Advances in Bioresearch
Adv. Biores., Vol 9 (1) January 2018: 92-101

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.9.1.92101

Advances in Bioresearch

ORIGINAL ARTICLE

Evaluation of native Rhizospheric and Phosphate Solubilising Microbes for Growth and Development of *Pongamia pinnata* under Nursery condition

Swatishree Pany, Swagatika Mishra, Nibha Gupta
Plant Pathology and Microbiology Division
Regional Plant Resource Centre, Bhubaneswar -751015 Odisha , India

ABSTRACT
To evaluate the plant growth promoting properties of native rhizospheric microflora of Pongamia pinnata, an important medicinal and biofuel plant, the present study had been planned. A systematic isolation, characterization and identification of rhizosphere soil, collected indigenously, produced 20 no. of fungi and 19 no. of bacteria, initially. Both the groups of organisms were characterized for their extracellular useful activity especially, phosphate solubilization potential and presented in terms of solubilization index, solubilization efficiency and total phosphate solubilized (µg/ml). Selected organisms (four fungi and 10 bacteria) with good phosphate solubilization activity were experimented under pot culture condition for the growth and development of Pongamia pinnata under nursery conditions. Data recorded on 120 days of plant growth exhibited the growth promotion of inoculated plants over control uninoculated experimental set. Results obtained on morphological and physiological growth parameter exhibited the significant effect of Aspergillus ustus and Aspergillus tamarii an enhancing plant height and biomass under treated conditions, indicated the possible role of Aspergillus tamarii in organic acid production, upgradation of mineral status of soil (especially P and K) as well as the establishment of plant in acidic soil and its growth and development. Hence, Aspergillus ustus and Aspergillus tamarii may be potent bioinoculants for the growth and establishment of plantation crops important for biomass, timber and medicinal properties. However, bacterial inoculants may also be useful for the important of growth of tree species, more elaborative experiments on its functional aspects is needed to establish its biofertilizer potential.
Keywords: *Pongamia pinnata,* Phosphate solubilization, fungi, bacteria, growth, forest trees

Received 02.09.2017 Revised 10.10.2017 Accepted 01.01.2018

How to cite this article:
S Pany, S Mishra, N Gupta. Evaluation of native Rhizospheric and Phosphate Solubilising Microbes for Growth and Development of *Pongamia pinnata* under Nursery condition. Adv. Biores., Vol 9 [1] January 2018.92-101.

INTRODUCTION

Microbial diversity varies on account of their harmful activity as pathogenic to different living system to their useful function as producers of enzymes, metabolites, organic acid by which indirectly play vital role in biogeochemical cycle on Earth. As a result soil fertility occurs. Since, most of the tropical soils are phosphate fixing, sometimes 'P' is not readily available for plant usage. Plant utilizes 0.1 % of the Phosphorous present in the Rhizospheric soil, rest is fixed in unavailable form. Presence of the mineral solubilizers in the variety of Rhizospheric environment of plants may be one of the useful strategies to improve the unavailability of 'P' in the vicinity of plant roots [1].

Plant rhizosphere is composite system having many beneficial microbial activities which participate in the plant growth and development in multiferous ways. Circumstantially the addition of new microbes though beneficial, may not be fruitful due to its adaptability towards new microenvironment. Hence, non native microbial inoculants may or may not function vigorously. The role of native microbial strains of different types endowed to the various useful activities are quite imperative in this regard. Application of chemical fertilizers for growth and development of economically important horticulture plants, agricultural crops and forestry is not viable due to its high cost and adverse effects on soil health. Hence, an alternative source of minerals has been explored the several researches [2]. In view, a systematic study has been planned to work out the bio fertilizer potential of native rhizospheric microbes of *Pongamia pinnata* towards growth promotion. This tree species is gaining lot of importance due to its biofuel

properties. Besides, it is useful as anti-oxidant, anti-inflammatory, anti-plasmodial, anti-diarrheal, anti-lipid peroxidated [3]. It is preferred species for controlling soil erosion, good shade tree, can tolerate moderate level of salinity, it is therefore, useful for reclamation of saline soil land.

MATERIALS AND METHODS

(I) Isolation of native microflora, Identification and Characterization:

Soil samples from the native plantation of *Pongamia Pinnata* was collected by digging 10 inches from soil upper layer of the stem/root interface of *Pongamia Pinnata* grows in Botanical Garden of the centre, were pooled and brought to polyethylene bags in the laboratory for analysis. Isolation of bacteria and fungi serial dilution and direct inoculation method was followed for the isolation of bacteria and fungi from the collected soil, [4, 5] on Sabouraud and Nutrient agar media. Scattered colony formed which were separated purified and maintained for the further analysis. Plate culture morphology and slide culture technique were followed for the characterization and identification of fungal cultures [4, 6]. Bacterial cultures were identified for the Gram staining properties and segregated accordingly. Both the groups of bacterial isolates were characterized for biochemical activities also.

(II) Screening of microbial isolates for the extracellular activity:

All bacterial and fungal isolates were evaluated for their beneficial extracellular activities like cellulase, amylase, xylanase, organic acid, lipase, growth hormone production, gelatinase and phosphate solubilisation. To do this individual organism were inoculated on specified media plates and after growth, tested accordingly.

(III) Analysis of Phosphate Solubilizing efficiency:

All microbial isolates were screened on Pikovaskya's plates for their solubilizing activity invitro condition [7]. The halozone forming cultures were selected further for their Phosphate content solubilisation under submerged culture condition by following Vandomolybdate method yellow colour method [8]. Solubilisation efficiency and solubilisation index was calculated as per the formula referenced by Elias *et al*. [9].

