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Effect of integrated nutrient management on growth and nutrient uptake in papaya (*Carica papaya* L.) At nursery level

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Abstract
A pot experiment was conducted in Green house of the Department of Agricultural Microbiology, IGKV, Raipur (Chhattisgarh) during 2016-17 to assess the effect of integrated nutrient management on growth performance of papaya seedling. The experiment was laid out in CRD with three replications and seven treatments involving organic manure, *Azotobacter* inoculation (Azoto 1 and Azoto 3) alone and along with PSB and 100% recommended dose of fertilizer and control. The results revealed that significantly highest plant growth was obtained under dual inoculation with *Azoto 3* + PSB as compared to uninoculated treatments and control. Dual inoculation with Azoto 3 + PSB + 75 % NP + 100 % K (T_7), treatment produced significantly maximum shoot dry biomass of 3.53 g/seedling against 2.92 and 2.67 g/seedling at 100% RDF and control respectively. Nitrogen uptake by papaya significantly increased from 25.62 at control to 43.76 mg per seedling. 32.21% increased in shoot dry biomass accumulation over control was found in papaya seedling receiving Azoto 3+ PSB + 75%NP + 100% K at 120 DAT. Inoculation treatment with Azoto 3 and PSB along with 75 %NP and 100% K considered most effective and may be adopted to improve vegetative growth of papaya in nursery.

Keywords: *Azotobacter,* PSB, organic manure, biomass, Nitrogen uptake, Microbial population

1. Introduction

Papaya (*Carica papaya* L.) is a good source of protein, carbohydrate, fibre, vitamin A and C, carotene and mineral like iron, calcium, phosphorus and potassium. It is one among the fruits, which has attained great popularity because of its quick returns, easy cultivation, above all, for its attractive and delicious taste, wholesome fruits have multifarious uses. The nutritional requirement of papaya is quite typical in view of its continuous growth behavior like vegetative, flowering and fruiting habit. Owing to continuous fruiting habits, nutrient requirement of papaya is high and use of large quantity of chemical fertilizers alone is not only feasible but also costly to the poor farmers, also pollute soil and ground water. However, biofertilizers offer an alternative to chemical inputs, which have an ability of mobilizing the nutrionally important elements from non-useable to useable form and known to increase yield [1].

Papaya being shallow rooted, organic amendment and soil microflora plays a major role in growth and productivity and soil health. Nitrogen fixing bacteria and phosphate solubilizer are the main biofertilizers for horticultural crops. The contribution of beneficial microbes like *Azotobacter*, PSB in increasing the growth, yield and in reducing the fertilizer requirement have been brought out by few workers in fruit crops [2].. *Azotobacter* is a free living heterotrophic nitrogen fixing bacteria not only provides nitrogen, but produces a variety of growth promoting substances [3].. *Azotobacter* sp. can also produce antifungal compounds to fight against many plant pathogens. Phosphate solubilizing microorganism group covers bacteria, and fungi which solubilize the unavailable forms of inorganic-P like tricalcium, iron, aluminum and rockphosphates into soluble forms by release of a variety of organic acids like succinic, citric, malic, fumaric, glyoxalic and gluconic acids [4]. Biofertilizers fixes 20-200 kg N/ ha/year, solubilizes phosphorous in the range of 30-50 kg P/ha/year and mobilizes P, Zn, Fe, Mo to varying extent. *Azotobacter* and PSB have been found to enhance growth and production of various fruit plants significantly [5].

Use of bio fertilizer in papaya becoming increasingly popular, it offers considerable benefits over conventional farming system particularly with respect to sustainable yield, better quality and hazard free produce. Apart from this, due to heavy use of inorganic fertilizers, fruits often eaten raw are more vulnerable to contamination with chemicals due to their residual toxicity as compared to cereals and pulses.

