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IMPACT OF *ARTHROBACTER MYSORENS*, *KUSHNERIA AVICENNIAE*, *HALOMONAS* SPP. AND *BACILLUS* SP. ON *HELIANTHUS ANNUUS* L. FOR GROWTH ENHANCEMENT

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ABSTRACT

Plant growth promoting rhizobacteria are very important in growth and initial development of plants. In the current work, PGPR treatment of *Helianthus annuus* L. seeds (Sunflower) was studied. *H. annuus* is an economically important crop grown for economic purposes such as to make oil for cooking, as medicine for animal feed etc. In the present study, five auxin-producing bacterial strains already isolated and identified by Ahmed (2011) were used. These strains were used to inoculate *Helianthus annuus* L. seeds var. premium. Plant growth experiments were conducted both in the laboratory as well as under wire house conditions. The microbial strains stimulated overall growth of the seedlings as indicated by various growth and biochemical parameters in different inoculated and non-inoculated treatments. Significant improvement in shoot length app. 136 and 106 % was recorded plants treated with *Bacillus* and *Arthrobacter* sp. respectively under field conditions. Similarly app. 51 and 57% increase was recorded in protein content of plants treated with *Halomonas venusta* and *Arthrobacter mysorens* compared to control treatment. These strains proved to be very efficient as biofertilizers and can be used for phytostimulation of crops instead of chemical fertilizers.

Key words: PGPR, *Arthrobacter*, *Kushneria*, *Bacillus*, *Halomonas.*

INTRODUCTION

Plant growth-promoting rhizobacteria (PGPR) are the bacteria living freely in the vicinity of plant roots. These aggressively form colonies in the root system and are studied as plant growth enhancers for improved growth of plants (Ravari and Heidarzadeh, 2014). However, it is not a single special genus or species of these bacteria that is involved in growth proliferation rather it is a consortium of bacteria having different PGP characters that improve plant growth (Bernard *et al.,* 2009; Pham *et al*., 2017; Franchi *et al*., 2017). These plant growth enhancing bacteria ameliorate the overall growth of treated plants by different direct or indirect processes (Laslo *et al*., 2012). Indirect mechanisms are those which are linked with the metabolite production e.g., siderophores which sequester iron crucial for the growth of pathogens and also synthesize certain metabolites against fungal contaminants resulting in growth improvement in plants (Ahmed and Hasnain, 2014). In contrast to this, some scientists also reported direct mechanisms including production of plant growth regulators (PGRs) i.e., auxins, gibberellins, cytokinins and polyamines which are involved in stimulation of plant growth at various stages, resulting in the improved nutrient uptake by plants especially phosphorus, facilitating nitrogen fixation thus helping the ion uptake or transport systems in plants (Ahmed and Hasnain, 2010; Bashan and De-Bashan, 2010; Taiwo *et al*., 2016). The present work aims to study the effect of phytohormone-producing bacterial strains *Kushneria avicenniae* (AHT), *Halomonas* sp. (AST), *Halomonas venusta* (APA), *Bacillus* sp. (AMP2) and *Arthrobacter mysorens* (AHA) on the growth of *Helianthus annuus* L. (Sunflower). Almost all the strains have shown positive impact on plant growth. It was observed that growth of inoculated plants was greater as compared to the control treatments.

MATERIALS AND METHODS

In the present study, five already isolated and identified auxin-producing rhizobacterial strains (PGPR) by Ahmed (2011) i.e., *Kushneria avicenniae* (AHT), *Halomonas* sp. (AST), *Halomonas venusta* (APA), *Bacillus* sp. (AMP2) and *Arthrobacter mysorens* (AHA) were used to inoculate *Helianthus annuus* L. var. premium seeds to evaluate the impact of bacterial inoculations on the growth of the treated plants by analyzing various growth and biochemical parameters.

Preparation of Bacterial Inoculum: Bacterial strains from 24 hours incubated fresh cultures at 37°C were taken and cells were harvested. The pellets were again suspended in autoclaved distilled water. The optical density of all the microbial strains was adjusted to the same value of 10^{-5} to 10^{-6} CFU at 600 nm.

