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Efficacy of Bio-inoculants Arbuscular Mycorrhizal Fungi and Phosphorus on Micronutrient Status of Leaves and Soil in Litchi (*Litchi chinensis* Sonn.) Layers in Nursery Condition

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Authors' contributions

This work was carried out in collaboration among all authors. Author PK carried out the research work under the supervision of authors RRS, RR and MS. Performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript by author PK. Author UK helping in drafting of the paper. All authors read and approved the final manuscript.

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ABSTRACT

Litchi (*Litchi chinensis* Sonn.) originated from South China, it is sub-tropical evergreen fruit crops, especially grown on the marginal climate of tropics and subtropics. It is delicious juicy fruit of India having excellent nutritional quality, pleasant flavoured, good amount of antioxidant and vitamins C, vitamin B-complex and phytonutrients flavonoids. It has a great potential to earn foreign exchange in the national and international market through export. Arbuscular mycorrhizal (AM) infection is a

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common association between plant roots and microorganisms. It is responsible for increasing plant nutrient uptake and also increases in macro and micronutrients in leaf. Therefore, the present work has been analyzed macro and micro nutrients from soil and leaf, after 60, 90 and 120 days after inoculation of two bio-inoculants with phosphorus (SSP) including nine treatments with three replications. After 120 days of inoculation both the species of mycorrhizal combination with phosphorus application were very effective. Highest Copper content is (10.99 ppm), Zinc (33.17 ppm), Iron (121.47 ppm) and Manganese (15.33 ppm) was recorded in case T5 (*G. mosseae* 10 g + Phosphorus 50 mg kg⁻¹ of soil) which is gradually increases. The soil nutrient content gradually decreased with time duration but no- significant difference was found among treatments after 120 days inoculation. After 120 days potting result was found that the Copper content is (1.70 ppm), Zinc (3.07 ppm), Iron (7.80 ppm) and Manganese (4.00 ppm) was recorded in case T5 (*G. mosseae* 10 g + Phosphorus 50 mg kg-1 of soil).this research was undertaken to find out whether Arbuscular mycorrhizal (AM) infection and phosphorus affect the micro-nutrient status of soil and leaves in nursery stage.

Keywords: Litchi chinensis; AM fungi; macro and micro nutrients.

1. INTRODUCTION

Litchi (Litchi chinensis Sonn.), a member of the Sapindaceae, is an important fruit crop that is widely cultivated in tropical and subtropical areas of the world. It is delicious juicy fruit of India having excellent nutritional quality, pleasant flavour and good amount of antioxidant and vitamins С, vitamin **B**-complex and phytonutrients flavonoids. And also, rich source of nutrients that required for the production of blood. It provides micro element such as Mg, Mn, Cu and Fe, that is required for the formation of RBC. Litchi originated in South China [1] and believed to be introduced in India in 18th century probably through North Eastern part of India and it is cultivated in initially spread along plains adjoining Himalayan foothills. India ranks second in the world next to china in litchi production with an area of 90 thousand-hectare, production 559 thousand MT and productivity 6 MT/ha [2]. Among important litchi growing states of India, Bihar contribution 40% of total litchi production. The total area under this crop in the state is 31.1 thousand hectares with annual production of 227 thousand tones, but the productivity of litchi in the state is only 6.2 tons per hectare which is quite low [2]. Though the productivity of litchi in India is better than some of countries including china, but this is far below the potential vield and there is scope of improvement in terms of yield as well as quality. The fruit are fleshy drupes with an edible aril surrounded by the pericarp.

Phosphorus is one of the important plant nutrients that involved and plays important role in plant functions like photosynthesis, movement of nutrient within the plant, transformation of sugars and starches and transfer of genetic characters from one generation to the next are mediated through phosphorus. The mycorrhizae increase the nutrient-uptake ability of the plant. The mycorrhizal symbiosis significantly improved plant growth performance, such as plant height, stem diameter, shoot, root or total dry weight [3]. The beneficial effect of AM fungi enhanced arowth. reduced phosphate seedlina requirements, increased resistance to fungal root pathogens and abiotic stresses consequently increased fruit production. Soil microbiota play significant role in solubilization, mobilization and mineralization of nutrients for proper growth and development of fruit trees. Innovative technologies like integrated nutrient management practices involving use of biofertilizers: Non-symbiotic N2-fixing Bacteria, Azospirillum brasiliense, AM fungi and Trichoderma viridae for enhancing plant growth is being used in horticultural crops because of higher cost and hazardous effect of chemical fertilizers [4]. Biofertilizers have attracted greater attention particularly in developing countries like India as a substitute for costly chemical fertilizers. They can be applied to seed, root or in order to soil mobilized the viability of nutrients by their biological activity and turn the soil health in general. They have ability to fix atmospheric nitrogen and mobilize phosphorus in soil from unavailable from to available forms. Biofertilizer as living cells of different types of microorganism (Bacteria, Algae and Fungi) which have an ability to mobilized nutritionally important elements from non- available form. It is considered as ecowhich friendly fertilizers, improves soil provide healthy and plant quality and better establishment especially during nursery stage.

