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Effect of indigenous beneficial rhizobacterial isolates on growth of Litchi seedlings

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ABSTRACT

A pot experiment was conducted to observe the effect of indigenous strains of rhizobacteria isolated from litchi orchards of East Champaran, Bihar (India) on the growth and survivability of litchi seedlings. Three efficient native rhizobacterial isolates belonging to genera *Pseudomonas, Azotobacter and Ochrobactrum* were used in seven different combinations and their effect on germination rate, shoot height, root length, number of leaves were observed and recorded in litchi seedlings. Pot 7 containing mixed combination of all the three isolates in equal proportion showed maximum root length (11.7 cm) and shoot length (12.5 cm), early germination as compared to control Pot P8 devoid of any isolates. Pot 4 (*Pseudomonas* + *Azotobacter*) and pot 5 (*Pseudomonas* + *Ochrobactrum*) also gave satisfactory result in all the parameters recorded. The results clearly state that, mixture of all the three rhizobacterial isolates as "Biomix" produced stimulatory growth results on litchi seedlings as compared to sole application and control. This "Biomix" could be used for formulating effective biofertilizers for litchi orchards in future.

Figures : 02 References : 15 Tables : 03
KEY WORDS : *Azotobacter*, Biofertilizer, Litchi seedling, *Ochrobactrum, Pseudomonas*, Rhizobacteria.

Introduction

Litchi (*Litchi chinensis*) is an important sub-tropical evergreen fruit crop belonging to family Sapindaceae. It is a rich source of vitamins, minerals and medicinal properties. Bihar is the leading producing state of Litchi. Litchi production is highly sensitive to soil and climatic conditions and short post harvest life. Both high and low temperatures[9], nutrients[12] and irrigation[7] may result undesirable loss of yield and fruit quality. Fertilizer is one of the most important inputs for improving production and productivity of litchi orchards. Inadequate nutrition often attributed to low yields in litchi[10] and poor fruit quality. Litchi is of tremendous export potential, hence its vast production is utmost important. Chemical fertilizers are mostly in use for their cultivation, which have some deleterious effect on fruit quality besides adverse effect on soil, water and environmental conditions[8]. To avoid such harmful effects, in recent years attention has been extended towards beneficial microbial consortia such as Plant Growth Promoting Rhizobacteria (PGPR). They are the group of soil rhizobacteria which aggressively colonize plant roots and bring about beneficial changes in plants[6] for easy and efficient uptake of nutrients, for biocontrolling phytopathogens, improved seed germination attributes, improved resistance mechanism in host, increased biomass production, plant growth and yield[11]. Soil amendment and biopriming seed treatment is gaining importance in improving seed quality, germination control of pathogen as an alternative to chemical fungicide[5]. Indigenous beneficial rhizobacterial isolates *i.e.* PGPR has gained important place in modern agriculture system acting as one of the active ingrediants of biofertilizers. These natural biofertilizers which when applied to plants, soil or composting pits, help in mobilizing various nutrients by their biological activity. Several literatures are available on growth enhancing ability of PGPR. There was reported increased plant height, dry weight of shoot and root in peach seedlings if inoculated with *Azotobacter*[1]. It was also observed the positive growth effect of PGPR on litchi plants producing increased plant height, leaves and root length[3]. The combined use of organic, inorganic, FYM and biofertilizer is fruitful for producing good quality, high yielding litchi plants[13]. The use of PGPR as one of the active ingrediants used for preparing best quality potting media for litchi plants was recommended.

Materials and Method

Experimental site : The experiment was carried out by using rhizobacteria collected from the litchi orchards situated in different blocks of East Champaran district

Formulaton of Biofertilizer

Three isolated native strains of rhizobacteria belonging to *Pseudomonas, Azotobacter* and *Ochrobactrum* were used for preparing biofertilizer in different combinations as liquid inoculum.

TABLE-1: Composition of NPK growth medium

Composition	Quantity/ litre
Glucose	15 g
Tricalcium phosphate	5 g
NaCl	0.5 g
KCl	0.2 g
$MgSO_4$	0.5 g
Yeast extract	0.5g
$(NH_4)_2SO_4$	0.5 g
Na_2MoO_4	0.001 g
$CaCl_2$	0.2 g
$FeSO_4$	0.1 g
$MnSO_4$	Trace
Double distilled water	1000ml

Preparation of mother culture

Nutrient broth (NB) was prepared and autoclaved for preparing mother culture of the isolates. Freshly grown rhizobacterial isolates were inoculated into autoclaved NB broth and then kept for incubation at room temperature on rotary shaker at 150 rpm for 48 hrs.