Thus, use of beneficial microbes in production of fruits is becoming more popular than other crops.
Hence, the present investigation is very important and opts for crops like papaya. As such judicious application of fertilizers are needed to meet out the nutritional requirement of the plants. The integrated nutrient nourishes papaya, which involves conjunctive use of chemical fertilizers and organic manures to sustain crop production and maintenance of soil health. The beneficial effect of bio-fertilizers is now well established in fruit crops like papaya [6].and banana [7]. In this context, the present investigation was under taken with an objective of finding out the effect of integrated nutrient management in papaya.

2. Materials and Methods

A green house experiment was conducted at Department of Agricultural Microbiology, Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhattisgarh) during the year 2016-2017 with Papaya (*Carica papaya* L.) The experiment was laid out in complete randomized design (CRD) with 7 treatments replicated thrice. Treatments were T_1 Control, T_2 100 % RDF, T_3 50 % N as organic manure + 50 % N as inorganic Fertilizer + 100 % PK, T_4 Azoto 1 + 75 % N+100 % PK, T_5 Azoto 3 + 75 % N + 100 % PK, T_6 Azoto 1 + PSB H27 + 75 % NP + 100 % K and T_7 Azoto 3 + PSB H27 + 75 % NP + 100 % K. Treatment details were given in Table 1. The *Azotobacter* strains (Azoto 1 and Azoto 3) and PSB strain (H-27) were used for inoculation. In RDF, the crop was fertilized with 200-200-200g NPK / plant/ year through urea, single super phosphate and muriate of potash, respectively. A well mixed 5 Kg mixture of soil, sand and compost in 3:1:1 ratio was filled in each polythene bag (12"x 10" size). The soil of the experimental site was vertisol having pH 7.3, organic carbon 6.2 g/kg soil, mineralizable Nitrogen status (204.3 kg/ ha.). The analysis was done as per Page *et al* (1982) [8].

Table 1: Treatment Details

Treatments	Treatment details
T_1	Control
T_2	100%RDF
T_3	50% N as OM+50% N+ 100% PK as RDF
T_4	Azoto 1 +75% N +100% PK
T_5	Azoto 3 + 75% N+100% PK
T_6	Azoto1 + PSB +75% NP +100% K
T_7	Azoto 3 +PSB +75% NP +100% K

Note: *Azotobacter* strains (Azoto 1 and Azoto 3) and PSB strain (H-27) were used for inoculation. OM: organic manure FYM

Uniform seedlings were selected for experiment purpose. Seedlings were inoculated with matured broth of *Azotobacter* and PSB prior to transplantation as per treatments. Efficient strains of both *Azotobacter* (Azoto 1 and Azoto 3) and PSB (H27) were obtained from culture deposit of Agril. Microbiology Deparment, CoA, Raipur. Isolates were activated by inoculating in Jensen's medium and Pikovskaya's media of respective pH (7.0) and temperature (28°C) and characterized for Gram staining [9]., colony morphology, acidity tolerance and temperature tolerance. 7 days old culture was used for morphology, colony characters and Gram's reaction.

After inoculation, papaya seedlings were transplanted so that a single healthy seedling was maintained in each polybag. In uninoculated pots, seedlings were dipped in same amount of nutrient broth but not inoculated with *Azotobacter* and PSB. Nitrogen and Phosphorus through urea and SSP were given respectively after seven days of transplantation of seedlings as per treatment description and Potassium were applied commonly to all polybags through MOP. The seedlings were allowed to grow up to 120 Days. The papaya seedling of variety: Red Lady was transplanted and treatments were given on 2nd November, 2016. Observations were taken at 30 days interval up to 120 DAT of papaya plant. The dry weight of crop plants was recorded after drying in the hot air oven at 70°C to constant weight. Fresh and dry matter yield (plant materials dried to constant weight) in shoot at different stages of transplanting were recorded. Total nitrogen content in shoot of papaya was determined by Kjeldahal method [10] by digesting sample in conc. H_2SO_4 followed by distillation and titration at 120 DAT. Rhizosphere soil samples from different treatments were collected after completion of experiment for enumeration of *Azotobacter* and PSB. Enumeration was done by serial dilution and plating technique[11]. After counting of colonies, the populations were expressed as cfu $g^{-1}$of dry soil using following formula [12].