(IV) Inoculation studies and pot experiments:

An experiment was carried out in Poly bags of size 12 × 16 cm contained red laterite soil at 35 ± 2 ^{0}C temperature with 60 % to 80 % humidity. The soil was loamy, sandy and having high content of iron, copper and manganese. Soil was analysed for physicochemical properties [10] and used for experiments after fumigation with 1 % Formalin (20 ml / bag) twice for 48 hours. The seeds were brownish in colour and soaked in dilute HCl (0.1 N HCl) overnight at room temperature and sown in poly bags pots. Single seedling was retained in each poly bag. Plants of 30 days old were used for inoculation studies. The experiments were set in two sets for fungal and bacterial inoculants separately. 2.5 ml of seven days old culture (liquid submerged culture) prepared in Sabouraud and Nutrient agar medium of pH 5.8 and 6, respectively and added to each pot containing single seedling of *Pongamia Pinnata* of 30 days old age. This supplementation of fungal culture was done thrice with monthly interval and fungal data was recorded for morphological analysis. Similarly, bacterial culture of 72 hours old where supplemented to the individual pot (25 ml / pot) and repeated at monthly intervals for 120 days. Data on Shoot Vigour Index, Root Vigour Index, NAR, LAR, RGR were also calculated as per the standard protocol [11, 12, 13, 14, 15].

RESULTS

I. Characterization of rhizospheric bacteria

Eighteen isolates obtained from rhizosphere of *Pongamia pinnata* were treated for gram staining properties and grouped into Gram positive and Gram negative bacteria. Among them, seven isolates were confirmed as Gram positive and remaining were Gram negative. Bacterial isolates were treated for to analyse different extracellular activity viz. cellulase, amylase, xylanase, organic acid production, lipase, IAA, gelatinase and phosphate solubilisation activities. Most of the bacterial isolates showed gelatinase activity. However, different bacterial cultures performed and exhibited variously as far as other enzymatic and the metabolic tests were concerned. The bacterial isolates exhibited phosphate solubilisation properties were mainly selected for further studies. Biochemical properties: Altogether 46 different biochemical tests were performed to characterize the selected bacterial isolates. Bacterial isolates PPB-9 and 17 were positive towards phenylalanine deamination tests and lysine. The other three bacterial isolates PPB-7, 6 and 4 had also shown their activity but these were different in arginine and nitrate reduction tests. PPB-4 was nitrate reductase positive where PPB-6 was able to utilize arginine. Bacterial isolate PPB-1 could be able to ferment esculine, glucose, rhamnose along with phenylalanine deamination activity. The two isolates namely PPB-2 and 12 both exhibited positive behaviour in 18 to 20

tests. Differences are due to indole production, arabinose and salicin utilization exhibited by bacterial isolate PPB-2 where as bacteria PPB-12 were malonate positive and showed fermentative properties for mannitol, fructose, raffinose and sucrose. Bacterial isolate PPB-3 was urease positive, ONPG and nitrate reduction positive. Whereas PPB-5 was high fermentative capacity for glucose, mannitol, raffinose, sucrose and xylose.

II. Characterization of rhizospheric fungi

Total 22 nos. of fungi were isolated from rhizosphere of the plant. All these fungi were described morphologically and identified. They belong to *Aspergillus ustus, Aspergillus tamarii, Aspergillus vesicolor, Asperigillus ruber, Aspergillus kanagawaensis*, *Fusarium poea*, *Tricoderma viride*, *Memononiella echinata, Cochliobolus spicifer* and mycelloid. Four fungal strains were remaining unidentified. All fungal isolates were screened for cellulase, amylase, xylanase, lipase, gelatinase, IAA, organic acid production and phosphate solubilisation. Almost all fungi were gelatinase and IAA positive. Mycelloid 1, *Aspergillus vesicular*, mycelloid 2, *Memononiella echinata* were lipase producers. Surprisingly, single fungus was organic acid producer, whereas 17 fungal isolates were phosphate solubilizer which was used further for the experimental studies.

III. Analysis of Phosphate solubilization activity

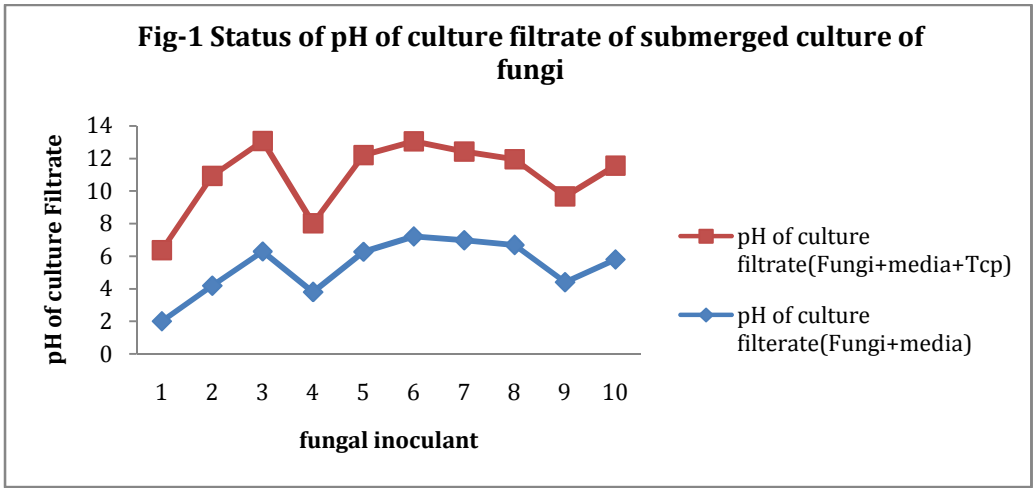
Phosphate solubilization was measured through plate culture for all selected bacteria and fungi. Interestingly, 10 bacteria and 10 fungi were able to produce clear zone in Pikovyaskya's medium containing 0.5 % TCP in solid Agar media. Comparatively, largest zone was observed in *Aspergillus tamarii* and spiny mycelloid fungi (29 mm and 21 mm, respectively) followed by *Aspergillus ustus* and mycelloid 4. The highest solubilization index i.e. 1.806 and 1.791 were exhibited by *Aspergillus tamarii* and spiny mycelloid, respectively. Both the fungi had shown highest phosphate solubilizing efficiency (%) also has been presented (Tab. 1). Almost all bacterial isolates have shown halo zone clearly in the Pikovyaskya's plates and exhibited solubilization potential. The solubilization index was ranged from 1.2 to 1.6 among bacterial isolates. The highest solubilization index was observed in PPB-9 i.e. 1.643 followed by PPB-12 (1.524) and PPB-06 (1.571). PPB-01, 02, 04, 07 performed well as far as Phosphate solubilization and halo zone formation is concerned. Similarly, the profile of solubilization efficiency was also calculated and PPB-09 exhibited highest 164.29 % followed by PPB-06 and PPB-12 (157.14 % and 152.38 %, respectively). Solubilization index and solubilization efficiency are strongly and positively correlated ($P < 0.05$).