Preparation of growth media for the inoculum

Growth medium used for transferring the isolates and formulating biofertilizer was prepared using the protocol used by Harsiddhi biotech India. Pvt. Ltd given in Table. The NPK growth media prepared contained the entire essential nutrients used for proper growth and survival of all kinds of bacteria *i.e.* nitrogen fixers, phosphate solubilizers and potassium solubilizers. Isolates grown overnight on rotary shaker were transferred to autoclaved NPK broth and were kept for 24-48 hrs for incubation on rotary shaker. After 48 hrs of incubation, quality check of the broth was done. The prepared liquid biofertilizer was used in different combinations for pot experiment.

Pot Experiment

The three isolated indigenous strains of rhizobacteria were used (Table-2) for conducting pot experiment. The media used for the experimental pots (P1 – P8) was soil mix which was composed of soil and Farm Yard manure in 1:1 ratio. Five germinated seeds of litchi were sown for each rhizobacterial treatment in

TABLE-2: Pots containing various combinations of isolates as biofertilizers

No .of Pots	Pot'scode	Isolates and their combinations / Control	Combination code
1	P1	*Pseudomonas*	B1
2	P2	*Azotobacter*	B2
3	P3	*Ochrobactrum*	B3
4	P4	*Pseudomonas + Azotobacter*	B4
5	P5	*Pseudomonas + Ochrobactrum*	B5
6	P6	*Azotobacter + Ochrobactrum*	B6
7	P7	*Pseudomonas + Azotobacter + Ochrobactrum*	B7
8	P8	Control	C

TABLE-3: Results of pot experiment, showing growth parameters in terms of root length, shoot length and number of leaves

Pot codes	Combination codes of isolates	Root length (cm)	Shoot length (cm)	No. of leaves
P1	B1	7.4	9.2	06
P2	B2	6.9	8.4	04
P3	B3	6.4	8.2	08
P4	B4	9.5	10.5	08
P5	B5	8.4	10.1	10
P6	B6	7.6	9.0	06
P7	B7	11.7	12.5	08
P8	B8	5.1	7.2	02

triplicate and kept under shade. The biofertilizers containing live rhizobacterial isolates (10ml/ treatment/ pot) were applied in seven pots (P1- P7) excluding control pot (P8). The biofertilizers were applied in three phases *i.e.* at the time of seed sowing, 15 DAS (days after sowing) and 30DAS. The seedling growth was observed time to time taking proper care. After 60 days of incubation in pots, seedlings were uprooted and length of roots and shoots were measured and number of leaves was counted. Statistical data analysis and graphs were plotted using

