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ORIGINAL ARTICLE **OPEN ACCESS**

Role of growth promoting microbes in growth improvement of *Holoptelia integrifolia* seedling for plantation on iron ore mine overburden

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ABSTRACT
The aim of present work to screen growth-promoting microbes (AM fungi, fluorescent Pseudomonas and Penicillium) to apply in Holoptelia integrifolia during seedlings production for plantation on iron ore mine overburden soil. Penicillium sp. 1 screened from 99 fungal isolates based on amylase and cellulase activities. Similarly, fluorescent Pseudomonas strain 1 was also screened. An experiment was conducted on Holoptelia integrifolia to test the role of selected microbes and AM fungi. Sapling growth was assessed by different parameters. Results revealed that inoculation of fluorescent Pseudomonas strain 1 + AM fungi was increased seed germination, shoot length, root length, N, P and Mg content in leaf. Collar diameter was maximum in fluorescent Pseudomonas strain 1+ Azospirillum sp. + AM fungi. Maximum leaf number was observed in fluorescent Pseudomonas strain 1 alone. Moisture contents of shoot and root were highest in AM fungi and Azospirillum alone, respectively. K and Ca contents in leaf were maximum in Azospirillum + AM fungi and AM fungi alone, respectively. Maximum root colonization was observed in AM fungi (93.3%). It is concluded that fluorescent Pseudomonas strain 1 + AM fungi have a synergistic effect and may be used as biofertilizer to increase the productivity of H. integrifolia in iron ore mine soil.
Keyword: *Afforestration, Biomass, companion fungus, Degraded land, enzymatic activity, Synergistic effect*

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INTRODUCTION

Opencast mining, caused several changes in physical, chemical and microbiological properties of soil [26]. Its creates much higher quantities of waste as compared to underground mining. Damage the topsoil layer and its effect caused several changes in physical, chemical and microbial properties of soils [46]. Soil fertility was increase and maintained by microorganism [53]. The microbial population present in the stress environment have adapted and become resistant to harsh condition [54, 27] may be used as biofertilizer [8].

Biofertilizer has positive quantitative effect on growth and vigor of plants [37]. They have a different form like organic manure, vermin compost, PGPR, AM fungi etc. Plant growth-promoting rhizobacteria (PGPR) are naturally-occurring, free-living soil bacteria that are capable of colonizing roots and enhancing plant growth when added to seeds or roots [49], accelerates the extent of nutrient availability, supplements the demand of chemical fertilizer [41].

Microbes degraded insoluble macromolecules like keratin, cellulose, collagen, lignin, chitin and casein depends on the secretion of extracellular enzymes with the ability to act on compact substrates, such microbial activity helps in the mineral recycling in nature [11]. Jamaluddin *et al.* [19] observed roots colonized by mycorrhizal fungi showed maximum acid phosphatase activity. Khokhar *et al*. [24] screened 17 fungal isolates, out of these 8 isolates were identified as cellulase producer. The best cellulase producer was *Trichoderma* sp. and *Penicillium* sp. followed by *Aspergillus* sp. [58].

H. integrifolia is an ornamental roadside tree and distributed in all tropical and temperate regions. Plant parts of this species were used in medicine (antibacterial effect, anti-inflammatory activity, anti-diarrheal

activity). The tree produced a large number of seeds, which are small, whitish, kidney-shaped (flat samara). The tree helps in regeneration of degraded land by spreading seeds through winds which attract animals and birds [36]. *H. integrifolia* was selected for nursery experiment to determine the effect of inoculation of microbial inoculants on seedling growth. These seedlings may be used for plantation in overburden mine land for better performance.

MATERIALS AND METHODS

Sample collection and isolation of microbes

Soil Sampling - Soil sample were collected from iron ore mined overburden (OB) dump [40]. In lab sample were homogenized and spread on paper to remove plant material, they are air dried, sifted with 2mm mesh sieve and stored at 4ºC used for experiment.