Table. 1- Solubilisation efficiency, index, and Phosphate solubilisation potential of Fungal and bacterial strains

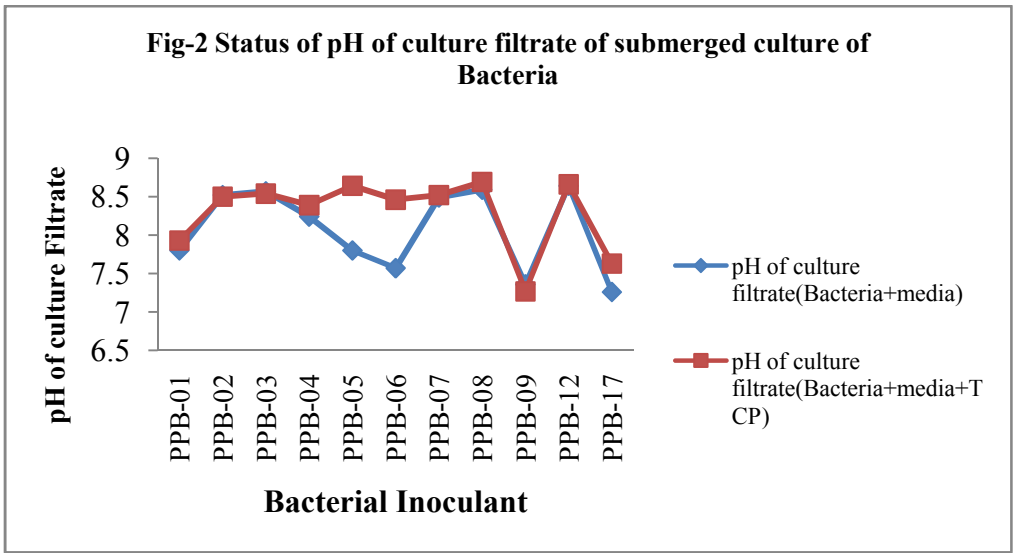
S.NO.	Fungal and bacterial culture	Solubilization index	Solubilization efficiency	Phosphate solubilization(µg/ml)
1	*Aspergillus ustus* (PPF-3)	1.44	143.75	42.15±0.021
2	*Aspergillus sp.* (PPF-4)	1.37	137.31	32.94±0.210
3	Spiny myceloid (PPF-5)	1.79	179.16	10.16±0.200
4	*Aspergllus tamarii* (PPF-8)	1.81	180.60	28.70±0.070
5	Mycelloid 1 (PPF-09)	1.28	127.58	00.25±0.030
6	Mycelloid 2 (PPF-13)	1.22	122.22	21.40±0.190
7	Mycelloid 3 (PPF-14)	1.48	147.61	06.20±0.050
8	Mycelloid 4 (PPF-15)	1.25	125.00	07.50±0.020
9	*Aspergillus kanagawaensis* (PPF-21)	1.13	112.90	44.06±0.090
10	*Fusarium oxysporum* (PPF-22)	1.10	110.00	04.55±0.003
11	PPB-01	1.43	142.86	00.15±0.003
12	PPB-02	1.47	147.37	00.11+0.002
13	PPB-03	1.21	121.43	00.28±0.010
14	PPB-04	1.42	141.67	00.13±0.004
15	PPB-05	1.23	123.08	03.06±0.030
16	PPB-06	1.57	157.14	00.93±0.040
17	PPB-07	1.32	131.59	00.12±0.003
18	PPB-08	1.43	142.86	00.10±0.002
19	PPB-09	1.64	164.29	00.08±0.001
20	PPB-12	1.52	152.38	00.13±0.040
21	PPB-17	1.31	130.77	00.03±0.050

Phosphate solubilization potential in submerged culture condition of all selected bacteria and fungi was also tested. The data recorded on pH of culture filtrate and Phosphate solubilization has been presented (Fig. 1, 2, Tab. 1). It is clearly evident that the most of the fungal culture are acid producers as they declined the pH of the normal medium after their growth in submerged liquid culture. However, no correlation was formed in between pH of the culture filtrate and total amount of phosphate solubilized ($R^2 = 0.6293$, $P < 0.05$). The *Aspergillus ustus* solubilized 42.15 ± 0.021 µg/ml phosphate into the media followed by *Aspergillus* sp. 4 (32.94 µg ml^{-1}) and *Aspergillus tamarii* (28.70 µg ml^{-1}). However, the solubilization potential of fungal culture in liquid submerged condition did not coincide with the plate culture tests.

On the other hand, bacterial isolates grown in submerged condition along with TCP did not decrease the pH even increase the final pH after 72 hrs of incubation at 37 ^{0}C. Though, all bacterial culture showed halo zone formation on Pikovyaskya's plates, their submerged culture phosphate solubilization capabilities were poor as compared to fungal cultures. PPB-5 could be able to solubilise 3.0 µg ml^{-1} of Phosphate in the liquid media. However, all fungal and bacterial cultures were used as inoculants for the further pot experiment to evaluate their potential in plant growth and development of *Pongamia pinnata* in nursery condition.



