Efficacy of bio-inoculants AMfungi and phosphorus on micronutrients statusofleaves and soil inlitchi (*Litchi chinensis* Sonn.)layers in nursery condition.

Abstract

Litchi (*Litchi chinensis* Sonn.) originated from South China, its sub- tropical evergreen fruit crops, especially grown on the marginal climate of tropics and subtropics. It's delicious juicy fruit of India having excellent nutritional quality, pleasant flavorand 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.However, most of the soils have very low plant available nutrients, such as phosphorus (P), zinc (Zn), iron (Fe), and manganese (Mn).Arbuscular mycorrhizal (AM) infection is a common association between plant roots and microorganisms and 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 micronutrients from soil and leafafter 60, 90 and120 days after inoculation of two bio-inoculants with phosphorus including nine treatments. Copper (Cu), Zinc (Zn), Iron (Fe) and Manganese (Mn) in leaves of litchi saplings in nursery stage. After120 days of inoculationboth the species of mycorrhizacombination with phosphorus application were very effective as evident from the results,highest Copper content is (10.99 ppm), Zinc (33.17 ppm), Iron (121.47 ppm) and Manganese (15.33 ppm) was recorded in case T_5(*G.mosseae*10 g + Phosphorus 50 mg kg^{-1} of soil) which is gradually increases. The soil nutrient content in soil gradually decreased with time duration but no- significant different was found among treatments after 120 days bio-inoculants inoculation. After 60 days potting result was found that the Copper content is (2.20 ppm), Zinc (3.37 ppm), Iron (8.17 ppm) and Manganese (4.40 ppm) was recorded in case T_5(*G.mosseae*10 g + Phosphorus 50 mg kg^{-1} of soil).

Key words:*Litchi chinensis*,VAM, macro and micronutrients.

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's delicious juicy fruit of India having excellent nutritional quality, pleasant flavor and good amount of antioxidant and vitamins C, 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 (Menzel *et al.*, 2002). and believed to be introduced in India in 18th century probably through North Eastern part of India and its 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 (NHB, 2015). Among important litchi growing states of indie, 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 7.3 tones per hectare which is quite low whereas, potentially of litchi productivity is as high as 15 tones per hectare. Though the productivity of litchi in India is better than some of countries including china, but this is far below the potential yield 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 in in plant Functions like photosynthesis, movement of nutrient within the plant, transformation of sugars and starches, and transfer of genetic characteristics from one generation to the next are mediated through phosphorus. The mycorrhizae thus 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 (Wu *et al.*, 2011).The beneficial effect of AM fungi includes enhanced seedling growth, reduced phosphate requirements increased resistance to fungal root pathogens and abiotic stresses consequently increased fruit production.Soil microbiodata play significant role in solubilization, mobilization and mineralization of nutrients for proper growth and development of fruit trees. Innovative technologies like integrated nutrient managementpractices involving use of biofertilizers: Non-symbiotic N_2-fixing Bacteria, *Azospirillumbrasiliense,*AM fungi and *Trichoderma viridae* (Bio-data agent) for enhancing plant growth is being used in horticultural crops because of higher cost and hazardous effect of chemical fertilizers (Wu and Zou, 2011). 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 availableforms. Biofertilizer as living cells of different types of microorganism (Bacteria, Algae, Fungi) which have an ability to mobilized nutritionally important elements from nonavailable form. It is considered as eco-friendly fertilizers, which improves soil quality and provide healthy plant and better establishmet especially during nursery stage. Chlorophyll (Chlorophyll- a and Chlorophyll-b) is a green pigment product which are found in cyanobacteria and the chloroplast of algae and plants. The plant forms chlorophyll in physiological process that occurs only in living cell (Momin and Kadam, 2011). it was hypothesized that by applying proper concentration of bio-inoculants in suitable rooting medium will help in the production of quality material with better root system of air layered,

increase concentration of nutrients and final survival of litchi air layered in nursery. This will further help in faster growth and better plant establishment in field as well as pot condition which in turn, will be beneficial for the farmer. Therefore, keeping the above facts the present investigation was undertaken the increases the concentration of micronutrients from leaf andsoil.