Isolation of microbes

Isolation, identification and screening of fungi - The population of different type of culturable fungi were isolated by using potato dextrose agar (PDA) media [3, 60]. Pure cultures of fungi isolates were identified with the help of literature Subramanian [51]; Barnett and Hunter [4]; Nagmani *et al*. [33]; Verma *et al*. [58]. After the identification pure culture was stored in the refrigerator for further use and preservation. Screening of fungi was done by starch agar medium (amylase activity) given by Ross, [45] and basal salt medium (cellulase activity) [17].

PSB sp. -Warcup techniques was used for isolation of PSB by using selective growth medium Kings B Medium. Colonies giving fluorescent were used for detection of PSB [25]. Fluorescent *Pseudomonas* sp. was further screened based on phosphatase enzymatic activity by an agar assay using National Botanical Research Institute's Phosphate (NBRIP) medium [35]. The pH of the NBRIP media was adjusted to 6.75 ± 0.25 before autoclaving. The halo and colony diameters were measured after 14 days of incubation of the plates at 25°C.

Azospirillum sp.

Azospirillum sp. was isolated from the roots of planted tree at OB spoil of mined area. The feeder roots were cut into 1cm pieces. Surface sterilized with H_2O_2 for 3 minutes washed with sterilized water and suspended in 15ml culture tube containing 5ml semisolid nitrogen-free bromothymol blue medium followed by incubation at 27ºC. After 3 days grey-white ring of 2mm diameter was observed on the surface of the root segment followed by a change in the medium colour from greenish-yellow to blue after one week indicating the growth of *Azospirillum* sp. [59].

Inoculum preparation of AM fungi

Feeder root were collected from the rhizospheric soil of tree and thoroughly washed in sterile water to remove all soil traces and external mycelium. About 5g of these feeder roots were planted in sterilized potting mixture containing soil, sand and organic matter in 2:2:1 ratio. Maize seed was sown on this potting mixture and watering was done twice a day using the fine rose cane. After three months, seedlings of the same trap plant were harvested washed and sheared to get the AM inoculums [54].

Collection of seeds and surface sterilization

To conduct pot experiment seeds of *H. integrifolia* was collected from the campus of Tropical Forest Research Institute Jabalpur, healthy seeds were sorted and surface sterilization of seed was done by 1% sodium hypochlorite (NaOCl) solution for 10 minutes, washed three times with distilled water and was subjected to hot water treatment [23].

Preparation of potential species of bacteria and fungi inoculums

Pure cultures of bacteria (PSB and *Azospirillum*sp.) were transferred to a specific medium in 250ml conical flask and make up the volume with diluted concentration (10^6), whereas fungus (*Penicillium* sp. 1) was inoculated on potato dextrose broth in 250ml conical flask and incubated for 7 days at 27ºC. Spore suspension and mycelial slurry of *Penicillium* sp. 1 was prepared in sterilized water by diluting broth and makeup the volume with diluted con. (10^6). Prepared solution 10^6 concentrations of bacteria and fungus solutions for field application.

Nursery experiment

Selected growth promoting microbes (both fungi and bacteria) were applied on forest tree species to study whether these microbes worked as biofertilizer and play a positive role in the establishment of seedling in nurseries or not.

Experimental design - For this purpose seeds of *H. integrifolia* were sown in the nursery in three types of soil mix, 1) vermicompost, 2) iron ore mine spoil soil and 3) nursery soil. All soils were mixed properly and sterilized with formaldehyde and filled in polythene bags. Seeds of *H.integrifolia* were sown in June 2015. The experimental *H.integrifolia* seedling received the following treatments and was arranged in RBD on a cemented platform: (1) Control, (2) fluorescent *Pseudomonas* strain 1, (3) *Azospirillum* sp., (4)

fluorescent *Pseudomonas* strain 1+ *Azospirillum* sp., (5) AM fungi, (6) fluorescent *Pseudomonas* strain 1 + AM Fungi, (7) *Azospirillum* sp. + AM fungi, (8) fluorescent *Pseudomonas* strain 1+ *Azospirillum* sp. + AM fungi, (9) *Penicillium* sp. 1, (10) fluorescent *Pseudomonas* strain 1+ *Penicillium* sp. 1, (11) *Azospirillum* sp. + *Penicillium* sp. 1, (12) fluorescent *Pseudomonas* strain 1+ *Azospirillum* sp. + *Penicillium* sp. 1. The percentage seed germination was recorded and after nine month of seed sowing plant height, shoot length, root length, collar diameter (using Vernier Calipers scale), number of leaf, moisture content of root and shoot, dry biomass of root and shoot were recorded [23].