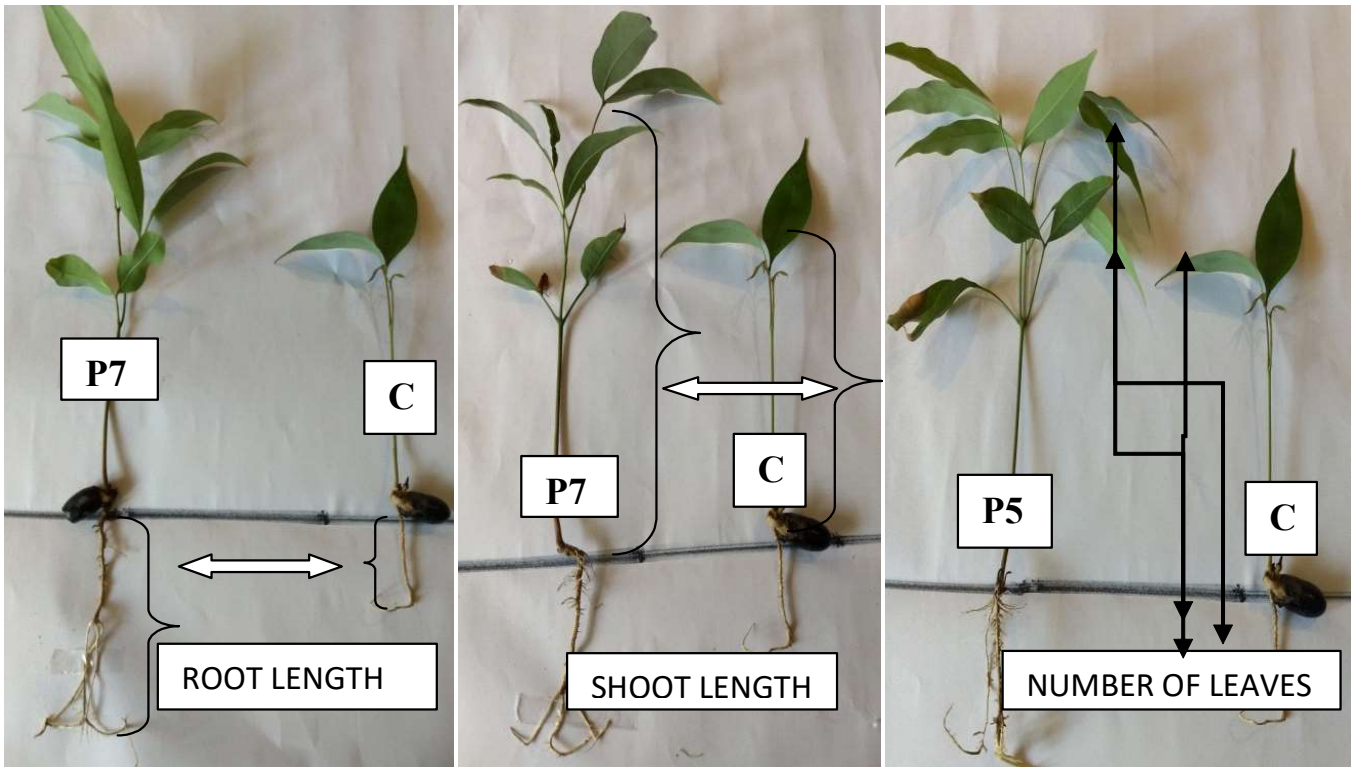

Fig. 1: Inoculated litchi seedlings showing enhanced growth as compared to control

Microsoft Excel, 2007.

Results and Discussion

In this experiment, variable results in all the three growth parameters *i.e.* shoot length, root length and number of leaves produced were observed. After 60 days, maximum root length (11.7cm) was observed in litchi seedlings of Pot7 (P7) containing mixture of *Pseudomonas*, *Azotobacter* and *Ochrobactrum.* Whereas, minimum root length (5.1 cm) was observed in pot 8 litchi seedlings (P8) as shown in Table 3. The increased root length of litchi seedlings clearly shows the indole acetic acid (IAA) producing ability of isolates. The phytohormone produced is responsible for changing the entire root morphology by enhancing their root length and root laterals[4]. A similar result on stimulatory effects of IAA on root length was obtained[3,14] on onion seeds and litchi plants respectively. Variation in shoot length was also observed in different combinations. The shoot length of litchi seedlings was observed in the range of 7.2 cm to 12.5 cm (Table-3). The minimum shoot length of litchi seedling was recorded in P8 (7.2 cm) and maximum shoot length (12.5cm) was recorded in Pot7 (P7) containing mixture of *Pseudomonas*, *Azotobacter* and *Ochrobactrum*. The increased shoot length observed in litchi seedlings is due to the combined effect of IAA and other growth promoting abilities like nitrogen fixation, phosphorus solubilization, potassium solubilzation *etc.* In the study, variable number of leaves was also observed in different combinations in the range of 2 - 10. The minimum number of leaves (2) was observed in control pot (P8) whereas maximum number of leaves (10) was observed in pot5 (P5) containing combination of *Pseudomonas* and *Ochrobactrum* shown in Fig 1. It can also be observed that the pots containing *Pseudomonas* alone (P1) or in combinations (P4, P5 and P7) showed significant effect on root length, shoot length and number of leaves. In control pot (P8), minimum growth results on litchi seedlings were observed (Table 3). The observations of pot experiment clearly reveal that out of all the rhizobacterial combinations (B1-B7), the B7 combination containing *Pseudomonas*, *Azotobacter* and *Ochrobactrum* gave best results in all the three growth parameters studied as shown in Fig 1. Significant results were also obtained by pot 4 (P4) and pot 5(P5) containing (*Pseudomonas* + *Azotobacter)* and (*Pseudomonas* + *Ochrobactrum*) respectively. Microbial consortia consisting of indigenous bacterial strains is very effective in plant growth as they are more adapted in that particular environment as compared to other foreign microorganisms. In the present study, the microbial consortia consisting of *Pseudomonas*, *Azotobacter* and *Ochrobactrum* were marked as the best biofertilizer combination for obtaining maximum growth effect in litchi seedling which can be efficiently used as plant growth promoting rhizobacteria (PGPR) for litchi cultivation in future.