All observations were statistically analyzed using ANOVA for CRD. The significant difference were tested through F-test at 5% level of significance [13].

3. Results and Discussion

Cultural characteristics of *Azotobacter* and PSB Isolates

Azotobacter (Azoto1 and Azoto 3) and PSB (H 27) isolates used in this study were revived by inoculating *Azotobacter* in Jensen's medium and PSB in Pikovskaya's medium of pH (7.0) and temperature (28°C) each. Bacterial colonies were obtained and were observed for morphological and cultural characteristics. Plate 1. *Azotobacter* isolates (Azoto1 and Azoto 3) were gram negative, in jensen's medium isolate produced gummy, nearly round and convex, colony with entire margine, whitish in colour (Table 2, Plate 1). Colonies of PSB isolate in Pikovskaya's medium were found to be round and yellowish in colour, showing clearing zone. The isolate was Gram negative and belonged to *Pseudomonas* genera based on staining morphological and cultural tests according to Bergey's Manual of Systematic Bacteriology (Table 2 and Plate 1). *Pseudomonas* sp. act as efficient solubilizers of Phosphorous is in line with the findings of Komy [14].

Table 2: Cultural and Morphological characteristics of *Azotobacter* and PSB isolates

Isolates	Colony morplology	Gram Staining
Azotobacter isolate in Jensen's medium		
Azoto 1	Gummy, round and convex,entire margine,whitish in colour	Gram-ve
Azoto 3	Gummy, round and convex,entire margine,whitish in colour	Gram-ve
PSB isolate in Pikovskaya's medium		
PSB H27	Smooth round, entire margin, showing clearing zone,yellowish in colour	Gram-ve

The broth culture of these isolates were used as root inoculants for the experiment. Preparation of inoculated broth and colony morphology of this isolate were clearly depicted in plate-1. Understanding the ecology and characteristics of culture as a prerequisite for inoculant establishment and persistence. Characteristics such as: low pH tolerance, drought and high temperatures tolerance are considered for high adaptability in regions with adverse conditions.

Acidity and temperature tolerance behaviour of *Azotobacter* and PSB isolates

Effect of high temperature and pH on *Azotobacter* and PSB isolates were given in table 2. Both the isolates, Azoto1 and Azoto 3 were observed as acid tolerant isolates and survives as low as pH 4, 5 and 5.5. It was also observed that both the Azotobacter isolates showed most favourable growth at pH 8.0 like that of control. Jimenez *et al*., 2011 [15] also expressed similar views and mentioned that the genus *Azotobacter* is ubiquitous in nature and they can grow with pH ranging from 6.0 to 9.0. PSB H27 was observed as potent acidity tolerant isolates and survives as low as pH 4. However the isolate showed favourable growth at pH5.0 and 5.5. At pH 8 the isolate also showed survibility as that of control (Table 3).

Azotobacter isolates, Azoto1and Azoto3 and PSB isolate, H27 showed full growth at temperature 30 and 35ºC. Growth decreases while increasing temperature from 35ºC to 40ºC. They showed no growth at 55 ºC (Table 3).

As revealed by the results, PSB isolate was able to grow and solubilize P when subjected to stress conditions. It seemed the stress tolerant behaviour of isolates (Table 3), that the isolates have potential to solubilize phosphates at low pH and high temperature which can withstand to extreme dry and hot climate of Chhattisgarh. Ability to withstand against stressed conditions is an important factor which determines the growth and survival of microorganism in soil [16] and is certainly useful in order to formulate those cultures which are able to survive / persist for longer period and work more efficiently under climatic conditions of Chhattishgarh Plains.

