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RESEARCH ARTICLE

Assessment of carrot growth performance with inoculation of AsT-PGPR under arsenic infested zone

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ABSTRACT

In the present study, the maximum rhizobacterial population was observed in Nutrient Agar (NA) (average; Cfu=135×10^6) followed by King's-B (average; Cfu=57×10^6), Soil extract agar (SEA) (average; Cfu=11×10^6), and Trypticase soy agar (TSA) (average; Cfu=9×10^6). Screening of arsenic tolerant rhizobacterial isolate revealed that about 1% of the bacterial isolate was from Nutrient Agar and King's-B survived at 20ppb arsenic concentration, while 0.8% and 0.7% survived from TSA and SEA media respectively. 50ppb arsenic tolerant rhizobacteria were screened for plant growth-promoting activity such as IAA, Phosphate solubilization, Siderophore production, ACC deaminase activity. Maximum IAA activity was observed in rhizobacterial isolates, isolated from all different media. P- solubilizer, Siderophore producer, ACC deaminase, proline, and TSS activities were observed in the isolates of NA media followed by King's-B media. 50ppb tolerate best suitable PGP traits producing isolates were inoculated to observe carrot plant growth in the pot experiment, Interesting and significant ($p<0.05$) result were observed in King's-B media producer isolates; (*Pseudomonas*) induces plant length, chlorophyll-a and chlorophyll-b content of the plant after 60 days followed by 30 days.

Key words: Arsenic tolerant PGPR (AsT-PGPR); PGP traits; Plant growth performance

1) INTRODUCTION

Arsenic is a naturally occurring important global environmental toxicant. Many people have died and hundreds of millions are accessing at serious risk in countries throughout the world such as Bangladesh, India, China, Vietnam, Taiwan, Japan, Poland, Hungary, Romania, Slovakia, Belgium, Chile, Argentina, and North Mexico [1,2,3]. Generally free arsenic in groundwater comes from arsenopyrites by its oxidation [4]. This groundwater is used extensively for irrigation purposes thereby most of the soils in this area are contaminated with arsenic [5], which may lead to alteration of soil ecology and crop productivity [6]. Humans are affected by arsenic not only through drinking water but also from agricultural crop and vegetables [7]. Therefore, such contamination increasing the possibility of arsenic biomagnification in crop fields which may indirectly dangerous to human beings for the coming decades [4]. It is established that arsenic is highly toxic to microbes as it inhibits their metabolic pathway [8]. Soil microbial populations and productivity of the soil are how much affected by the arsenic still are not evaluated. Reports of arsenic toxicity against diazotrophic and phosphate solubilizing bacteria are also inadequate. It is evident that PO_4^- uptake is competitively inhibited by arsenic and is actively transported into the plant root system [7]. Remediation of arsenic from contaminated agricultural soil is an urgent need, and bioremediation is an effective eco-friendly tool to mitigate these problems [6]. Bacterial activities reduce the concentration of arsenic in the soil through various mechanisms such as sorption, biomethylation, complex action, and redox reaction [9]. Mobility of arsenic in soil affected by several bacterial species; mediated through a redox reaction, the biochemical mechanism to exploit arsenic oxyanions, either as the electron donor (As Vth stage) or acceptor (As IIIrd stage) [10,11]. Despite its toxicity, several microorganisms are capable of using either the oxidized form of inorganic arsenic, As (V), or the reduced form, As (III), in their metabolism and even some microorganisms are capable of resisting arsenic toxicity through the *ars* genetic system [6]. Several bacteria such as *Escherichia coli* [12], *Staphylococcus aureus* [8], *Pseudomonas aeruginosa* and *Rhizobium* sp. Carrasco et al., [13] have shown their ability to uptake arsenic and to become resistant to a significant level of arsenic. It is also evident from the