Materials and Methods

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 - 19conducted in Complete Block Randomized Design (CRD)with a promising one years old uniform sized of litchi cultivar Purbiat 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. Uniform potting mixture of soil and Coco peat (2:1) 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 in soil, the black poly bags were $1/3^{rd}$ filled with potting mixture and inoculums of different treatments concentration*viz.,*two AM fungi species *viz., Glomus mosseae* and *Glomus coronatum* with Phosphorus that is T_0 Control (Uninoculated), T_1 *G. mosseae* @10 gm kg^{-1} of soil , T_2 *G. coronatum,* @10 gm kg^{-1} of soil, T_3 Phosphorus @ 50 mg kg^{-1} of soil, T_4 Phosphorus @ 75 mg kg^{-1} of soil, T_5 *G. mosseae* 10 gm + Phosphorus 50 mg kg^{-1} of soil, T_6 *G. mosseae* 10 gm + Phosphorus 75 mg kg^{-1} of soil, T_7 *G. coronatum* 10 gm + Phosphorus 50 mg kg^{-1} of soil, T_8 *G. coronatum* 10 gm + Phosphorus 75 mg kg^{-1} of soilwas placed in the polybags and spread a layered of soil with requisite inoculants. Then the freshy cut litchi layers of *cv.* Purbi seedling were transplanted in the black poly bag followed by light irrigation.The experiment was laid out in Complete Randomized block design with three replications including nine treatments and regular monitoring was done. Firstly, soil sample were collected at 3-5 cm depth (rhizospheric zone) from the pot/polybags of three tagged plants of each treatments after 60, 90 and 120 days inoculation.

Estimation ofmicro nutrients from the soil: Lindsay and Norvell (1978) proposed 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. 20 ml DTPA solution was added and stopped the tubes. it was shok for 2 hrs. at 25 ˚C and filtered the content with filtered

paper.**Estimation of micronutrients (Zn, Cu, Fe and Mn) from leaf**: The 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:

[AvailableMicronutrient (ppm)= Reading of AAS × Dilution factor]

Result and discussion

S.No.	Treatments (Doses Per kg of soil)	Copper (Cu)*	Iron (Fe^{+})*

		60 DAI	90 DAI	120 DAI	60 DAI	90 DAI	120 DAI
T_0	Control	8.78	8.96	9.11	108.67	109.33	113.00
T_1	*G. mossae* 10 g	9.58	9.63	9.90	115.33	117.33	118.00
T_2	*G.coronatum* 10 g	9.30	9.33	9.70	114.33	116.67	117.33
T_3	Phosphorus 50 mg	9.25	9.25	9.43	112.83	115.00	116.19
T_4	Phosphorus 75 mg	8.91	9.04	9.32	110.33	114.67	115.33
T_5	*G. mossae* 10 g + Phosphorus 50mg	10.77	10.95	10.99	118.00	120.13	121.47
T_6	*G.mossae* 10 g + Phosphorus 75 mg	10.18	10.35	10.40	117.80	118.47	119.13
T_7	*G. coronatum* 10 g + Phosphorus 50 mg	10.25	10.38	10.43	117.33	118.56	119.22
T_8	*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**

Table 1: Effect of bio-inoculants leaf micronutrient (Cu and Fe) content of layered litchi plants cv. Purbi.

*-Showed that the mean value of three replications, DAI- Date After Inoculation.

Leaf nutrient status with respect to Copper (Cu) content in laves air layered litchi plants gradually increases and varies with passage of time interval under different treatments concentration.So, obtained has been depicted in (Table 1).The data table sowed that the highestCopper in caseT_5 (10.99 ppm) which was at par with T_6 (10.40 ppm), T_7 (10.43 ppm) and T_8 (10.37 ppm). The next effective treatments were T_1 (9.90 ppm) which was statistically similar with other rest treatment. The lowest content of copper was recorded in T_0 Control (8.78 ppm) after 120 days potting of bio-inoculants. And highest Iron content was recorded with (121.47 ppm) was found in T_5which was at par with all treatment except control. The significantly minimum Iron content in leaf with (113.00 ppm) was obtained in case of T_0 control. The previous studies observed that mycorrhizal hyphae could take part in Cu and Mn uptake (Li *et al.*, 1991; Giovanneti, 2008). 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 (Caris*et al.*, 1998; Wang and Xia, 2009).Similarly soil and leaf nutrient maximum in sour orange reported by Ortas*et al.*, (2002) 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 insolublenutrient to soluble form and increased their availability to the plants resulting in increasednutrient contents of leaf and plant.