Plant Experiment: Certified seeds of *Helianthus annuus* L. var. premier were procured from research institute of Punjab Seed Corporation, Lahore, Pakistan. The seeds were sterilized using 0.1% $HgCl_2$ and after several washings by using distilled water, these seeds were

treated with inoculum of bacterial isolates [*Kushneria avicenniae* (AHT), *Halomonas* sp. (AST), *Halomonas venusta* (APA), *Bacillus* sp. (AMP2) and *Arthrobacter mysorens* (AHA)] that is prepared after adjusting optical density to 10^{-5} to 10^{-6} CFU at 600 nm, separately. For control treatment, seeds were treated with autoclaved distilled water for similar time period without any bacterial inoculation. Both control and treated seeds were then sown in pots which contain 188.6g sieved soil at the rate of six seeds per pot and then allowed to grow at photoperiod of 16 hours at 25±2°C. For laboratory trial, the treated plants were harvested after 30 days and different growth parameters were studied. The experiment was repeated thrice. In case of wire house experiment, plants were grown upto maturity and then the flowers produced were harvested and various parameters such as flower size, flower weight and flower diameter were recorded. Biochemical analysis was done by estimating auxin content of the plants following Mahadevan (1984). Protein content and pigment analysis of the plants was also done following Lowry *et al*. (1951) and Lichtenthaler and Wellburn (1983), respectively. This study was conducted at the Department of Botany, PU.

Statistical Analysis: The data obtained were statistically analyzed using the software SPSS v.16 using Duncan's multiple range test.

RESULTS

Plant Experiment: Laboratory Trial: In the laboratory experiment, significant increase in germination percentage of seeds was observed in bacterial treatments when compared with control treatment with the exception of plants treated with *Halomonas* sp. (AST) and *Bacillus* sp. (AMP2). The isolates *Halomonas venusta* (APA), *Kushneria avicenniae* (AHT) and *Arthrobacter mysorens* (AHA) have shown significant increase of 9.7, 16.1 and 24.2% in germination over control. Bacterial inoculations also enhanced shoot length in comparison with the non-inoculated treatment with the exception of *Kushneria avicenniae* (AHT) and *Bacillus* sp. (AMP2) which caused reduction in shoot length when compared with control. The bacterial isolates *Halomonas* sp. (AST), *Halomonas venusta* (APA) and *Arthrobacter mysorens* (AHA) have shown significant increase i.e., 29.2, 34.6 and 52.2% respectively, in the shoot length of treated plants. All bacterial isolates caused increase in root lengths of treated plants with the exception of *Halomonas* sp. (AST) and *Bacillus* sp. (AMP2) which have shown reduction in root length. The bacterial isolates *Kushneria avicenniae* (AHT), *Halomonas venusta* (APA) and *Arthrobacter mysorens* (AHA) have shown significant increase of 9, 19.5 and 35.7% in root length of inoculated plants as compared to non-inoculated control treatments, respectively (Table 1).

Improvement in the leaf number was observed in plants which are treated with bacteria in comparison to control plants. The bacterial isolates *Kushneria avicenniae* (AHT), *Halomonas* sp. (AST), *Halomonas venusta* (APA), *Bacillus* sp. (AMP2) and *Arthrobacter mysorens* (AHA) have shown 3.1, 7.6, 1.6, 5.9 and 13.3% rise in the number of leaves, respectively. Effect of different bacterial cultures was recorded on fresh weight of inoculated and non-inoculated plants. All bacterial treatments caused increment in fresh weight of plants except *Halomonas venusta* (APA) which caused 24.7% reduction in fresh weight of treated plants as compared to the non-inoculated plants. The bacterial isolates *Kushneria avicenniae* (AHT), *Halomonas* sp. (AST), *Bacillus* sp. (AMP2) and *Arthrobacter mysorens* (AHA) have shown 44.2, 70.2, 80.5 and 129.2%significant increase in fresh weight of treated plants as compared to the control (Table 1).