Root colonization by AM fungi

The feeder roots of seedlings treated with AM fungi were washed and stained for rapid assay for mycorrhizal colonization [42].

Estimation of N, P, K and Ca, Mg in leaves

Healthy representative of leaf sample from each treatment were kept in a paper enveloped, labelled and dried in an oven, powdered and used for acid digestion, after that phosphorous, nitrogen, potassium, calcium and magnesium were analyzed [28].

Statistical Analysis

Data obtained on, seed germination, plant biomass, plant height, diameter at collar height, nitrogen, potassium, phosphorus, calcium and magnesium content in leaves, growth indices were analyzed by one way analysis of variance (ANOVA), means were separated when 'F' values were significant at $P = 0.05$ was computed using statistics software, SPSS 16.

RESULT

In mine land, plantation was done by planting the saplings raised in nursery. To study the potentials of microbes isolated, nursery experiments were conducted using one tree species. Sterilized seeds were treated with different microbes and sown in polyethylene bags. After eight months seedlings were uprooted and different parameters, height, diameter, number of leaves, moisture contents, dry biomass and leaf N, P, K, Mg and Ca were measured.

Screening of Fungi

A total of 99 fungi were obtained from the soil samples. Out of 99, only 21 test fungi were found to produce amylase activity as indicated by production of zone on starch agar medium around the fungal colonies grown at pH 7 after 5 days of incubation (28±3°C). Among these fungal isolates, 46 fungal isolates were identified as cellulase producer. But only 11 fungi were able to hydrolyze both cellulose and starch by releasing cellulase and amylase enzymes. While, only *Penicillium* sp. 1 have highest zone value as compare to other fungi (data not shown).

Screening of phosphorus solublizing fluorescent *Pseudomonas* sp.

In the present investigation solubilization of phosphorus were studied by using randomly selected three strains of fluorescent *Pseudomonas* sp., isolated from iron ore mined OB dump. The fluorescent *Pseudomonas* strain 1can more solubilize the phosphorus rock material as compared to other two strains (data not shown).

Measurement of growth parameter

Seed germination

Overall 3600 seeds were sown out of which 2568 (71.3%) seeds were germinated in nursery. When the individual treatments were considered the best germination was recorded in treatment fluorescent *Pseudomonas* strain 1+ AM fungi (89.3%). AM fungi alone were placed in second order (83.0%). Fluorescent *Pseudomonas* strain 1+ *Azospirillum* sp. + AM fungi (70.0%) and fluorescent *Pseudomonas* strain 1+ *Azospirillum* sp. + *Penicillium* sp. 1 (66.3%) were placed in third and fourth order respectively (Table 1).

Root length, shoot length and diameter of seedlings

Maximum shoot length was observed in fluorescent *Pseudomonas* strain 1+ AM fungi which were 2.9 times more that control followed by AM fungi alone. Maximum root length was observed in fluorescent *Pseudomonas* strain 1+ AM fungi which was 2.7 times more that control followed by fluorescent *Pseudomonas* strain 1+ *Azospirillum* sp. + AM fungi. The maximum collar diameter was observed in fluorescent *Pseudomonas* strain 1+ *Azospirillum* sp. + AM fungi which was 2.8 time more that control followed by fluorescent *Pseudomonas* strain 1+ AM fungi. Maximum number of leaf was observed in fluorescent *Pseudomonas* strain 1alone which was 2.3 times more that control followed by fluorescent *Pseudomonas* strain 1+ *Penicillium* sp. 1 (Table 1).