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earlier reports that a group of bacteria exhibited biotransformation ability of highly toxic arsenite [As (III)] to less toxic arsenate [As (V)], but a very few bacteria can eliminate arsenic directly as a form of volatile methyl arsenious acid [8]. There is an urgent need to explore the arsenic level of the crop fields in concerning bacterial diversity specially diazotroph and phosphate solubilizing bacteria. Possible microbial agents to be identified for reduction of arsenic level by transformation, which will be a potential biofertilizer to recover the soil health. Rhizospheric bacteria are closely associated with plant roots and mostly act as plant growth-promoting activity, such bacteria known as PGPR plant growth promoting rhizobacteria [14,15]. The close association of PGPR showed the best coping mechanism between plant and microbes, including the surrounding environment [16]. The coping behaviors of PGPR against metal stress develop several mechanisms such as immobilization, mobilization, or transformation of metals rendering them inactive to tolerate the uptake of toxic metal [17,18]. Based on earlier reports [19], we hypothesized that the transport of arsenic in plants reduces through arsenic tolerant plant growth-promoting rhizobacteria (AsT-PGPR) as shown in figure 1. The penta stage of arsenic is an analog of phosphate and its travel by phosphate transport mechanism and 3rd stage of arsenic travel through aquaglyceroporins [20,19]. Considering the deleterious effect of arsenic pollution, the aims of the present work are (i) selection of arsenic infested agricultural zone, (ii) isolation and screening AsT-PGPR, and (iii) evaluation of carrot plant growth by inoculation of AsT-PGPR.

2) MATERIALS AND METHODS

Sample Collection and Isolation of arsenic tolerant PGPR: The root adhering soil (RAS) samples were collected from rooted vegetables grown field at 30 days of growth. The RAS sampled from different locations of district Ballia and were kept in plastic bags for isolation of rhizospheric bacteria. Analysis of physicochemical and arsenic concentrations in soil samples was examined as earlier described [5]. For the isolation of rhizospheric bacteria, the roots were shaken to remove excess soil and 10 g of closely associated rhizospheric soil from each sample was added to 90 ml of sterile water and shaken for 30 min on a mechanical rotary shaker. Tenfold dilutions were made and plated on to five media, Jensen's N free medium, King's-B (KB) medium, nutrient agar (NA), trypticase soy agar (TSA), and soil extract agar (SEA) as earlier described [21, 27]. Bacterial cultures were maintained on the respective slants and stored at 4^0C until further use. The intrinsic resistance of the rhizobacteria isolates against Arsenic tolerance was evaluated by observing the growth on NA medium amended with various concentrations of Na_2HAsO_4-$7H_2O$ (10, 20, 30, 40, 50, 60, 70,80,90 and 100ppb). The control plate was also maintained with 0.05% Na_2HAsO_4-$7H_2O$ (w/v). The plates were incubated for 48 h at 28 ± 2^0C and the growth on Na_2HAsO_4-$7H_2O$ amended broth was compared with control plates. All the isolates that showed Arsenic tolerance up to 50 ppb, were screened for the expression of plant growth-promoting attributes at different Na_2HAsO_4-$7H_2O$ concentrations (10, 20, 30, 40, 50, 60ppb). The PGP attributes were estimated as total cellular protein by the Bradford method. IAA production and gibberellins were estimated by colorimetric methods as described earlier [21,15], phosphate solubilization was observed on Pikovskaya's agar plates, ACC deaminase activity was analyzed by using DF Salt minimal medium with ACC as the sole source of nitrogen [22] and siderophore production was also examined [23].

AsT-PGPR inoculation and plant growth: Pot experiment: The tested topsoil samples were mixed thoroughly with Na_2HAsO_4-$7H_2O$ (50ppb) and filled into plastic 2 kg pot. Seeds of carrot were surface sterilized in 1% (w/v) sodium hypochlorite for 3 min, washed several times with sterilized distilled water (SDW), and soaked in SDW overnight [24]. Twenty soaked seeds were sowed directly into the 0.8% agar plate and incubated for three days in a dark room [16,24,25]. Three similar sizes of sprouted seed were placed in each treatment pots in the greenhouse with natural light (10–12h; photoperiod) and temperature (16–30^0C), and after 12h, 5 ml of incubated (24 h) arsenic tolerant PGPR isolated broth were