2. MATERIALS AND METHODS

2.1 Plant Materials and Experimental Design

The present study was undertaken to evaluate the response of AM fungi on layered litchi under pot condition during the 2018-19. The experiment was conducted in Complete Block Randomized Design (CRD) with a promising one years old uniform sized of litchi cultivar Purbi taken from the Research Farm of Bihar Agricultural University, Sabour, Bhagalpur, India. The inoculums of two species viz., Glomus mosseae and Glomus coronatum were procured from Tata Energy Resource Institute (TERI), New Delhi, India. Number of spores was found 80/100 gm of inoculum. Uniform potting mixture of soil and vermicompost (3:1) was prepared and filled in black poly bags of size 9×12 cm having a capacity of 3.5 kg of potting mixture. For the treatments application the poly bags were 1/3rd filled with potting mixture and 10 g/kg of soil AM fungi was put in the bag in such a way that root of the layer was just above the inoculum. For phosphorus application, quantity of SSP to be added under each treatment was calculated for each treatment and weighed on electronic balance. The quantity was dissolved in 250 ml of water and the water containing required quantity of phosphorus was added to the poly bag after planting. the different treatments concentration viz., two AM fungi species viz., Glomus mosseae and Glomus coronatum with Phosphorus that is To Control (Uninoculated), T1 G. mosseae @10 gm kg⁻¹ of soil , T2 *G. coronatum*, @10 gm kg⁻¹ of soil, T3 Phosphorus @ 50 mg kg⁻¹ of soil, T4 Phosphorus @ 75 mg kg⁻¹ of soil, T5 *G. mosseae* 10 gm + Phosphorus 50 mg kg⁻¹ of soil, T6 G. mosseae 10 gm + Phosphorus 75 mg kg⁻¹ of soil, T7 G. coronatum 10 gm + Phosphorus.

50 mg kg⁻¹ of soil, T8 *G. coronatum* 10 gm + Phosphorus 75 mg kg⁻¹ of soil was placed in the polybags and spread a layered of soil with requisite inoculants. Then the freshy cut litchi layers of cv. Purbi were transplanted in the black poly bag followed by light irrigation.

Observation recorded after 60, 90 and 120 days after inoculation. Soil sample were collected at 3-5 cm depth (Rhizospheric zone) from the pot/polybags of three tagged plants of each treatments. Leaf samples were also collected at the different date of observation from middle portion of third leaf from the top. Leaf samples were collected from the same tagged plants from where soil and root samples were taken. Leaf samples were washed, dried hot air oven at 68°C for 72 hours or till constant weight [5]. The dried leaf samples were ground in stainless steel blender and stored in paper bags for further analysis.

The mean difference was tasted by F-test at (5%) level of significance. Critical difference at 5% level of significance was used for comprising among the treatments.

Chemical analysis of micro nutrients of soil and leaf: Diethylenetriaminepentaacetic acid (DTPA) method for extraction of available micronutrients in soil. 10 g of air-dried soil sample were taken and transferred it into 100 ml polyethylene tubes. Air-dried soil 10 g of each sample + 20 ml DTPA of extracting solution were added shaken during 2 hrs. Soil extracts (extractant/soil ratio) were obtained (0.005 with DTPA-TEA mol L^{-1} diethylenetriaminepentaacetic acid + 0.1 mol L^{-1} triethanolamine + 0.01 mol L⁻¹ CaCl2) solution at pH 7.3 and maintain 25°C as described by Lindsay & Norvell [6]. This is the method used by some Brazilian laboratories, adopting the "IAC System of Soil Analysis [7].

The leaves micro nutrient element will be analyzed by using the diacid digested material using Atomic Absorption Spectrophotometer. For the estimation of Zn, Cu, Fe and Mn by using formula:

[Available Micronutrient (ppm) = Reading of AAS × Dilution factor

3. RESULTS AND DISCUSSION

The litchi plants responded positively to the application of varying concentrations of AMF and phosphorus.