Moisture content and dry biomass of seedlings

Maximum moisture content of shoot was observed in AM fungi alone was 3.5 times more than control followed by fluorescent *Pseudomonas* strain 1alone. Maximum moisture content of root was observed in

Azospirillum sp. alone was 2.01 times more than control followed by fluorescent *Pseudomonas* strain 1 + *Azospirillum* sp. + AM fungi treatment. Maximum shoot dry biomass was observed in fluorescent *Pseudomonas* strain 1alone which was 2.44 times more than control followed by *Penicillium* sp. 1 alone. Maximum root dry biomass was observed in *Azospirillum* sp. alone which was 1.81 times more than control followed by fluorescent *Pseudomonas* strain 1+ *Azospirillum* sp. + AM fungi (Table 1 and Fig1).

Table 1: Effect of growth promoting microbes (biofertilizers) on growth of *Holoptelea integrifolia*

S.No.	Treatments	PSG	Shoot length (cm)	Root length (cm)	Collar diameter (cm)	Number of leaf	Moisture content		Dry Biomass	
							Shoot (g)	Root (g)	Shoot (g)	Root (g)
1	Control	21.67^{f}	18.28^{f}	16.38^{f}	0.45^{e}	19^{f}	15.3^{e}	19.31^{f}	26.82^{i}	22.63^{h}
2	fluorescent *Pseudomonas* sp.	37.33^{def}	32.12^{de}	21.43^{e}	0.79^{cd}	44.88^{a}	42.3^{b}	26.87^{e}	65.58^{a}	25.99^{efg}
3	*Azospirillum* sp.	29^{ef}	33.85^{cde}	32.85^{bc}	0.72^{d}	33.17^{b}	18.71^{de}	40.6^{a}	47.81^{d}	41.01^{a}
4	fluorescent *Pseudomonas*sp. + *Azospirillum* sp.	32.67^{ef}	33.35^{cde}	32.04^{bc}	0.98^{bc}	23.26^{cd}	23.03^{cd}	32.37^{bc}	43.13^{f}	33.01^{c}
5	AM Fungi	82.67^{ab}	47.78^{ab}	34.56^{b}	0.99^{bc}	24.8^{cd}	53.53^{a}	35.13^{b}	51.17^{c}	32.86^{c}
6	fluorescent *Pseudomonas*sp. + AM Fungi	89.33^{a}	53.44^{a}	45.4^{a}	1.06^{b}	34.49^{b}	23.99^{cd}	28.17^{de}	42.54^{f}	29.1^{d}
7	*Azospirillum*sp. + AM Fungi	50^{cde}	31.06^{de}	28.25^{d}	0.96^{bc}	19.96^{ef}	19.12^{de}	28.45^{de}	36.39^{g}	25.6^{fg}
8	fluorescent *Pseudomonas*sp. + *Azospirillum*sp. + AM Fungi	70.0^{abc}	38.47^{bcd}	34.69^{b}	1.27^{a}	22.22^{de}	25.22^{c}	40.12^{a}	44.89^{ef}	36.29^{b}
9	*Penicillium* sp. 1	49^{cde}	36.15^{cd}	22.86^{e}	0.89^{bcd}	26.06^{c}	28.09^{c}	33.19^{b}	55.6^{b}	26.96^{def}
10	fluorescent *Pseudomonas*sp. + *Penicillium* sp. 1	42.67^{def}	25.69^{ef}	30.18^{cd}	0.81^{cd}	35.32^{b}	29.29^{c}	20.8^{f}	33.61^{h}	23.67^{gh}
11	*Azospirillum*sp. + *Penicillium* sp. 1	56^{cd}	31.34^{de}	32.13^{bc}	0.81^{cd}	24.37^{cd}	23.68^{cd}	32.48^{bc}	43.18^{f}	34.61^{bc}
12	fluorescent *Pseudomonas*sp. + *Azospirillum*sp. + *Penicillium* sp. 1	66.33^{bc}	42.11^{bc}	29.79^{cd}	0.82^{cd}	25.23^{cd}	28.15^{c}	30.06^{cd}	46.93^{de}	28.49^{de}
$CD_{0.05}$		11.564	7.32	2.507	0.17	2.32	4.05	2.18	2.24	2.17
SE		3.79	1.33	0.89	0.03	0.91	1.3	0.78	1.18	0.66