Table 3: Acidity and Thermal tolerance behaviour of *Azotobacter* and PSB isolates used as bio fertilizer in papaya

(A) Acidity tolerance behavior of *Azotobacter* and PSB isolates

Growth of *Azotobacter* in Jensen's media of different pH					
Isolate	**4pH**	**5pH**	**5.5pH**	**8pH**	**Control**
Azoto 1	+	+	+	++	++
Azoto 3	+	+	+	++	++
Growth of PSB in Pikovskaya's of different pH					
PSB H27	+	++	++	++	++

Table (B): Thermal tolerance behavior of *Azotobacter* and PS Bisolates

Growth of *Azotobacter* in Jensen's media of different temperature					
Isolate	**30 °C**	**35 °C**	**40 °C**	**55°C**	**Control**
Azoto 1	++	++	+	-	++
Azoto 3	++	++	+	-	++
Growth of PSB in Pikovskaya's of different temperature					
PSB H27	++	++	+	-	++

(++ Very good growth, + good growth, -No growth)

Biomass accumulation in Papaya plants as affected by different treatments

The data on fresh and oven dried shoot biomass per seedling was given in table 4. Seedlings inoculated with *Azotobacter* and PSB inoculation showed significantly higher shoot biomass accumulation over control and uninoculated ones. Shoot fresh weight g/seedling was observed maximum at T_7 (32.42g / seedling) followed by T_6 (30.17 g/seedling) at 120 DAT. Inorganic fertilization T_2 (16.74 g / seedling) is also significantly differ from control while minimum was found at control 13.82 g/ seedling at 120 DAT. Fresh and dry Biomass accumulation in shoot of papaya plant ranged from 13.82 to 32.42 g/seedling and 2.67 to 3.53 g/seedling at 120 DAT respectively under different treatments. *Azotobacter* (Azoto 3) inoculation either alone (3.36 g/ seedling)or in combination with PSB (3.53 g/seedling) significantly increased the shoot dry biomass over control. (Table 4 and Fig.1). Dual inoculation with Azoto 3 + PSB H27 + 75 % NP + 100 % K (T7), treatment produced significantly maximum shoot dry biomass of 3.53g/seedling against 2.92 and 2.67 g/seedling at 100% RDF and control respectively. 32.21% increased in shoot dry biomass accumulation was found by papaya seedling at 120 DAT in Dual inoculation with Azoto 3 (T7)as against the control. Inoculation of *Aztobacter (Azoto 3)* and PSB (H 27) recorded the highest values of DMA, being 3.53 g plant-1 at 120 DAT. There were 1.32 fold increase in DMA as compared with control at 120 DAT. Shiva Kumar *et al.* (2012) [17] reported papaya yield to increase by 10.2% in organic practices, compared to that under 100% recommended dose of fertilizers.

Seedlings inoculated with *Azotobacter* and PSB inoculation showed significantly higher shoot biomass accumulation over control and uninoculated ones. Further application of organic manure there was also significant increase in shoot biomass over control. These results corroborated with the findings of Sukhada *et al.* (1995) who reported that papaya plants inoculated with *G. mosseae* exhibited an increased dry matter (26.6%) under sterile conditions.

Table 4: Effect of different treatments on dry biomass accumulation and N content in shoot of papaya plant at 120 DAT

Treatments	Shoot Fresh weight g / Plant	Shoot Dry weight g / Plant	Concentration of Nitrogen %	Nitrogen uptake (mg /seedling)
T_1	13.82	2.67	0.96	25.62
T_2	16.74	2.92	1.07	31.24
T_3	20.82	3.07	1.08	33.14
T_4	24.07	3.14	1.21	37.98
T_5	26.14	3.36	1.21	40.64
T_6	30.17	3.42	1.24	42.41
T_7	32.42	3.53	1.24	43.76
SEm(±)	0.003	0.003	-	0.004
CD (5%)	0.009	0.011	-	0.013