Plant Experiment: Wire-House Trial: In the wire-house experiment, considerable improvement in percentage of germination was observed in all inoculated plants except plants treated with *Halomonas* sp. (AST) which caused reduction in germination percentage. The bacterial isolates *Kushneria avicenniae* (AHT), *Halomonas venusta* (APA), *Arthrobacter mysorens* (AHA) and *Bacillus* sp. (AMP2) have shown increase in germination percentage i.e., 12, 12.02 and 8.01, 20%, respectively over control. Regarding shoot length of the plants, one of the isolate *Kushneria avicenniae* (AHT) has shown reduction in shoot length of plants while all other bacterial strains including *Halomonas* sp. (AST), *Halomonas venusta* (APA), *Bacillus* sp. (AMP2) *Arthrobacter mysorens* (AHA) caused significant increment of 23.7, 67.4, 136.2 and 106.7% in shoot length of plants. Considerable increase in leaf number was also observed in all inoculated treatments as compared with the non-inoculated control except the isolate *Kushneria avicenniae* (AHT). The bacterial strains *Bacillus* sp. (AMP2), *Halomonas* sp. (AST), *Arthrobacter mysorens* (AHA) and *Halomonas venusta* (APA) have shown significant increase in the leaf number i.e., 16.5, 19.6, 35.6 and 52.2%, respectively over control. At maturity, flowers produced were also collected and flower diameter was measured to observe the growth promoting impact of bacterial isolates on flower size in inoculated and control treatments. Bacterial inoculations caused increase in flower diameter as compared to non-inoculated control treatment except *Halomonas venusta* (APA). *Arthrobacter mysorens* (AHA) caused significant increase i.e., 31.4% in flower diameter as compared to non-inoculated control treatment. Inoculation with the isolate *Bacillus* sp. (AMP2) has shown 1.4% increase in flower diameter over control while treatment with the

Halomonas sp. (AST) and *Kushneria avicenniae* (AHT) showed significant increase of 15.2 and 23% in the diameter of flower in comparison with non-inoculated control treatment (Table 2). Inoculation with all the strains caused increase in number of ray florets of flowers in treated plants as compared to the non-inoculated control treatment except the isolate *Bacillus* sp. (AMP2). Treatment with *Kushneria avicenniae* (AHT) caused slight increase (3.7%) in number of ray florets while *Arthrobacter mysorens* (AHA), *Halomonas* sp. (AST) and *Halomonas venusta* (APA) have shown 18.2, 27.4 and 15.1% increase in the number of ray florets when compared with control. Similarly all bacterial strains caused increase in number of disc florets of flowers in treated plants in comparison to the non-inoculated control treatment except the *Kushneria avicenniae* (AHT) which caused reduction in the number of disc florets. The bacterial isolates *Halomonas venusta* (APA), *Halomonas* sp. (AST), *Bacillus* sp. (AMP2) and *Arthrobacter mysorens* (AHA) have shown significant increase of 21.9, 30.1, 36.9 and 51.8%, respectively, in the number of disc florets of flowers as compared to the non- inoculated control treatment (Table 2).

Biochemical analysis of the inoculated and non-inoculated plants was also carried out. Generally inoculation with bacterial strains enhanced chlorophyll 'a' content of the plants. All bacterial strains caused increase in chlorophyll 'a' content of plants in comparison to the non-inoculated control treatment except the strain *Halomonas* sp. (AST). The isolates *Kushneria avicenniae* (AHT), *Bacillus* sp. (AMP2), *Arthrobacter mysorens* (AHA) and *Halomonas venusta* (APA) have shown significant increase of 19.8, 40.3, 72.7 and 86.6% in the chlorophyll 'a' content of the plants over control. Bacterial strains also caused increase in chlorophyll 'b' content of plants in comparison with non-inoculated control treatment except *Halomonas* sp (AST) and *Halomonas venusta* (APA). The bacterial strains *Kushneria avicenniae* (AHT), *Bacillus* sp. (AMP2) and *Arthrobacter mysorens* (AHA) have shown 12.05, 38.7 and 55.8% significant increase in the chlorophyll 'b' content of treated plants as compared to the control treatment. The bacterial isolates (APA, AST, AHT, AHA and AMP2) have shown significant increase (18.8, 29.8, 58.9, 53.8 and 90.6% respectively) in the carotenoids content of the treated plants over control (Table 3). *Halomonas* sp. (AST), *Arthrobacter mysorens* (AHA), *Bacillus* sp. (AMP2) and *Halomonas venusta* (APA) treatments have also shown significant increase of 15.4, 27.2, 36.0 and 48.5% in the amount of auxin in treated plants as compared to the control. Improvement in protein content was also observed with all bacterial treatments except *Halomonas* sp. (AST) which caused decrease in protein content of the plants. Inoculations with *Kushneria avicenniae* (AHT), *Bacillus* sp. (AMP2), *Halomonas venusta* (APA) and *Arthrobacter mysorens* (AHA) have shown significant enhancements in the protein content of the plants i.e., 6.3, 35.8, 51.5 and 57.8%, respectively as compared to the control (Table 3).