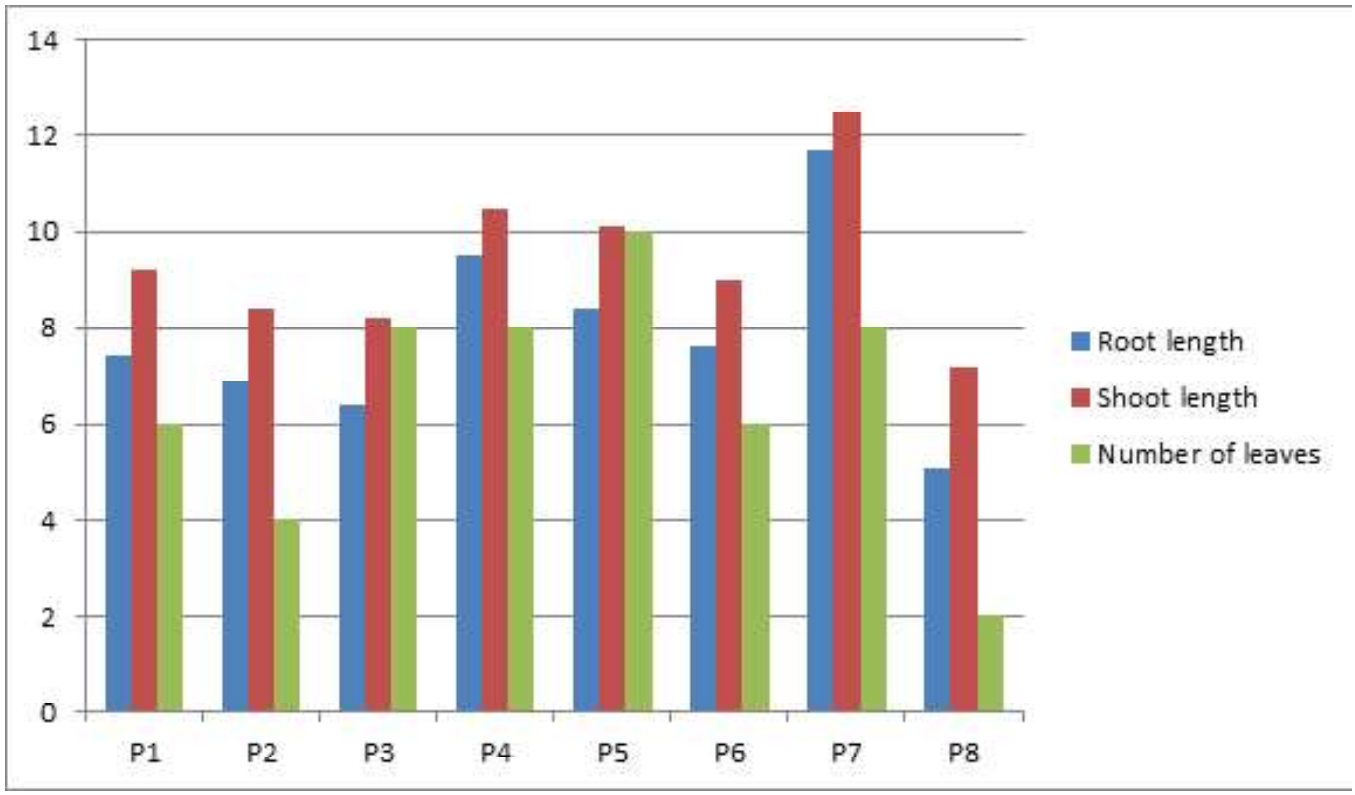

Fig. 2 : Showing growth of litchi seedlings in terms of root length, shoot length and number of leaves

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