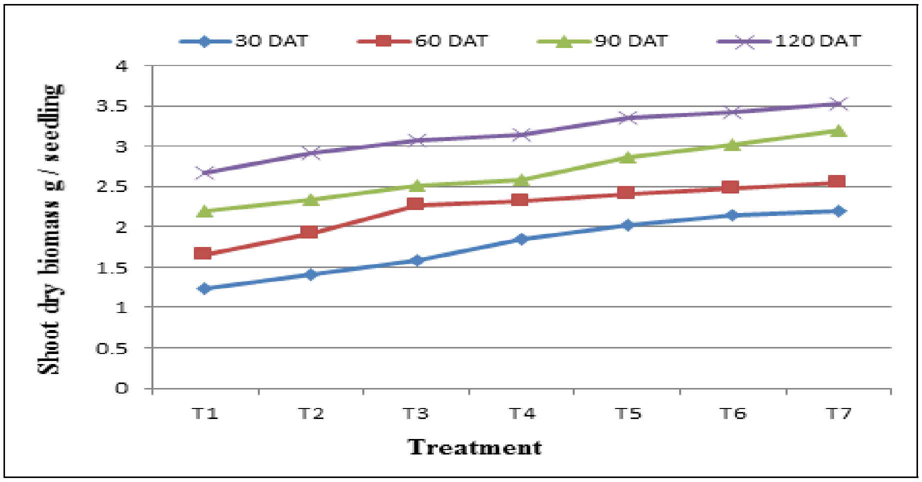


Fig 1: Effect of *Azotobacter* and PSB inoculation on dry biomass accumulation in papaya at different stages

Nitrogen status in papaya plants

Concentration of nitrogen as well as accumulation of N in shoot of papaya plants at 120 DAT was given in (Table 4). Application of *Azotobacter* alone and along with PSB showed higher nitrogen concentration in seedlings than only inorganic fertilization and control. (Table 4). Highest nitrogen concentration in shoot (1.24 %) was found in T7 and T6 followed by T5 and T4 (1.21 %), T3 (1.08 %), T2 (1.07 %) whereas in control it was 0.96 % at 120 DAT. The N content in papaya shoot ranged from 25.62 to 43.76 mg/seedling under different treatments at 120 DAT. The N uptake in shoot was found significantly maximum in T7 (43.76 mg/seedling) followed by T6 (42.41 mg/seedling), T5 (40.64 mg/seedling), T4 (37.98 mg/seedling) and T3 (33.14 mg/seedling) which were significantly higher from control 25.62 mg/seedling at 120 DAT. Significantly highest N uptake in shoot were observed when inoculated with Azoto3+PSB isolate with 75% NP followed by inoculation with Azoto1, PSB and 75% NP. Similar trend in nutrient concentration was reported by Kaushik *et al.*, (2003) [18]. The application of bio fertilizer also increased the uptake of nutrients, which enhanced the growth and development of seedling.

***Azotobacter* and PSB population in rhizosphere soil of papaya plants**

Effect of inoculation on population dynamics of *Azotobacter* and PSB in rhizosphere soil of pot grown papaya was seen in Fig.2. *Azotobacter* population density at 120 DAT increased significantly over control under different treatments. However, highest population density of *Azotobacter* in soil was observed due to inoculation treatments. At 120 DAT *Azotobacter* population density was observed in between 28.62 × 10^4 to 58.22 × 10^4 g^{-1} of soil among treatments being significantly maximum in T7. These study results have close conformity with findings of Torres *et al.* (2000) [19]. This is ascribed due to efficient recycling of nutrients under this treatment. However, significantly highest population density of PSB in soil was observed due to inoculation with PSB H27 and Azoto 3. The PSB population was significantly maximum recorded 8.85× 10^3 was found in T7. Results of present investigation are in confirmation with the findings of Walpola and Yoon (2013) [20]. who reported that remarkable increase in the PSB population was observed in PSB-inoculated rhizosphere soil when compared with uninoculated soil.