Table1. Effect of bacterial inoculations on growth parameters including germination percentage, shoot length, root length, number of leaves and fresh weight of *Helianthus annuus* L. (sunflower) under laboratory conditions.

S.#.	Treatment	Germination percentage	Shoot length (cm)	Root length (cm)	No. of Leaves	Fresh weight (g)
1	Control	68.90+4.26 $^{(ab)}$	14.90+0.26 $^{(b)}$	4.78+0.27 $^{(b)}$	4.47+0.47 $^{(a)}$	1.23+0.04 $^{(b)}$
2	AHT	75.56 + 3.94 $^{(abc)}$	10.74+0.20 $^{(a)}$	5.21+0.29 $^{(bc)}$	4.60+0.40 $^{(a)}$	1.77+0.04 $^{(c)}$
3	AST	62.24+ 5.00 $^{(a)}$	19.27+0.28 $^{(c)}$	3.80+0.45 $^{(a)}$	4.80 +0.29 $^{(a)}$	2.09+0.04 $^{(d)}$
4	APA	80.01+4.05 $^{(bc)}$	20.07+0.31 $^{(c)}$	5.71+0.24 $^{(cd)}$	4.54+0.35 $^{(a)}$	0.93+0.06 $^{(a)}$
5	AMP2	67.79 +7.17 $^{(ab)}$	14.07 +0.39 $^{(b)}$	3.07+0.28 $^{(a)}$	4.74+0.47 $^{(a)}$	2.22+0.06 $^{(c)}$
6	AHA	85.56 +3.58 $^{(c)}$	22.70+0.30 $^{(d)}$	6.48+0.23 $^{(d)}$	5.07+0.50 $^{(a)}$	2.83+0.07 $^{(f)}$

Mean of three replicates. Different letters within same column indicate significant difference between strains using Duncan's multiple range test ($P \leq 0{\cdot}05$).

Table 2. Effect of bacterial inoculations on growth parameters including germination percentage, shoot length, root, number of leaves flower diameter, number of ray florets and number of disc florets of *Helianthus annuus* L. (sunflower) under wire-house conditions

S. #.	Treatment	Germination percentage	Shoot length (cm)	No. of leaves	Flower diameter	No. of ray florets	No. of disc florets
1	Control	69.45+5.12 $^{(ab)}$	22.5+1.38 $^{(b)}$	26.17+2.03 $^{(ab)}$	6.91+0.25 $^{(b)}$	38.08+4.08 $^{(b)}$	108.34+3.20$^{(b)}$
2	AHT	77.79+5.55 $^{(ab)}$	15.5+1.25 $^{(a)}$	22.00+1.03 $^{(a)}$	8.51+0.20 $^{(cd)}$	39.50+1.60 $^{(b)}$	81.67+3.64 $^{(a)}$
3	AST	63.89+5.12 $^{(a)}$	27.8+0.83 $^{(c)}$	31.34+1.47 $^{(bc)}$	7.96+0.14 $^{(c)}$	48.50+1.23 $^{(c)}$	141.00+1.77$^{(cd)}$
4	APA	75.01+7.13 $^{(ab)}$	37.6+2.04 $^{(d)}$	35.50+1.78 $^{(cd)}$	4.75+0.20 $^{(a)}$	43.83+3.03 $^{(bc)}$	132.17+6.09 $^{(c)}$
5	AMP2	83.34+6.08 $^{(b)}$	53.1+2.34 $^{(f)}$	30.50+2.04 $^{(bc)}$	7.01+0.17 $^{(b)}$	27.50+2.04 $^{(a)}$	148.34+3.49 $^{(d)}$
6	AHA	77.80+3.52 $^{(ab)}$	46.5+2.21 $^{(e)}$	39.84+1.84 $^{(d)}$	9.08+0.30 $^{(d)}$	45.00+2.55 $^{(bc)}$	164.50+3.10 $^{(e)}$

Mean of three replicates. Different letters within same column indicate significant difference between strains using Duncan's multiple range test ($P \leq 0{\cdot}05$).