Leaf nutrient status with respect to Copper (Cu) content in leaves of air layered litchi plants gradually increases and varies with passage of time interval under different treatments concentration. So, data has been depicted in (Table 1). The data table sowed that the highest Copper in case T5 (10.99 ppm) which was at par with T6 (10.40 ppm), T7 (10.43 ppm) and T8 (10.37 ppm). The next effective treatments were T1 (9.90 ppm) which was statistically similar with

other rest treatment. The lowest content of copper was recorded in T0 Control (8.78 ppm) after 120 days after potting. Timmer and Leyden reported that mycorrhizal inoculated plants had higher Cu concentration than non-mycorrhizal plants.

Highest Iron content was recorded with (121.47 ppm) was found in T5 which was at par with all treatment except control. The significantly minimum Iron content in leaf with (113.00 ppm) was obtained in case of T0 control. The previous studies observed that mycorrhizal hyphae could take part in Cu and Mn uptake [8,9]. These data indicated that the Fe increment due to bio-inoculants is dependent of both fungal and plant species, and might to be result of both the external hyphae and the increase of root Fe (III) chelate reductase activities [10,11]. Similarly soil and leaf nutrient maximum in sour orange

reported by Ortas et al. [12]. Leaf and plant nutrient increased because mycorrhizal plants are able to extract more nutrient from soil through their extramatricular mycelium and thus helped easier absorption in lower levels of fertility. AM fungi are release growth substances, growth regulators, hormone and enzyme in the rhizosphere, which help in the change of insoluble nutrient to soluble form and increased their availability to the plants resulting in increased nutrient contents of leaf and plant.

The data pertaining to the AM fungi and phosphorus on the Zinc and Manganese content in the leaves of litchi layers depicted in (Table 2) show that the gradual increment and varies with lapse of time interval under the different treatments. After 120 DAI higher value for the Zinc was recorded in case T5 (33.17 ppm) which was at par with T6 (31.33ppm), T7 (32.00 ppm),

Table 1. Effect of bio-inoculants and	phosphorus on leaf micronutrient (Cu and Fe)
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S. no.	Treatments (Doses per kg of soil)	С	opper (Cu)*	lron (Fe [⁺])*			
		60	90	120	60	90	120	
		DAI	DAI	DAI	DAI	DAI	DAI	
T0	Control	8.78	8.96	9.11	108.67	109.33	113.00	
T1	<i>G. mosseae</i> 10 g	9.58	9.63	9.90	115.33	117.33	118.00	
T2	G. coronatum 10 g	9.30	9.33	9.70	114.33	116.67	117.33	
Т3	Phosphorus 50 mg	9.25	9.25	9.43	112.83	115.00	116.19	
T4	Phosphorus 75 mg	8.91	9.04	9.32	110.33	114.67	115.33	
T5	G. mosseae 10 g + Phosphorus 50 mg	10.77	10.95	10.99	118.00	120.13	121.47	
T6	G. mosseae 10 g + Phosphorus 75 mg	10.18	10.35	10.40	117.80	118.47	119.13	
Τ7	G. coronatum 10 g + Phosphorus 50 mg	10.25	10.38	10.43	117.33	118.56	119.22	
T8	G. coronatum 10 g + Phosphorus 75 mg	10.20	10.30	10.37	116.00	117.67	119.00	
	CD (0.05)	0.79	0.71	0.64	6.20	8.87	8.27	
	CV (%)	4.83	4.30	3.82	3.18	4.48	4.12	

*-Showed that the mean value of three replications, DAI- Day after Inoculation

	Table 2.	Effect	of bio-	inoculants	and p	hosphorus	on leaf	micronutrients	(Zn and	d Mn)
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S.	Treatments (Doses per kg of soil)	Ziı	Zinc (Zn) ppm*			Manganese (Mn) ppm*		
no.		60	90	120	60	90	120	
		DAI	DAI	DAI	DAI	DAI	DAI	
T0	Control	27.67	28.00	29.00	11.67	12.17	12.39	
T1	<i>G. mosseae</i> 10 g	29.33	30.17	31.17	13.22	13.33	13.50	
T2	<i>G. coronatum</i> 10 g	29.17	29.50	30.33	13.00	13.22	13.39	
T3	Phosphorus 50 mg	28.30	29.30	30.13	12.67	13.00	13.19	
T4	Phosphorus 75 mg	28.17	29.22	29.55	12.00	12.26	12.46	
T5	<i>G. mosseae</i> 10 g + Phosphorus 50 mg	31.33	32.50	33.17	13.87	14.33	15.33	
T6	<i>G. mosseae</i> 10 g + Phosphorus 75 mg	30.29	31.00	31.33	13.17	13.50	14.00	
T7	G. coronatum 10 g + Phosphorus 50 mg	30.67	31.33	32.00	13.33	13.50	14.17	
T8	G. coronatum 10 g + Phosphorus 75 mg	30.15	30.48	30.82	13.00	13.33	13.83	
	CD (0.05)	0.82	1.91	2.63	1.08	0.95	1.12	
	CV (%)	1.63	3.71	5.01	4.98	4.24	4.86	