Abbreviation: 1,*Asperigillus ustus,*2,*Asperigillus sp*,3,Spiny myceloid,4,*Asperigillus tamarii,*5,Myceloid,6, Myceloid,7, Myceloid,8, Myceloid,9, *Asperigillus kanagawaensis,*10, *Fusarium oxysporum.*



(IV) Evaluation of Phosphate solubilizing microbial isolates under pot experiment:-

Inoculation of different fungal strains has resulted in different pattern of plant growth as compared to uninoculated control. Tab. 2 presents the data of shoot height measurements of seedlings in the nursery. It is evident that there are prominent and significant differences in seedlings height of control and those

of inoculated seedlings. The application of *Aspergillus ustus* and *Aspergillus tamarii* showed maximum plant height as compared to other two treatments (P < 0.01). However, all inoculants exhibited growth promoting effect over control seedlings. The effect of some fungal application like *Aspergillus kanagawaenesis* and *Fusarium oxysporum* and their establishment is quite visible and significantly higher than the uninoculated control, hence, all four fungal inoculation resulted in 39.51 to 59.25 % higher shoot length as compared to uninoculated control.

Table. 2- Growth parameters of *Pongamia pinnata* grown under (fungal) experimental conditions

Sl.No.	Growth Parameters	Control	*Asperigillus ustus*	*Asperigillus tamarii*	*Asperigillus kanagawaensis*	*Fusarium oxysporum*
1	Length of shoot (in cms)	35.63 ± 06.89	110.66 ± 25.60**	107.18 ± 29.99**	94.83 ± 27.30	96.94 ± 29.44
2	No.of leaves	22.60 ± 04.44	028.60 ± 10.94	029.33 ± 12.83	31.73 ± 10.18*	25.40 ± 08.84
3	No.of branches	13.15 ± 01.32	015.47 ± 04.57NS	014.93 ± 04.73NS	15.87 ± 03.74NS	13.00 ± 03.18NS
4	Fresh weight of shoots(gms)	49.53 ± 10.01	068.62 ± 18.49**	057.93 ± 14.93*	69.20 ± 21.36**	70.67 ± 23.05**
5	Dried weight of shoots(gms)	31.70 ± 10.38	045.46 ± 12.84**	043.65 ± 14.49**	52.26 ± 21.35**	54.28 ± 21.22**
6	Length of roots(cms)	43.82 ± 14.76	061.33 ± 15.33*	057.40 ± 05.58*	63.73 ± 11.23*	65.17 ± 05.84*
7	Fresh weight of root(gms)	38.58 ± 06.93	056.15 ± 07.22**	061.42 ± 05.71**	60.37 ± 02.66**	57.67 ± 03.44**
8	Dried weight of roots(gms)	24.97 ± 4.747	040.39 ± 2.55**	047.60 ± 06.59**	45.95 ± 01.46**	35.84 ± 06.99**
9	Shoot vigour index	2842.62 ± 328.30	4527.10 ± 1101.80	4384.73 ± 1226.91	3879.36 ± 1116.80	3965.82 ± 1246.80
10	Root vigour index	1793.90 ± 603.90	2509.15 ± 0627.25	2348.23 ± 0228.07	2607.17 ± 0459.14	2665.97 ± 0238.80
11	Net assimilation rate	384.08 ± 122.90	2299.42 ± 0632.45	2379.05 ± 0663.39	3247.67 ± 0171.33	0945.88 ± 0016.23
12	Leaf area ratio	36.22 ± 09.48	40.40 ± 10.84	35.26 ± 8.81	28.44 ± 2.07	13.33 ± 1.93
13	Relative growth rate	0.44 ± 00.14	00.94 ± 00.29	00.98 ± 0.31	01.38 ± 0.06	01.18 ± 0.10

± = implies Standard deviation of 15 replications; **= significant at P<0.01; * =significant at P<0.05; NS- Not significant

The inoculation of phosphate solubilizing fungi also yielded good root growth in supplemented seedlings as compared to non supplemented plants. Variable but significantly longer root had been observed in supplemented plants as compared to the non-supplemented control (P < 0.05). *Fusarium oxysporum* has yielded 48.72 % longer roots followed by *Aspergillus kanagawaenesis* that has given 45.48 % longer roots than uninoculated control. Similar differential responses due to different inoculations were observed in leaf and branches numbers in supplemented seedlings over control. However, no significant difference was observed for the number of branches in supplemented plants over control, though *Aspergillus kanagawaenesis* enhanced the leaf number by 40.31 % as compared to the control (P < 0.05).

Mean Biomass (fresh and dry) measured after four months indicated the maximum increment in growth of plants inoculated with these fungi. It is apparent that, in the term of biomass, the seedlings inoculated with different fungi showed a higher production and superiority over control. On the other hand, most of the fungal strains exhibited higher fresh and dry biomass of shoot and root in supplemented seedlings as compared to the uninoculated control. It is evident that seedlings inoculated with *Aspergillus ustus* and *Aspergillus tamarii* showed better shoot growth and *Aspergillus kanagawaenesis* and *Fusarium oxysporum* showed highest biomass as compared to other two treatments (P < 0.01). *Fusarium oxysporum* and *Aspergillus kanagawaenesis* produced 83.84 % and 64.85 % higher dry biomass of shoots whereas *Aspergillus tamarii* helped in enhancing root dry biomass by 90.6 % over control (P < 0.01). Relative growth rate (RGR) was also changed due to the enhancement, stem height and leaf area in supplemented