Table 3. Effect of bacterial inoculations on biochemical parameters including pigment content, auxin content and protein content of *Helianthus annuus* L. (sunflower) under laboratory conditions.

S.#.	Treatment	Chlorophyll 'a' (µg/g)	Chlorophyll 'b' (µg/g)	Carotenoids (µg/g)	Auxin content (µg/g)	Protein content (µg/g)
1	Control	1.45+0.08 (ab)	2.38+0.18 (b)	0.38+0.01 (a)	56.34+6.11 (b)	6.89+0.62 (ab)
2	AHT	1.73+0.09 (bc)	2.67+0.16 (b)	0.61+0.03 (b)	16.34+1.85 (a)	7.33+0.78 (bc)
3	AST	1.22+0.04 (a)	2.37+0.10 (b)	0.50+0.02(ab)	65.00+4.72 (bc)	4.68+0.37 (a)
4	APA	2.69+0.17 (d)	1.83+0.19 (a)	0.46+0.04 (a)	83.67+7.12 (d)	10.44+0.78 (d)
5	AMP2	2.02+0.06 (c)	3.31+0.09 (c)	0.74+0.07 (c)	76.67+4.97 (cd)	9.36+0.75 (cd)
6	AHA	2.49+0.07 (d)	3.71+0.06 (c)	0.59+0.02 (b)	71.67+5.55(bcd)	10.87+0.95 (d)

Mean of three replicates. Different letters within same column indicate significant difference between strains using Duncan's multiple range test ($P = 0{\cdot}05$).

DISCUSSION

Plant growth promoting rhizobacteria cause enhancement of plant growth via direct or indirect mechanisms (Tailor and Joshi, 2014). These growth proliferating microbes also boost up the mineral ion uptake due to increase in specific ion fluxes at the root surface. Direct mechanisms for plant growth improvement involve nitrogen fixation, production of phytohormones, release of certain enzymes and mobilization of different nutrients whereas indirect mechanisms involve the plant tolerance towards stress, inducing host resistance or pathogen suppression etc. These all mechanisms work independently or synchronize with each other (Kong *et al*., 2015). Plant growth enhancing bacterial strains improve growth of the treated plants. In the present study, growth enhancement potential of already isolated and identified five rhizobacterial strains [*Kushneria avicenniae* (AHT), *Halomonas* sp. (AST), *Halomonas venusta* (APA), *Bacillus* sp. (AMP2) and *Arthrobacter mysorens* (AHA)]was evaluated using *Helianthus annuus* var. premier (Sunflower). Inoculation of *H. annuus* seeds with plant growth promoting rhizobacteria significantly enhanced the germination percentage of sunflower seeds. The isolates AHT (9.7& 12%), APA (16.1& 12%) and AHA (24.2& 8%) improved germination percentage significantly both under laboratory and wire house conditions. *Bacillus* sp. (AMP2) improved percentage germination (20%) only under wire house conditions compared to control. These findings have been attributed to the improved synthesis of hormones like auxin, cytokinins and gibberellins which can cause enhanced activity of specific enzymes that promoted germination at early stage such as amylase which resulted in increased availability of starch content. Significant increase in seedling vigour may result due to increased synthesis of auxins (Grobelak and Hiller, 2017).

Halomonas venusta (APA) and *Arthrobacter mysorens* (AHA) treatment caused increase in shoot length [34.6 & 67.4%; 52.2 & 106.7% respectively] both under laboratory and wire house conditions. Also these two strains (APA & AHA) caused improvement in root length (19.5 and 35.7% respectively), under laboratory conditions. *Halomonas* sp. (AST) affected shoot length positively both under laboratory and wire house conditions [29.3 & 23.7%] in comparison to control (Table 1&2). In case of wire-house experiment, treatment with *Bacillus* sp. (AMP2) has also shown 136.2% significant increase in shoot length. Bacterial inoculations greatly increase the production of all types of roots including primary, secondary and tertiary roots and also the total primary root length. Root volume has also been reported to be significantly increased by bacterial treatment (Ahmed and Hasnain, 2014; de Souza *et al*., 2015). Indole- 3 -acetic acid (IAA) produced by plant growth promoting rhizobacteria is proposed to increase root growth and length, modifying the plant morphological functions so that it will uptake more nutrients from the soil (Das *et al*., 2013). Improved mineral transport and nutrient uptake ability has been observed as a result of bacterial treatment which caused enhanced plant growth (Ahmed and Hasnain, 2014; Agbodjato *et al*., 2016). It is proposed that tryptophan is supplied by the root exudates to these working bacteria which play a vital role in microbial auxin synthesis.