Mean in columns followed by the same letter are not significantly different at $P = 0.05$;PSG = Percentage of seed germination

N, P, K, Mg and Ca content in leaf

Biochemical analysis for all treatments in dry leaf biomass of *H.integrifolia* was determined. Dry leaf samples were grinded and digested for analysis. The maximum nitrogen was observed in fluorescent *Pseudomonas* strain 1+ AM fungi which was 37.5 time more than control followed by fluorescent *Pseudomonas* strain 1+ *Azospirillum* sp. Maximum phosphorus was observed in AM fungi alone which was 172.2 time more that control followed by fluorescent *Pseudomonas* strain 1+ AM fungi. Maximum potassium was observed in *Azospirillum* sp. + AM fungi which was 2.1 time more than control followed by fluorescent *Pseudomonas* strain 1+ *Azospirillum* sp. The maximum Mg was observed in fluorescent *Pseudomonas* strain 1+ AM fungi which was 2.99 time more than control followed by fluorescent *Pseudomonas* strain 1+ *Azospirillum* sp. + *Penicillium* sp. 1. Maximum Ca was observed in AM fungi alone which were 38.84 times more that control followed by fluorescent *Pseudomonas* strain 1+ AM fungi (Table 2).

Root colonization in sapling

Arbuscular mycorrhizal root colonization (RC) was determined in the nursery experimental plants (Table 3). Maximum colonization was observed in AM fungi (93.3) with arbuscules, vesicles and hyphae followed by *Azospirillum* sp. + AM fungi (53.3) with vesicles and hyphae and fluorescent *Pseudomonas* strain 1+ AM fungi (53.3).

Economic of raising biofertilizer applied saplings

Economic of different treatment was calculated on the basis of cost of materials used, in local market at Jabalpur. Application of fluorescent *Pseudomonas* strain 1+ AM fungi and other cost including sterilization of soil, vermicompost, etc., were calculating total cost for raising saplings of both species (Table 4).

Table 2: Biochemical analysis of *Holoptelea integrifolia* leaf

S. No.	Treatment	N	P	K	Mg	Ca
1	Control	0.088^{e}	0.262^{f}	0.010^{c}	0.931^{ef}	0.107^{j}
2	Fluorescent *Pseudomonas* sp.	0.853^{de}	4.051^{c}	0.020^{a}	2.432^{ab}	2.760^{d}
3	*Azospirillum* sp.	1.258^{cde}	3.029^{d}	0.017^{abc}	1.547^{cd}	1.600^{h}
4	Fluorescent *Pseudomonas* sp. + *Azospirillum* sp.	2.920^{ab}	3.166^{d}	0.021^{a}	0.904^{ef}	3.160^{c}
5	AM Fungi	0.508^{de}	45.125^{a}	0.017^{abc}	2.307^{b}	4.156^{a}
6	Fluorescent *Pseudomonas* sp. + AM Fungi	3.310^{a}	41.404^{b}	0.017^{abc}	2.787^{a}	3.533^{b}
7	*Azospirillum* sp. + AM Fungi	0.794^{de}	2.626^{de}	0.023^{a}	0.733^{f}	2.431^{ef}
8	Fluorescent *Pseudomonas* sp. + *Azospirillum* sp. + AM Fungi	1.736^{bcd}	3.354^{cd}	0.019^{ab}	0.032^{g}	3.142^{c}
9	*Penicillium* sp. 1	2.780^{ab}	2.065^{e}	0.018^{abc}	1.251^{de}	1.916^{g}
10	Fluorescent *Pseudomonas* sp. + *Penicillium* sp. 1	1.059^{de}	3.008^{d}	0.011^{bc}	1.349^{de}	2.373^{f}
11	*Azospirillum* sp. + *Penicillium* sp. 1	1.721^{bcd}	2.615^{de}	0.012^{abc}	1.824^{c}	2.702^{de}
12	Fluorescent *Pseudomonas* sp. + *Azospirillum* sp. + *Penicillium* sp. 1	2.516^{abc}	2.956^{d}	0.017^{abc}	2.485^{ab}	1.262^{i}
$CD_{0.05}$		1.260	0.743	0.007	0.421	0.288
SE		0.157	0.911	0.002	0.149	0.234