seedlings. However, *Aspergillus kanagawaenesis* and *Fusarium oxysporum* showed higher RGR, with the inoculation of *Aspergillus kanagawaenesis* and *Fusarium oxysporum* where growth of seedlings was maximum. *Aspergillus kanagawaenesis* and *Aspergillus tamarii* showed higher Net assimilation rate followed by *Aspergillus ustus.* Net assimilation rate (NAR) measured 3247.67 and 2379.05 gm^{-2} d^{-1}, respectively. *Aspergillus ustus* and *Aspargillus tamarii* showed quite higher LAR (leaf area ratio) as compared to the uninoculated seedlings. Data recorded on Root vigour index (RVI) presented in Tab. 2 exhibited the superior response of *Aspergillus kanagawaenesis* and *Fusarium oxysporum* over control and Shoot vigour index (SVI) showed superior response of *Aspergillus ustus* and *Aspergillus tamarii* while *Aspergillus kanagawaenesis* and *Fusarium oxysporum* improved the Root vigour index under inoculated conditions.

Inoculation of different bacterial strains has resulted in different pattern of plant growth as compared to uninoculated control. Tab. 3 presents the data of shoot height measurements of seedlings in the nursery. It is evident that there are prominent and significant differences in seedlings height of control and those of inoculated seedlings. The application of PPB-9 and PPB-12 followed by PPB-1 showed maximum plant height as compared to other six treatments ($P < 0.01$). However, all inoculants exhibited growth promoting effect over control seedlings. The effect of some bacterial application like PPB-9 and PPF-4 followed by PPB-1 and 12 and their establishment is quite visible and significantly higher than the uninoculated control. Both the bacterial strains 9 and 12 enhanced the plant shoot length by 53.24 and 51.61 % over control. However, PPB-9 also contributed in improving the branches development as well as increasing no. of leaves of the plant. Due to this dry biomass of shoot was also upgraded by 83.54 %. Similarly, PPB-12 also helped in biomass enhancement overall.

The inoculation of phosphate solubilizing fungi also yielded good root growth in supplemented seedlings as compared to non supplemented plants. Variable but significantly longer root had been observed in supplemented plants as compared to the non-supplemented control. PPB-1 bacterial strain poised to be best among all 10 strains inoculated in the present study. It helped in development of root length and enhancement of fresh and dry biomass of roots. Similar differential responses due to different inoculations were observed in leaf and branches numbers in supplemented seedlings over control. However, significant difference in PPB-4 was observed for the number of leaves in supplemented plants over control ($P < 0.05$).

Mean Biomass (fresh and dry) measured after four months indicates the maximum increment in growth of plants inoculated with these bacteria (Tab. 3). It is apparent that, in the term of biomass, the seedlings inoculated with different bacteria showed a higher production and superiority over control ($P < 0.01$). On the other hand, most of the bacterial strains exhibited higher fresh and dry biomass of shoot and root in supplemented seedlings as compared to the uninoculated control. It is evident that seedlings inoculated with PPB-1 and PPB-5 showed better growth and PPB-1 and PPB-5 showed highest biomass as compared to other six treatments. Net assimilation rate (NAR) was also changed due to the enhancement in dry biomass, stem height and leaf area in supplemented seedling. However, PPB-9 and PPB-17 showed higher NAR. Net assimilation rate (NAR) measured 323.59 and 239.71 gm^{-2} d^{-1}, respectively. PPB-6 showed quite higher LAR (leaf area ratio) as compared to the control. Data recorded on RVI presented in table-3 exhibited the superior response of PPB-1 and PPB-2 followed by PPB-4 and PPB-3 over control and SVI showed superior response of PPB-9 and PPB-12.

The soil used in the experiment was red laterite soil having acidic pH and poor in mineral content. The texture was also rough, dry and porous nature. The physiochemical analysis of experimental soil represented in the Tab. 4 exhibited the decline in pH of soil used in inoculation experiment of fungal inoculants. Among four fungal inoculations implemented during the pot experiment revealed the maximum decrease of pH in test soil inoculated with *Aspergillus ustus*. The soil pH recorded was acidic in nature and it was ranged 5.20 to 5.53. The Present study revealed the role of phosphate solubilizing fungi in changing the pH status of soil due to its organic acid production. These organism had also been exhibited the organic acid production in plate culture test when inoculated in the sabourauds medium along with the blue colored dye "Bromothymol blue". In all, all four inoculated used in the experiment were organic acid producers especially, PPF-3 and PPF-8 followed by PPF-21 and PPF-22. In case of bacterial inoculations, the treated soil especially of PPB-2, showed 4.99 pH. All bacterial treated soil exhibited acidic pH except PPB-12 which increased the pH 6.12. As far as the EC is concerned, all treated soil showed varied results ranged from 0.02 to 0.15 d S m^{-1} ($P < 0.01$). Similarly, comparatively higher OC (g kg^{-1} soil) was recorded in all fungal and bacterial inoculated soil over control except the soil inoculated with PPB-1 and *Fusarium oxysporum*.

Analysis of NPK of the soil collected from different experimental plant rhizosphere showed significant differences as compared to the control ($P < 0.05$). Almost all treatment exhibited the enhanced quality of

N in the soil except *Aspergillus kanagawaensis* and Fusarium oxysporum and PPB-6. All fungal treated soil qualified for phosphorous showed less value as compared to the control (86.00 ± 4.24 kg ha^{-1}). In similar way, PPB-2 showed 41.75 ± 003.9 (kg ha^{-1}) P in the soil. Other bacterial treatment showed either enhanced level of P or decreased amount of P in soil. As far as quantity of K is concerned, all treated soil less amount of K as comparision to the uninoculated control soil. *Aspergillus tamarii* and F significantly decreased the K content i.e. 251.00 ± 67.90 and 286.50 ± 57.30 (kg ha^{-1}), respectively ($P < 0.05$). Bacterial inoculation also affected the soil K content significantly at $P < 0.05$ level. It was 295.00 ± 18.00 and 243.00 ± 46.70 (kg ha^{-1}) in case of PPB-1 and PPB-8, respectively.