Bacterial treatments did not affect the number of leaves under laboratory conditions compared to control. The isolate *Arthrobacter mysorens* (AHA), however, enhanced number of leaves upto 52.2% under wire house conditions compared to control. Almost all of the bacterial strains caused increment in fresh weight of plants with maximum increase observed in treatment with *Arthrobacter mysorens* (AHA) i.e., 129.2%, respectively over control (Table 1). Bacterial treatments remarkably promoted flower growth with reference to their diameter. Maximum increase (31.4%) in flower diameter was observed in treatment with isolate *Arthrobacter mysorens* (AHA), when compared with control. Maximum increase in the number of ray florets (27.4%) was observed in case of treatment with the isolate *Halomonas* sp. (AST) that caused significant increase over control. Also maximum increase in the number of disc florets was observed in

Arthrobacter mysorens (AHA) by 51.8% over non-inoculated control treatment (Table 2).

Considerable increase in chlorophyll 'a' was observed in case of treatment with isolates *Halomonas venusta* (APA) and *Arthrobacter mysorens* (AHA) i.e., 86.6, and 72.7% respectively, over control. Similarly, treatment of seeds with *Bacillus* sp. (AMP2) and *Arthrobacter mysorens* (AHA) caused significant increase in the chlorophyll 'b' content by 38.8 and 55.8%, respectively when compared with control. Almost all of the bacterial isolates positively affected carotenoid content of the plants. Maximum enhancement in carotenoid content was observed by inoculation with *Bacillus* sp. (AMP2) that caused 90.5% increase, in comparison to the control (Table 3). The increase in chlorophyll content of the leaves resulted in higher photosynthetic rates, higher yield and nutrient content of leaves which means improved plant biomass (Hidri *et al*., 2016). Vafadar and his coworkers (2013) also observed maximum amount of chlorophyll in *Punica granatum* due to bacterial treatments after four months of growth. The biochemical analysis of both treated and non-treated plants showed that inoculations of bacterial cultures caused enhancement in auxin production of the treated plants significantly. Treatment with isolates *Halomonas venusta* (APA), *Bacillus* sp. (AMP2), *Arthrobacter mysorens* (AHA) and *Halomonas* sp. (AST) have shown significant increase of 48.5, 36.0, 27.2 and 15.4%, in auxin biosynthesis respectively, when compared with control (Table 3). Application of PGPR enhanced the protein content of the treated plants which can be correlated to the high yield of seeds. Treatment with isolates *Kushneria avicenniae* (AHT), *Bacillus* sp. (AMP2), *Halomonas venusta* (APA) and *Arthrobacter mysorens* (AHA) have shown significant increase of 6.2, 35.8, 51.5 and 57.7% in protein content respectively, when compared to the non-inoculated control treatment (Table 3). In addition to this, other workers have also reported that PGPR are involved in improvement of the nutritive value of beans by enhancing their soluble protein (Stefan *et al*., 2013). Bacterial treatments improved water and nutrient uptake of plants which, indirectly or directly, affects the overall physiology of the plants. All the isolates used in the current study are auxin-producing and auxin is the master phytohormone which, directly or indirectly, affects all the plant processes, thus, inoculation with auxin-producing bacteria resulted in better chlorophyll content and higher protein content compared to non-treated plants, thereby, exhibiting the growth promotional potential of these isolates.

Conclusion: In conclusion, treatment with the selected five bacterial isolates have shown increase in growth and development of *H. annuus* plants by improving various growth and biochemical parameters of the inoculated plants thereby, increasing the total yield, biomass and vigour of the plants. Inoculation of seeds resulted in enhanced auxin production which increased the secondary and tertiary roots thereby, increasing the surface area of growing roots leading to more efficient absorption of nutrients by the plant. Thus, these bacteria can be further utilized as biofertilizers for plant growth improvement. Moreover, developing economies should use more products that are based on local isolates because no microbial inoculants can be used universally for all ecosystems.

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