Table 2: Effect of bio-inoculants leaf micronutrient (Zn and Mn) content of layered litchi plants cv. Purbi.

S. NO.	Treatments (Doses Per kg of soil)	Zinc (Zn) ppm*			Manganese (Mn) ppm*		
		60 DAI	90 DAI	120 DAI	60 DAI	90 DAI	120 DAI
T_0	Control	27.67	28.00	29.00	11.67	12.17	12.39
T_1	*G. mossae* 10 g	29.33	30.17	31.17	13.22	13.33	13.50
T_2	*G.coronatum* 10 g	29.17	29.50	30.33	13.00	13.22	13.39
T_3	Phosphorus 50 mg	28.30	29.30	30.13	12.67	13.00	13.19
T_4	Phosphorus 75 mg	28.17	29.22	29.55	12.00	12.26	12.46
T_5	*G. mossae* 10 g + Phosphorus 50mg	31.33	32.50	33.17	13.87	14.33	15.33
T_6	*G.mossae* 10 g + Phosphorus 75 mg	30.29	31.00	31.33	13.17	13.50	14.00
T_7	*G. coronatum* 10 g + Phosphorus 50 mg	30.67	31.33	32.00	13.33	13.50	14.17
T_8	*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- Date After Inoculation.

The data pertaining to Zinc and Manganesecontent in the leaves of litchi sampling 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 caseT_5 (33.17 ppm) which was at par with T6 (31.33ppm),T_7 (32.00 ppm), T_8 (30.82 ppm), T_1 (31.17 ppm) and T_2 (30.33 ppm). The next effective T_3 (30.13 ppm)treatments was at par with other treatments. However lower value was recorded in case T_0Control(29.00 ppm). And Manganesewas found significantly highest in caseT_5 (15.33 ppm) which was at par with T_6(14.00 ppm),T_7 (14.17 ppm),T_8 (13.83 ppm),T_1 (13.50 ppm) and T_2 (13.39 ppm). The next effective T_3 (13.19 ppm)treatments was at par with other treatments. The minimum content of manganese was recorded in case T_0 control(12.39 ppm) after 120 days bio-inoculants inoculation.The previous studies observed that mycorrhizal hyphae could take part in Cu and Mn uptake (Li *et al.,* 1991; Giovanneti, 2008). It has also been proven that mycorrhizal symbiosis can improve Zn nutrition as a secondary consequence of P nutrition (Subramanian *et al.,* 2009). 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 Marques *et al.,* (2006) 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 bio-inoculants was gradually increases with lapse oftime.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 (Adivappar*et al.,* 2004). 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 (Turk et al., 1996). 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 (Azcon-Aguilar and Barea, 1996).

Table 3: Effect of bio-inoculants on soil micronutrient (Copper and Zinc)content of layered litchi plants cv. Purbi.

S.No.	Treatments (Doses Per kg of soil)	Copper (Cu)*			Zinc (Zn)*		
		60 DAI	90 DAI	120 DAI	60 DAI	90 DAI	120 DAI
T_0	Control	2.03	1.93	1.87	2.13	2.10	2.07
T_1	*G. mossae* 10 g	2.20	2.06	1.93	3.07	3.00	2.90
T_2	*G.coronatum* 10 g	2.17	2.05	1.93	3.00	2.93	2.87
T_3	Phosphorus 50 mg	2.10	2.00	1.90	2.90	2.86	2.79
T_4	Phosphorus 75 mg	2.17	2.07	1.97	2.87	2.83	2.80
T_5	*G. mossae* 10 g + Phosphorus 50mg	2.20	2.00	1.70	3.37	3.23	3.07
T_6	*G.mossae* 10 g + Phosphorus 75 mg	2.17	2.07	1.93	3.07	2.97	2.90
T_7	*G. coronatum* 10 g + Phosphorus 50 mg	2.16	2.00	1.80	3.24	3.13	3.00
T_8	*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**

*-Showed that the mean value of three replications, DAI- Date After Inoculation.