Table-3: Growth parameters of *Pongamia pinnata* grown under (bacterial) experimental conditions:

Sl.No.	Growth parameters	Control	PPB-1	PPB-2	PPB-3	PPB-4	PPB-5	PPB-6	PPB-7	PPB-8	PPB-9	PPB-12	PPB-17
1	Length of shoot(cms)	35.63± 6.89	50.6±9.97**	39.10±5.62	42.60 ±5.50	39.08 ±4.95	48.2 ±4.87	44.7 ±7.51	31.24 ±16.86	29.7 ±6.22	54.6±11.99**	54.02±10.42**	46.84±7.08
2	No. of leaves	22.80± 4.06	29.8±10.47	31.80±10.21	25.00 ±4.06	36.4±11.08**	26.8 ±6.46	24.6 ±5.60	22.4 ±10.07	19±4.53	33.4 ±7.34**	30.2 ±5.72*	28.2 ±6.38
3	No. of branches	11.40± 1.25	13.60 ±3.05	13.80±03.35	13.40 ±1.34	16.4 ±3.21	14±1.87	12.6 ±2.07	11.4 ±3.65	10.8 ±1.30	15.2 ±2.05	14.4 ±2.30	14.6 ±2.07
4	Fresh weight of shoot(gms)	17.77± 2.94	22.08 ±2.84**	20.79 ±1.83**	20.41 ±2.20**	19.29 ±1.47	22.8±4.16	20.28 ±3.99	17.18 ±10.55	18.74 ±4.35	22.56±2.68**	24.21±2.58**	22.62 ±2.98**
5	Dried weight of shoot(gms)	09.54± 1.94	12.68±3.03**	10.51 ±01.41	11.08 ±1.46	10.1 ±3.08	8.568 ±1.63	5.609 ±3.55	9.498 ±8.39	10.078 ±3.82	17.51±1.49**	14.67 ±2.69**	12.9 ±3.66**
6	Length of root(cms)	18.39± 5.74	36.23 ±5.32**	28.50±3.12*	27.50 ±6.50*	27.77±5.64*	20.1 ±1.35	19.5 ±3.50	23.6 ±2.25	13.57 ±4.52	25.77 ±1.23	24.6 ±3.15	27.5±1.23**
7	Fresh weight of root(gms)	16.90± 1.34	25.21±1.79	23.09±02.43	23.05 ±3.84	24.06 ±1.28	18.84 ±2.72	15.7 ±1.56	20.21 ±1.11	15.92 ±2.29	16.19 ±2.16	18.6 ±2.29	22.73 ±1.78
8	Dried weight of root(gms)	08.81± 2.16	14.36±2.09	10.74±02.87	12.37 ±3.67	12.35 ±1.72	10.21 ±3.07	7.82 ±1.97	9.51 ±0.62	7.23 ±1.22	9.14 ±1.94	12.01 ±1.54	14.43 ±2.98

9	Shoot vigour index	1212.57±466.35	2070.05±407.66	1640.49±300.07	1742.77±227.04	1598.76±202.63	1971.86±199.16	1828.68±307.37	1278.03±689.53	1215.01±254.56	2233.69±490.58	2209.96±426.33	1916.22±289.72
10	Net assilmilation	137.28±18.32	231.79±34.50	0163.21±031.27	0179.17±43.67	155.997±11.13	196.007±29.45	116.507±40.80	199.24±106.58	125.297 ±0.404	323.59 ±31.7	183.8 ±7.77	239.71 ±48.99
11	Root vigour index	481.37±151.22	1482.31 ±217.49	1165.94±127.74	1125.03±265.92	1135.93±230.56	1015.93±336.41	797.75 ±147.19	965.48 ±92.21	555.01 ±185.06	1054.11 ±61.55	1006.39 ±157.81	1125.03 ±61.37
12	Leaf area rate	63.87 ±7.31	56.319±9.74	61.627 ±11.6	54.917 ±10.63	56.293 ±15.72	80.74 ±18.37	89.593 ±16.76	71.527±25.34	70.313 ±3.83	70.64 ±11.51	44.67 ±0.49	48.92 ±7.12
13	Relative growth rate	0.16 ±0.02	0.232±0.04	0.187±0.04	0.209 ±0.05	0.196 ±0.04	0.173 ±0.04	0.124 ±0.04	0.196 ±0.10	0.149 ±0.01	0.23 ±0.03	0.24 ±0.01	0.25 ±0.03

± = 15 Replication; ** = Significant at P<0.01; * = Significant at P<0.05

Table. 4- Physiochemical properties of experimental soil:

Microbial inoculants	pH	EC (dSm^{-1})	OC (g/kg soil)	Avl. N (kg/ha)	Avl. P (Kg/ha)	Avl K (kg/ha)
Control	5.53 ± 0.05	0.08 ± 0.026	6.08 ± 2.69	408.50 ± 71.42	086.00 ± 004.24	590.75±18.44
Aspergillus ustus (PPF-3)	5.20 ± 0.01	0.15 ± 0.005	6.59 ± 0.13	513.50 ± 74.10	050.75 ± 013.80	528.00±00.00
Aspergillus tamarii (PPF-8)	5.52 ± 0.62	0.02 ± 0.017	6.78 ± 1.70	537.00 ± 32.50	068.50 ± 026.20	251.00±67.90
Aspergillus kanagawaensis (PPF-21)	5.56 ± 0.01	0.02 ± 0.002	8.08 ± 1.97	389.00 ± 66.50	075.50 ± 007.80	337.00±31.10
Fusarium oxysporum (PPF-22)	5.34 ± 0.15	0.02 ± 0.008	3.44 ± 1.18	350.50 ± 77.10	074.00 ± 012.70	286.50±57.30
PPB-1	5.52 ± 0.21	0.02 ± 0.002	4.93 ± 1.18	560.50 ± 88.40	071.00 ± 004.20	295.00±18.00
PPB-2	4.99 ± 0.15	0.15 ± 0.064	6.60 ± 1.18	428.00 ± 21.60	041.75 ± 003.90	338.00±29.70
PPB-3	5.58 ± 0.69	0.08 ± 0.008	6.50 ± 0.27	474.50 ± 33.20	083.00 ± 018.40	437.00±07.10
PPB-4	5.11 ± 0.11	0.15 ± 0.055	6.52 ± 0.25	498.50 ± 21.90	131.00 ± 035.40	514.00±63.60
PPB-5	5.50 ± 0.18	0.12 ± 0.052	8.05 ± 1.39	584.00 ± 55.20	104.00 ± 018.40	492.50±38.90
PPB-6	5.67 ± 0.05	0.12 ± 0.020	6.59 ± 1.97	397.00 ± 32.50	069.00 ± 009.90	473.50±12.00
PPB-7	5.52 ± 0.37	0.04 ± 0.001	6.97 ± 0.66	522.50 ± 53.00	093.00 ± 008.50	438.00±66.50
PPB-8	5.38 ± 0.15	0.04 ± 0.020	5.67 ± 0.39	490.00 ± 55.20	087.00 ± 022.60	243.00±46.70
PPB-9	5.84 ± 0.08	0.02 ± 0.005	6.13 ± 1.05	498.00 ± 43.80	105.50 ± 026.20	510.50±50.20
PPB-12	6.12 ± 0.37	0.03 ± 0.005	7.62 ± 1.58	405.00 ± 00.00	139.50 ± 020.50	369.00±56.60
PPB-17	5.76 ± 0.23	0.11 ± 0.092	7.90 ± 5.09	513.50 ± 65.80	253.50 ± 004.90	429.00±42.20

DISCUSSION

In order to evaluate the impact of rhizospheric indigenous microflora endowed with useful extracellular activity especially phosphate solubilizing fungi and bacteria, an experiment under pot culture condition was carried out. The samples of rhizosphere of *Pongamia pinnata* was red laterite soil and having low 'P' content and acidic in nature. Hence, the possibilities of occurrence of Phosphate solubilization and their

phosphate solubilizing potential invitro are quite imperative. It is confirmed that the rhizosphere of the *Pongamia pinnata* is a good source of inhabitant bacteria and fungi which are full of enzymatic and mineral solubilizing potential. Many of them were confirmed as good and potential Phosphate solubilizer under submerged liquid culture condition. However, the fungi isolated from rhizospheric soil were Phosphate solubilizing as compared to bacterial isolates. Hence, we could expect their plant growth promoting properties as well. Microbial inoculants are recommended in the form of liquid culture to raise healthy seedlings of forest trees at nursery conditions [16]. The cost effective technology as an alternative like mineral solubilizer bioinoculants are also in demand due to the high cost of chemical fertilizer as well as its limited supply or availability to the crop plants. To this context, the present experiment set up on *Pongamia pinnata* under nursery conditions along with different treatments has also exhibited the promising and effective microbial potential in the growth improvement. Under experimental conditions *Asperigillus sp.* was found to be very promising in increasing plant growth including (shoot height, no. of leaves, no. of branches, fresh weight of shoots, dried weight of shoots, shoot vigour index, Root vigour index, RGR, NAR, LAR). However, *Fusarium oxysporum* fungus was also prominent in improving length of shoot, length of roots and weight of roots of seedlings of *Pongamia pinnata*. Variations in plant growth due to fungal inoculation may be credited to their Phosphate solubilization potential and availability of Phosphate as well. Survivals of plants are upto 120 days. Without any fertilizer treatment except bioinoculants indicate the positive role of fungus towards seedling establishment and survival. It could happen due to the availability of nutrients [17].

Phosphate solubilizing fungi *Aspergillus* and *Pencillium* were reported as Phosphate solubilizers as well as growth promoters for host plants [18]. Present studies also confirm the inoculation of *Asperigillus sp.* and PPB-1 in the rhizosphere of *Pongamia pinnata* is effective. Significant difference in morphological and physiological growth has been observed in the experimental plants. The present study has also been corroborated with the findings of the response of fungal inoculation on forest trees like *Dalbergia sissoo* and *Acacia auriculiformis* [19,20].

Several reports are available on the role of organic acid in the phosphate solubilization in vitro condition. It is difficult to analyze and evaluate the organic acid production capacity of these fungi in field condition. However, decline in the pH of experimental rhizosphere soil, indicate the presence of these factors responsible for change in the pH of the medium.

Question arises that whether acid production is correlated with the 'P' content of the soil or not. It is clearly evident from the present study that *Aspergillus ustus* decline the pH of the soil and same had been found with low amount of 'P' as compared to the uninoculated control soil. The low amount of 'P' content in the soil of experimental plant rhizosphere indicate the availability of the soluble Phosphorus and its subsequent utilization by the host plants, as the experimental plants of these sets were produced maximum plant height and biomass as compared to the control.

The soil 'P' content of soil of experiment was also correlated with the plant shoot height, no. of leaves, no. of branches and root height. Biomass at $p < 0.05$ level and it was significant. In contrast, the decline of pH in the bacterial experimental set was not found in the present study. All the experimental soil treated with the bacterial inoculation was observed as alkaline. It is also very clear that 'P' content of the experimental soil was low as compared to control soil. But not significantly at par with the fungal inoculated soil.

It is evident from the present study that bacterial inoculations were not effective for the plant growth and development of *Pongamia pinnata* as compared to fungal inoculation. We may interpret the responsible factor the organic acid for the 'P' solubilization, as the low amount of the 'P' content in soil was present as compared to control. We may opined the utilization of more 'P' content by *Pongamia pinnata* plants in the experimental conditions. Indirectly, it helps in enhancing the growth of plants. Hence, it had lowered down the 'P' content in soil of surrounding of plants.