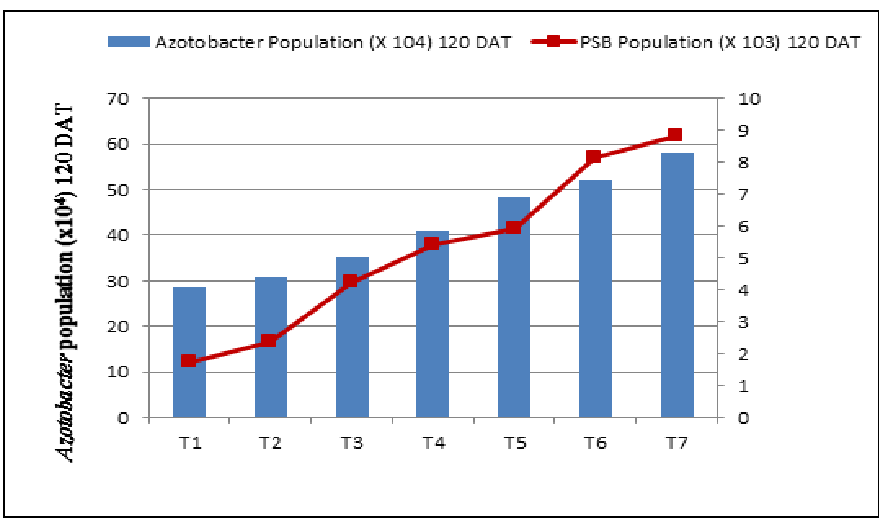
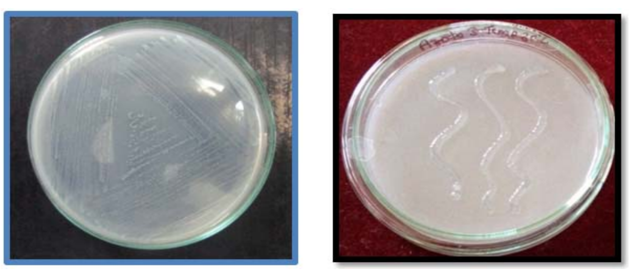


Fig 2: *Azotobacter* and PSB population at 120 DAT

The present findings showed that both *Azotobacter* and PSB application along with 75 %N is comparatively better for improving growth and development of papaya seedling as it influences other process such as nutrient transformation, uptake of trace element and plant hormones. Plate 2. The growth parameters of papaya such as dry weight, N uptake and microbial count were significantly increased by biofertilizer application and 75% NP when compared to inorganic fertilization and control. It is well established fact that dual inoculation of *Azotobacter* and PSB is better as compared to single inoculation of either and obviously over uninoculated treatments. Plate 2. The higher growth in dual inoculation of *Azotobacter* and PSB treated seedling might be due to synergistic effect of PSB with *Azotobacter* inoculation. Such increase in seedling growth might have been attributed due to beneficial effect of PSB on *Azotobacter* which probably induced to the synthesis of growth promoting substances which could stimulate the root growth and elongation, thereby bringing about more nitrogen fixation and crop growth. Further P intake by PSB enhanced BNF, which mediated the efficient uptake of N in seedlings. Such synergistic effect of increased uptake of one nutrient facilitated by other nutrients also reported by Prasad, (1998) [21].

These results were in agreement with earlier works of Ravishankar *et al.* (2008) [22] and Shivakumar *et al* (2012) [17] in papaya cvs. Coorg Honey Dew and Surya respectively. Shiva Kumar *et al.* (2012) reported papaya yield to increase by 10.2% in organic practices, compared to that under 100% recommended dose of fertilizers. Shukhada (1989) [23] also conducted similar glass house studies with papaya to see the response biofertilizer to papaya seedlings. As per Yadav *et al.*, 2011[24] biofertilizer inoculation to papaya seedlings increased in shoot dry matter over control, also increased N content in papaya.



PSB Azotobacter (Azoto 3)

Plate 1: Revive of culture

Plate 2: Better growth of inoculated papaya plant

Conclusion

Dual inoculation involving Azotobacter strain, Azoto 3 and PSB along with 75% NP and 100% K dose significantly effective in increasing growth performances and nitrogen uptake by papaya plants in nursery. This practice may be adopted to improve vegetative growth of papaya in nursery which will decrease in production costs and increase in yield with reduction of diseases in papaya by maintaining healthy plant growth in nursery.

The study suggested that though the co-inoculation had favourable effect, selection of effective strains which were more compatible to *Azotobacter* would be necessary for obtaining the meaningful benefits from co-inoculation. However, further studies in field condition is recommended to ensure the long term growth performance of papaya in response of inoculation under different soils towards reaching a valid conclusion.

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