*-Showed that the mean value of three replications, DAI - Day after Inoculation

T8 (30.82 ppm), T1 (31.17 ppm) and T2 (30.33 ppm). The next effective T3 (30.13 ppm) treatments was at par with other treatments. However lower value was recorded in case T0 Control (29.00 ppm). The results obtained by Krikun and Levy [13] showed that mycorrhizal-inoculated citrus rootstocks had high Zn content than non - inoculated plants.

Manganese was found significantly highest in case T5 (15.33 ppm) which was at par with T6 (14.00 ppm), T7 (14.17 ppm), T8 (13.83 ppm), T1 (13.50 ppm) and T2 (13.39 ppm).

The next effective T3 (13.19 ppm) treatments was at par with other treatments. The minimum content of manganese was recorded in case T0 control (12.39 ppm) after 120 days bioinoculants inoculation. The previous studies observed that mycorrhizal hyphae could take part in Cu and Mn uptake [8,9]. It has also been proven that mycorrhizal symbiosis can improve Zn nutrition as a secondary consequence of P nutrition [14]. The present study all bio-inoculants significantly increase the concentration of Zn and gradually increase with passage of time. The result is consistent with the finding of Margues et al. [15] who observed that inoculation with G. claroideum or G. intraradices enhanced the Zn accumulation in the tissues of Solanum nigrum plants. Fe concentration in the leaves of layered litchi plants under the different treatment of bioinoculants was gradually increases with lapse of time. AMF are also known to release growth substances, growth regulators, hormones and enzymes (acid phosphatase) in the rhizosphere, which help in the conversion of insoluble nutrients to soluble form and increase their availability to the plants resulting in increased contents of major nutrients like N, P and K and micronutrients like Fe, Mg, Mn, Mo and Co [16]. Normally, acquisition of those nutrients with low mobility in the soil, such as P, Zn and Cu, may be enhanced in the plants by AM inoculation by Turk et al. [17]. In plants, particularly those with weak root system, hyphal connections act as a bridge between roots and nutrient sites in soil and facilitate efficient uptake of immobile nutrients by host plant [18].

The critical examination of data pertaining to available copper content was estimate in soil under different time interval depicted in (Table 3). It was revealed from data copper content gradually decreased with time duration but no- significant different was found among treatments. The (Table 3) reveals that there was a distinct variation in available Zn in soil due to treatment application on various date of observation. It clearly indicated that available Zn content decreased gradually after time interval and treatments also differed significantly. On ward 60 DAI the maximum available Zn was found in T₅ (3.37 ppm) which was at par with T₇ (3.24 ppm). However, minimum have been available in case T0 (2.13 ppm) was recorded. After 120 days of treatment the highest level of Zn (3.07 ppm) was recorded in T5 which was statistically equal with T7 (3.00 ppm) treatments. However, significantly minimum Zinc (2.07 ppm) was recorded in control. The present study supported by Abbasi and Yousra, [19] considerable effect of treatment on soil nutrient content regarding Zn and Fe was noted. Application of bio-inoculants of AM fungi and Azospirillum spp. was able to maintain high content of Zn and Fe of soil. According to them, concentration of Fe. Mn. Cu and Zn in soil were highest after application of biofertilizers with poultry manure, with no significance difference among them. This relative increase in soil micronutrients due to the application of biofertilizers is attributed to the contribution of microorganism is the decomposition of organic wastes and residues present in the soil or applied through organic materials, thereby releasing more nutrients from these substrates in the soil [20].