The critical examination of data pertaining to available copper content was estimate in soil under different time interval after 120 DAI. The data depicted in (Table-3) It is evident from the data that irrespective of the treatments soil nutrient content in soil gradually decreased with time duration but no- significant different was found among treatments. The maximum Copper content was observed 60 days after potting with (2.20 ppm) in case T_5 which was equal to found in case $T_1$with (2.20 ppm). However lowest was recorded in T_0 with (2.03 ppm). 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. Onward 60 DAI the maximum available Zn was found in T_5 (3.37 ppm) which was at par with T_7 (3.24 ppm). However, minimum have been available in case T_0 (2.13 ppm) was recorded. After 120 days of treatment the highest level of Zn (3.07 ppm) was recorded inT_5 which was statistically equal with T_7(3.00 ppm) treatments. However, significantly minimum Zinc (2.07 ppm) was recorded in control. The present study supported by (Abbasi and Yousra, 2012) 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 Feof soil. According to them, concentration of Fe, Mn, Cu and Zn in soil were highest for biofertilizers alone with poultry manure. 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 (Javid, 2006).

Table 4: Effect of bio-inoculants on soil micronutrient (Manganese and Iron)content of layered litchi plants cv. Purbi.

S. No.	Treatments (Doses Per kg of soil)	Manganese (Mn)*			Iron (Fe^{+})*		
		60 DAI	90 DAI	120 DAI	60 DAI	90 DAI	120 DAI
T_0	Control	3.23	3.17	3.10	7.23	7.17	7.00
T_1	*G. mossae* 10 g	4.17	4.07	3.87	7.99	7.86	7.70
T_2	*G.coronatum* 10 g	4.13	4.03	3.87	7.87	7.73	7.63
T_3	Phosphorus 50 mg	3.93	3.85	3.73	7.53	7.47	7.37
T_4	Phosphorus 75 mg	3.90	3.83	3.73	7.47	7.40	7.30
T_5	*G. mossae* 10 g + Phosphorus 50mg	4.40	4.20	4.00	8.17	8.00	7.80
T_6	*G.mossae* 10 g + Phosphorus 75 mg	4.13	4.00	3.83	7.93	7.83	7.67
T_7	*G. coronatum* 10 g + Phosphorus 50 mg	4.17	4.00	3.80	8.00	7.87	7.70
T_8	*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- Date After Inoculation.

Soil nutrient status with respect to Mn content was estimated at different time interval and data so obtained has been 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 but no- significant different was found among treatments. As a similar result was foundthat 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 T_5*G. mosseae* + Phosphorus 50 mg (8.17 ppm) which was at par with other treatments accept T3 and T_4 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 T_5 which was 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 T_5*G. mosseae* + Phosphorus 50 mgwhichwas statistically similar with T_7*G. coronatum* 10 g + Phosphorus 50 mg(4.17 ppm) and observed in untreated T_0(3.23 ppm).Similar study was reported by (Abbasi and Yousra, 2012) 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.According to them, concentration of Fe, Mn,Cu and Zn in soil were highest for biofertilizers alone with poultry

manure. 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 (Javid, 2006).

Conclusion

The influence of phosphorus (50 mg and 75 mg) mycorrhiza (*G. mosseae* and *G. coronatum*) alone and in combination. The treatment significantly influenced the micronutrients viz., Copper (Cu), Zinc (Zn), Iron (Fe) and Manganese (Mn) in leaves of litchi saplings in nursery stage. After120 days of inoculationboth the species of mycorrhiza combination with phosphorus application were very effective as evident from the results,highestCopper content is (10.99 ppm), Zinc (121.47 ppm), Iron (33.17 ppm) and Manganese (15.33 ppm) was recorded in case T_5(*G.mosseae*10 g + Phosphorus 50 mg kg^{-1} of soil). Hence, the treatment*G. mosseae*10 g + Phosphorus 50 mg can be used as the best treatment to increase the healthy planting material and survival of litchi cv.Purbi without hampering the soil fertility status.

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