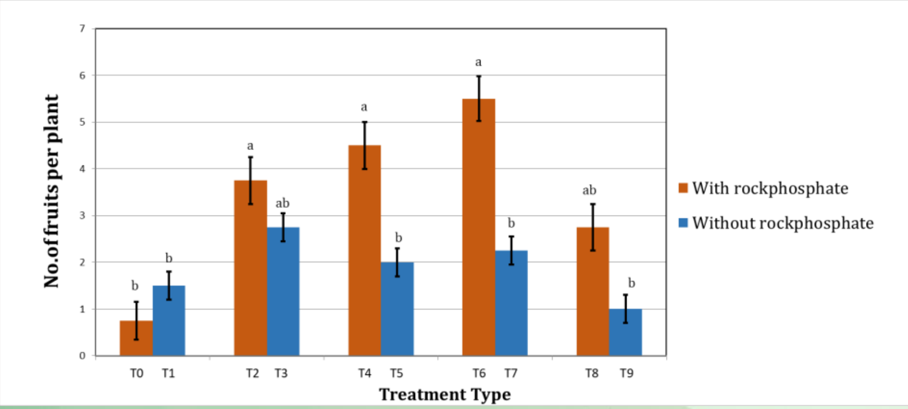

Figure 2. Effect of the different treatments for number of fruits per plant. Means share a same letter denoted no significant difference at $p < 0.05$.

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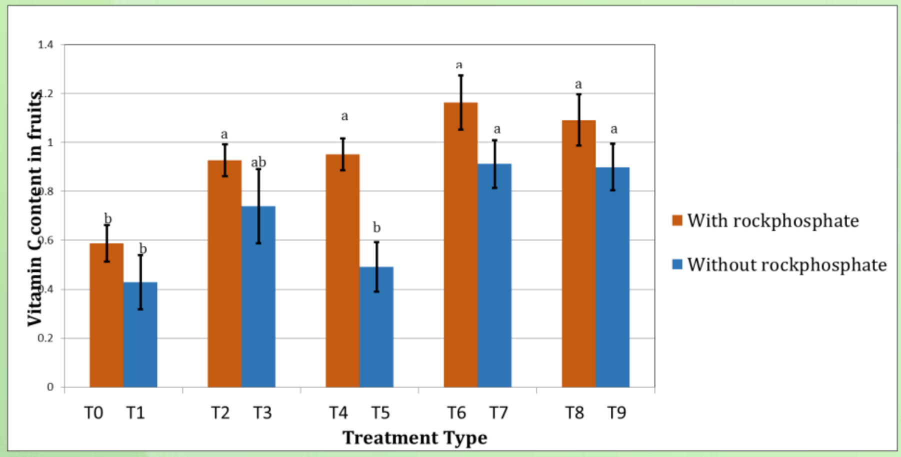

Figure 3. Effects of the different treatments for vitamin C content of fully matured fruits
Means share a same letter denoted no significant difference at $p < 0.05$.

The vitamin C content of the sampled fresh fruits of *Capsicum annuum* L. varied from 1.72mg/cm 3 to 0.40 mg/cm 3 to fresh weight of 0.5mg. It was significantly ($p<0.05$) high in all the treatments except T_0, T_1 and T_5 (Figure 3). However, there was no significant difference ($p \geq 0.05$) observed for final plant height (cm), number of leaves per plant, leaf area index, number of branches per plant, number of flowers per plant, and weight of fruits (g) and photosynthesis rate (Table 2).

Table 2. Mean values of growth parameters, yield parameters and photosynthesis rate for different treatments. Means share a same letter denoted no significant difference at $p < 0.05$.

Treatment No	Treatment type	Growth Parameters				Yield parameters		Photosynthesis rate (µmol $m^{-2}S^{-1}$)
		Plant Height (cm)	No. of leaves per plant	Leaf Area Index (cm^2)	No. of branches per plant	No. of Flowers per plant	Fruit weight (g)	
T0	Field soil only	23.68 ± 1.7^{a}	18	140.57 ± 15.3^{a}	12	3	$1.51\pm.30^{b}$	95.5 ± 9.8^{b}
T1	Field soil and rock phosphate	18.90 ± 0.9^{b}	17	107.04 ± 15.9^{b}	13	3	1.62 ± 0.25^{b}	95.5 ± 11.8^{b}
T2	Field soil and AMF	$27.03\pm1.^{a}$	20	132.26 ± 7.1^{a}	12	2	2.31 ± 0.3^{a}	122.75 ± 37.0^{b}
T3	Field soil, AMF and rock phosphate	27.13 ± 1.8^{a}	18	123.55 ± 11.2^{a}	15	5	2.09 ± 0.26^{ab}	175.5 ± 33.3^{ab}
T4	Field soil and biochar	$23.37\pm2.^{b}$	17	101.82 ± 8.7^{b}	16	3	1.83 ± 0.0^{b}	337 ± 28.4^{a}
T5	Field soil, biochar and rock phosphate	25.32 ± 1.3^{a}	12	107.94 ± 7.7^{b}	13	4	2.84 ± 0.28^{a}	106.25 ± 24.3^{b}
T6	Field soil and compost	25.62 ± 1.6^{a}	24	135.01 ± 25.3^{a}	16	3	2.71 ± 0.27^{a}	154.25 ± 33.2^{b}
T7	Field soil, compost and rock phosphate	27.87 ± 2.4^{a}	26	137.59 ± 18.9^{a}	17	5	2.90 ± 0.13^{a}	270.25 ± 56.1^{a}
T8	Field soil and inorganic fertilizer	$22.25\pm2.^{b}$	14	$108.46\pm11.^{b}$	16	2	1.86 ± 0.24^{ab}	263 ± 32.5^{a}
T9	Field soil, ino. fer. and rock phosphate	26.75 ± 2.7^{a}	26	136.99 ± 5.4^{a}	17	5	2.76 ± 0.18^{a}	151.75 ± 41.2^{ab}

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Conclusion

Considering growth and yield parameters addition of standard compost, biochar and AMF, combination with rock phosphate separately can successfully replace the use of inorganic fertilizer in chili cultivation in dry zone, Sri Lanka. Organic fertilizer with rock phosphate positively influenced on vitamin C content of chili fruits.

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