Mean in columns followed by the same letter are not significantly different at $P = 0.05$

Table 3: Root colonization in different treatments and control saplings

S. No.	Treatments	*Holopteleaintegrifolia*			
		RC (%)	A	V	H
1	AM fungi	93.3	√	√	√
2	Fluorescent *Pseudomonas* sp. + AM fungi	53.3	-	√	√
3	*Azospirillum* sp. + AM fungi	53.3	√	√	-
4	Fluorescent *Pseudomonas* sp. + A*zospirillum* sp. + AM fungi	46.7	-	√	-
5	Control	20.0	-	√	-

A= arbuscules, V= vesicle, H= hyphae, RC = root colonization by AM fungi

Table 4: Economic of raising sapling with consortium of growth promoting microbes

S. No.	Material	Amount (g)	Estimated (Rs)
1	Solarised soil	1000	2.00
2	Vermicompost	1000	4.00
3	Polythene bag	per piece	1.00
4	Fluorescent *Pseudomonas* sp. culture	10ml	0.48
5	AM Fungal spore multiplication	1000	4.00
Total cost per seedling			11.48

*Cost is based on the survey of local market at Jabalpur (M.P.)

Fig. 1: Growth response of *Holoptelea integrifolia* seedling under different treatments at forest pathology nursery, Tropical Forest Research Institute, Jabalpur (MP) (A) Seedling after 4 months; (B) Length of different treatment plants after uprooting of seedlings (C) Presence of vesicles (D) Presence of arbuscules (E) Presence of hyphae

DISCUSSION

On the basis of data presented in table 1 application of combined biofertilizer fluorescent *Pseudomonas* strain 1+ AM fungi were used to boost the growth of *H.integrifolia* seedling in nursery. This combination was show maximum seed germination percentage, shoot and root length. These may be due to enhanced AM spore production and enhanced spreads of AM hyphae were reported [21, 56]. The enhancement in growth and diameter of seedlings inoculated with AM fungi may be due to activities of these fungi [13] and other microorganisms, whose population increase due to their interaction in the mycorhizosphere of

these plants [2]. Many plant growths promoting rhizobacteria was reported as mycorrhization helper bacteria [15]. The dual inoculation of phosphorus solubilising bacteria and AM fungi, the phosphorus solubilising bacteria rendered more P soluble, while AM fungi enhanced phosphorus uptake [16]). Thus combined inoculation of phosphorus solubilising bacteria and AM synergistically affects the plants health and growth. Prasad [43] has reported the positive synergistic effect of AM fungi and phosphate solubilising bacterium on growth of *Azadirachta indica* in nursery soil.

Collar diameter of seedling was maximum observed in combination of fluorescent *Pseudomonas* strain 1+ *Azospirillum* sp.+ AM Fungi, these results were found due to application of AM fungi along with *Azospirillum* sp. showed a synergistic effect on growth, root colonization and shoot p content. *Azospirillum* sp. and AM fungi solubilise the insoluble phosphorus of rhizosphere soil and may be responsible for enhancement in growth and dry biomass of seedlings [20]. Enhancement in growth of seedlings treated with *Azospirillum* sp. may also be due to production of some growth promoting substance by the bacterium [48]. Effect of PSB in collar diameter was also observed by Bhrigu *et al*. [9] and observed non significant increase. The seedlings treated with fluorescent *Pseudomonas* sp. exhibited increase in root and shoot length and higher biomass as compared to seedlings treated with other microbial inoculants and un-inoculated one. It may be due to phosphorus solubilisation activity of PSB [50].