The present study based on the inoculation studies on growth and development of *Pongamia pinnata*, indicated the good effect of bioinoculation of fungi and bacteria of phosphate solubilizing nature. The fungal inoculants exhibited potential effect on 'P' solubilization and ultimately on growth of plants. Bacterial inoculants were poor comparatively. Though, these organisms were native to *Pongamia pinnata* and obtained for its own rhizosphere also proved the importance and usefulness of native microbes. An elaborative experiment on the effect of fertilizer, its combination with biological inoculants, seasonal variation; edaphic and other environmental factor, stress factor on the performance of bioinoculants, followed by plant growth is needed to develop any biofertilizer components on consortium. However, the data obtained through present experimental work is useful for the development of quality planting materials for different forest plantations as well as commercial use as their species endowed with biofuel and medicinal properties.

The experimental results obtained from these studies have clearly evidenced that application of Bioinoculants is safe and useful for amendments of nutritional studies of soil, improvement of acidic soil properties and for increasing the growth of plantation seedlings to significant extent.

ACKNOWLEDGEMENTS

The financial assistant obtained from the forest and environment dept., Govt. of Odisha (State plan 2016-17) is gratefully acknowledged.

REFERENCES

1. Nayak, S.R., Panigrahi, M.R., Gupta, N. (2016). Inoculation of *Pongamia pinnata* with Phosphate solubilizing Fungi: Effect on plant growth and transplantation establishment. International journal of Pharmaceutical and Biological research, 1(5):14-21.
2. Abbasniayzare, S.K., Sedaghathoor, S., Dahkaei, M.N.P. (2012). Effect of Biofertilizer application on growth parameters of *Spathiphyllum illusion.* American-Eurasian Journal of Agricultural and Environmental Science, 12(5):669-673.
3. Chopade, V.V., Tankar, A.N., Pande, V.V. *et al*. 2008. *Pongamia pinnata*: Phytochemical constituents, traditionaluses and pharmalogical properties. International Journal of Green Pharmacy, 2:72-5.
4. Nagamani, A., Kunwar, I.K., Manoharachary, C. (2013). Handbook of Soil fungi.. I.K. International, Pvt. Ltd. S-25 Green Park Extension, New Delhi.
5. Zhu, F., Qu, L., Hong, X., Sun, X. (2011). Isolation and Characterization of a Phosphate-Solubilizing Halophilic Bacterium *Kushneria* sp. YCWA18 from Daqiao Saltern on the Coast of Yellow Sea of China. Evidence–Based Complimentary and Alternative Medicine, 2011:6.
6. Barnett, H.L., Hunter, B.B. (1972). Identification of Fungi. Illustrated Genera of Imperfect Fungi.
7. Karpagam, T., Nagalahskmi, P.K. (2014). Isolation and characterization of Phosphate Solubilizing Microbes from Agricultural soil. International Journal of Current Microbiology and Applied Sciences, 3(3):601-614.
8. Tandon, H.L.S. (1999). Methods of analysis of soils, plants, waters and fertilisers. Publ. Fertiliser development and consultation organization, New Delhi, India pp.13-35.
9. Elias, F., Woyessa, D., Muleta, D. (2016). Phosphate Solubilization Potential of Rhizosphere Fungi Isolated from Plants in Jimma Zone, Southwest Ethiopia. International Journal of Microbiology, 2016:11.
10. Asema, S.U.K., Tanveer, S.T., Sultan, S. (2015). Analysis of Soil Samples for its Physico-Chemical Parameters from Aurangabad City. International journal of innovative research and development, 4(13).
11. Leopold, A.C., Kriedemann, P.E. (1975). Plant Growth and Development. McGraw-Hill Book Co., New York.
12. Noggle, G.R., Fritz, G.J. (1986). Introductory Plant Physiology. Prentice Hall of India Private Ltd., New Delhi, 1986.
13. Basak, U.C., Gupta, N., Rautaray, S., Das, P. (2004). Effects of salinity on the growth of mangrove seedlings. Journal of Tropical Forest Science, 16:437-439.
14. Tiwari, M.R., Khanal, S., Shrestha, B., Jha, R.K. (2006). Nutritional variation of different feed ingredients and compound feed found in different parts of Nepal. Nepal Agric. Res. J., 7:75-81.
15. Dickson, A.A., Leaf, L., Hosner, J.F. (1960). Quality appraisal of white spruce and white pine seedling stock in nurseries. Forestry Chronicle, 36:10-13.
16. Odee, D.W., Haukka, K., McInroy, S.G., Sprent, J.I., Sutherland, J.M., Young, J.P.W. (2002). Genetic and symbiotic characterization of rhizobia isolated from tree and herbaceous legumes grown in soils from ecologically diverse sites in Kenya. Soil Biol Biochem., 34:801–811.
17. Nenwani, V., Doshi, P., Saha, T., Rajkumar, S. (2010). Isolation and characterization of a fungal isolate for phosphate solubilization and plant growth promoting activity. Journal of yeast and fungal research, 1(1):009-014.
18. Dastager, S.G., Deepa, C.K., Pandey, A. (2010). Isolation and characterization of novel plant growth promoting Micrococcus sp NII-0909 and its interaction with cowpea. Plant physiology and biochemistry, 48(12):987-992.
19. Dash, S., Mohapatra, A.K., Gupta, N. (2013). Growth response of *Dalbergia sissoo* Roxb. to mineral solubilizing bacteria and fungi in nursery conditions. International society of tropical ecology, 54(1):109-115.
20. Dash, S., Gupta, N. (2011). Microbial bioinoculants and their role in plant growth and development. International journal for biotechnology and molecular biology research, 2(13):232-251.