Soil nutrient status with respect to Mn content was estimated at different time interval and data depicted in (Table 4). It is evident from the data that irrespective of the treatments soil nutrient content in soil gradually decreased with time duration. After 60 DAI maximum Mn was observed in T5 (4.40 ppm) and minimum observed in non-inoculated (3.23 ppm). The maximum Mn recorded in T5 (4.00 ppm) and lowest in T₀ (3.10 ppm) after 120 days inoculation. As a similar result was found that the distinct variation in available Fe in soil due to treatment application on various date of observation. It clearly indicated that available Fe content decreased gradually after time interval and treatments also differed significantly. On 60 DAI the maximum available Fe was found in T5 G. mosseae + Phosphorus 50 mg (8.17 ppm) which was at par with other treatments accept T3 and T4 and untreated control which have minimum available Fe (7.23 ppm) was recorded. After 120 days of treatment the highest level of Fe (7.80 ppm) was noted in T5 which was

S. no.	Treatments (Doses per kg of soil)	Copper (Cu)*				Zinc (Zn)*		
		60	90	120	60	90	120	
		DAI	DAI	DAI	DAI	DAI	DAI	
T0	Control	2.03	1.93	1.87	2.13	2.10	2.07	
T1	<i>G. mosseae</i> 10 g	2.20	2.06	1.93	3.07	3.00	2.90	
T2	<i>G. coronatum</i> 10 g	2.17	2.05	1.93	3.00	2.93	2.87	
T3	Phosphorus 50 mg	2.10	2.00	1.90	2.90	2.86	2.79	
T4	Phosphorus 75 mg	2.17	2.07	1.97	2.87	2.83	2.80	
T5	<i>G. mosseae</i> 10 g + Phosphorus 50 mg	2.20	2.00	1.70	3.37	3.23	3.07	
T6	G. mosseae 10 g + Phosphorus 75 mg	2.17	2.07	1.93	3.07	2.97	2.90	
T7	G. coronatum 10 g + Phosphorus 50 mg	2.16	2.00	1.80	3.24	3.13	3.00	
Т8	G. coronatum 10 g + Phosphorus 75 mg	2.13	2.00	1.87	3.00	2.90	2.83	
	CD (0.05)	NS	NS	NS	0.17	0.19	0.15	
	CV (%)	3.42	3.22	4.76	3.37	3.95	3.07	

	Table 3.	Effect of bio-inoculants	and phosphorus on	soil micronutrient	(Cu and Zn)
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*-Showed that the mean value of three replications, DAI - Day after Inoculation

Table 4. Effect of bio-inoculants and phosphorus on soil micronutrient (Mn and Fe)

S.	Treatments (Doses per kg of soil)	Manganese (Mn)*				Iron (Fe ⁺)*		
no.		60	90	120	60	90	120	
		DAI	DAI	DAI	DAI	DAI	DAI	
T0	Control	3.23	3.17	3.10	7.23	7.17	7.00	
T1	<i>G. mosseae</i> 10 g	4.17	4.07	3.87	7.99	7.86	7.70	
T2	<i>G. coronatum</i> 10 g	4.13	4.03	3.87	7.87	7.73	7.63	
T3	Phosphorus 50 mg	3.93	3.85	3.73	7.53	7.47	7.37	
T4	Phosphorus 75 mg	3.90	3.83	3.73	7.47	7.40	7.30	
T5	<i>G. mosseae</i> 10 g + Phosphorus 50 mg	4.40	4.20	4.00	8.17	8.00	7.80	
T6	<i>G. mosseae</i> 10 g + Phosphorus 75 mg	4.13	4.00	3.83	7.93	7.83	7.67	
Τ7	G. coronatum 10 g + Phosphorus 50 mg	4.17	4.00	3.80	8.00	7.87	7.70	
T8	G. coronatum 10 g + Phosphorus 75 mg	4.10	3.97	3.80	7.87	7.77	7.60	
	CD (0.05)	0.23	0.19	0.20	0.50	0.65	0.61	
	CV (%)	3.34	2.91	3.12	3.77	5.00	4.78	

*-Showed that the mean value of three replications, DAI- Day after Inoculation

statistically equal with rest treatments. Significantly minimum Fe (7.00 ppm) was recorded in control and after 60 DAI highest 4.40 ppm available Mn found in T5 *G. mosseae* + Phosphorus 50 mg which was statistically similar with T7 *G. coronatum* 10 g + Phosphorus 50 mg (4.17 ppm) and observed in untreated.

T0 (3.23 ppm). Similar study was reported by Abbasi and Yousra [19] considerable effect of treatment on soil nutrient content regarding Zn and Fe was noted.

4. CONCLUSION

Our results showed that mycorrhizal inoculation with phosphorus generally increased with micro nutrient status of leaves and decreased soil micro nutrient content of the litchi layers in in potted condition. Hence the treatment T5 (*G. mosseae* 10 g + Phosphorus 50 mg) can be used as the best treatment to increase the micronutrient status without hampering the soil fertility. AMF and phosphorus have lead to the development of healthy planting material in the nursery which could reduce the time taken by the young plants to reach the maturity; hence early fruiting can be observed bringing monetary benefits to the farmer.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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