Moisture content of shoot was highest in AM fungi because fungi have also been found to increase water uptake and/or otherwise alter the plant physiology to reduce stress response to drought and salinity. The improved nutrient uptake and better water utilization in endomycorrhizal plants reduce the transplant shock, quick recovery after temporary wilting and survival after transplanting [31]. AM fungi effect is the increase in resistance and tolerance of AM fungi infected plants to soil pathogens. *Azospirillum* sp. is work as helper bacteria its primary effect is on increased root development (higher surface area, higher root hair, increase execration of root exudates) [6]. Improved nutrition through mycorrhiza has been demonstrated by Hatch [18]; Mitchell *et al*. [32]; Rosendahl [44]; McComb and Griffith [29] among other. By using radio-isolates techniques, Melin [30] demonstrated that the fungal symbionts are able to transfer carbon (C) 14, Nitrogen (N) 15, Phosphorus (P) 32, Sodium (Na) 22, Calcium (Ca) 45 from the nutrient solution into the plant in considerably larger quantities as compared to non-mycorrhizal roots. *Azospirillum* species improve plant growth is through the production of indole acetic acid (IAA), a plant hormone, mineral or water uptake, or specific enzymatic activity; changes in membrane function, or a combination of small mechanisms affecting the plant in concert [5, 6]. Auxin is an important phytohormone, which promotes root cell division, root initiation, and cell enlargement [47]. Indole-3-acetic acid (IAA) produced by PGPR are reported to increase root growth and length, modifying the plant (morphological functions) to uptake more nutrients from the soil. Gibberellins also can alter the plant morphology by the elongation of stem tissues.

Lowering plant ethylene levels: both abiotic and biotic factors affect the agricultural crops and its yield under stress conditions [14].The increase in fresh root weight in inoculated plants could be either with increased mycorrhizal colonization or due to the formation of external mycelial network around the roots by AM fungi. The results are in agreement with the findings of earlier investigators [22] who observed improved root biomass production in different plants due to AM fungi inoculation. *Azospirillum* sp. was increased photosynthetic pigments in inoculated plants is solely recorded as enhancement of total chlorophyll content of the inoculated plants [52]. It was assumed that increased production of photosynthates enhanced plant growth and yield [37, 1]. Dry biomass of seeding was high as compared to control.

Panhwar *et al*. [38] proved PSB strains were able to colonize the surface and interior roots. The highest bacterial population was found in the rhizosphere. Naher *et al*. (2009) where the inoculated PSB manifested intercellular growth and formed aggregated cells and mucilaginous materials that were involved in the root attachment. Plant root surface attachment with bacterial flagella has been shown to play a significant role in colonization [10]. The production of extracellular polysaccharide and cell aggregation might help in colonization process.

Biochemical analysis on leaf of *H. integrifolia*

Biochemical analyses were done on leaf of *H. integrifolia* (Table 2). K was highest observed in combination of fluorescent *Pseudomonas* strain 1+ *Azospirillum* sp. + *Penicillium* sp. 1. Uptake of P and Ca was highest in fluorescent *Pseudomonas* strain 1+ *Penicillium* sp. 1. Because these combinations were mineralize insoluble P present in the soil and make available to plants for their growth and development [57]. *Penicillium* with *Pseudomonas* sp. show maximum uptake these may be due to companion fungus (*Penicillium* sp.) [13]. Other microorganism, which population increase due to their interaction in the mycorhizosphere of these plants [2]. N was highly utilized by fluorescent *Pseudomonas* strain 1+

Azospirillum sp. Bashan *et al*., [6] were also observed uptake of mineral by *Azospirillum* sp. Ca was maximum uptake by fluorescent *Pseudomonas* strain 1+ *Rhizobium* sp.

CONCLUSION

Biofertilizers consortium including fluorescent *Pseudomonas* + AM fungi can be used for raising of seedlings of *Holoptelia integrifolia* in nursery for plantation in iron mine overburden soil to increase productivity of this species.

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