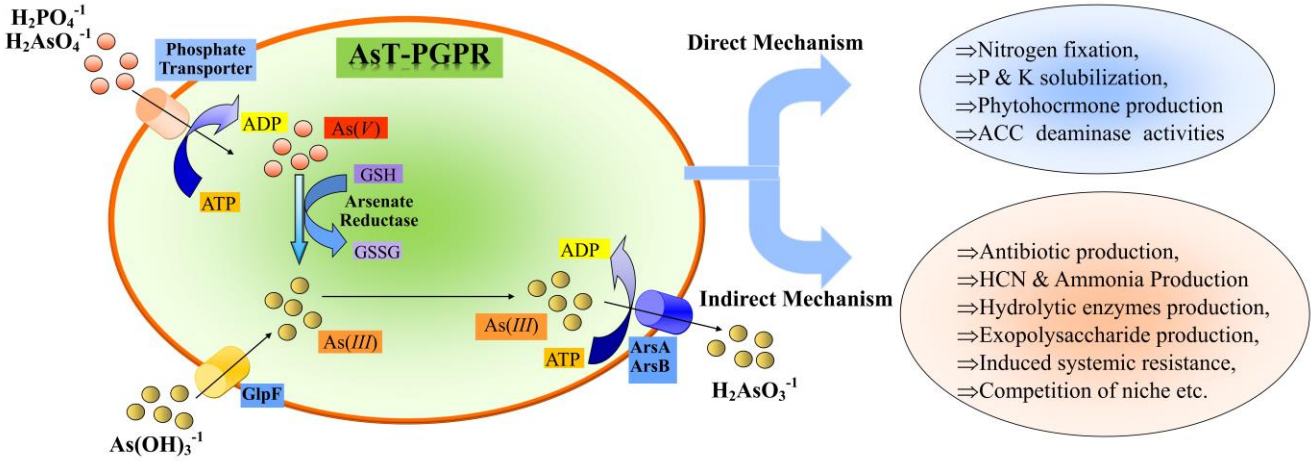


Figure 1: Schematic mechanism of arsenic tolerant plant growth-promoting rhizobacteria (AsT-PGPR) and its plant growth- promoting traits [14, 17]

inoculated in each pot. The plants were harvested after 30, 60 DAS (days after showing) for further analysis.

***Physico-chemical analysis of plant*:** Edible part (EDP), Leaf (L) of the plant were examined for their length, fresh weight (FW) and dry biomass (DW) were examined after 30 and 60 DAS. Leaf chlorophyll a (Chl-a) and b (Chl-b) were estimated by spectrophotometrically (A663nm and A645nm) by method [26]. The content of glucose, fructose, and sucrose was determined by the ferrocyanide, Proline content (µg mg^{-1} FW) was determined according to Bates (1973) in the 0.5 gm fresh weight (FW) of leaf tissue at 520nm.

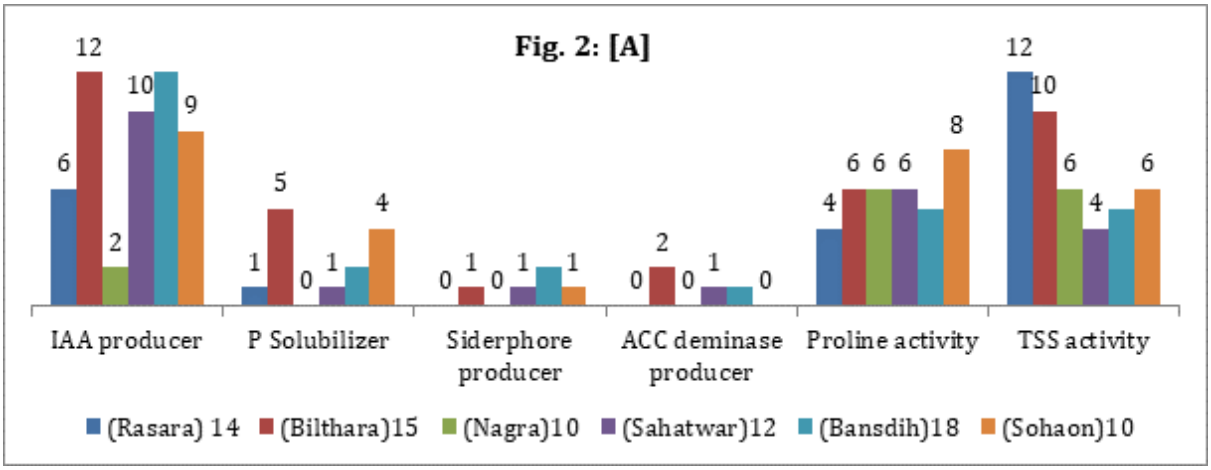
Statistical analyses: The data obtained were subjected to ANOVA, and means were compared with Duncan's multiple range test. Pearson correlation coefficients were used to assess the significance of interrelationships between the different study parameters of wheat. All statistical analyses were conducted using SPSS (Version 14; IBM, Armonk, NY, USA).

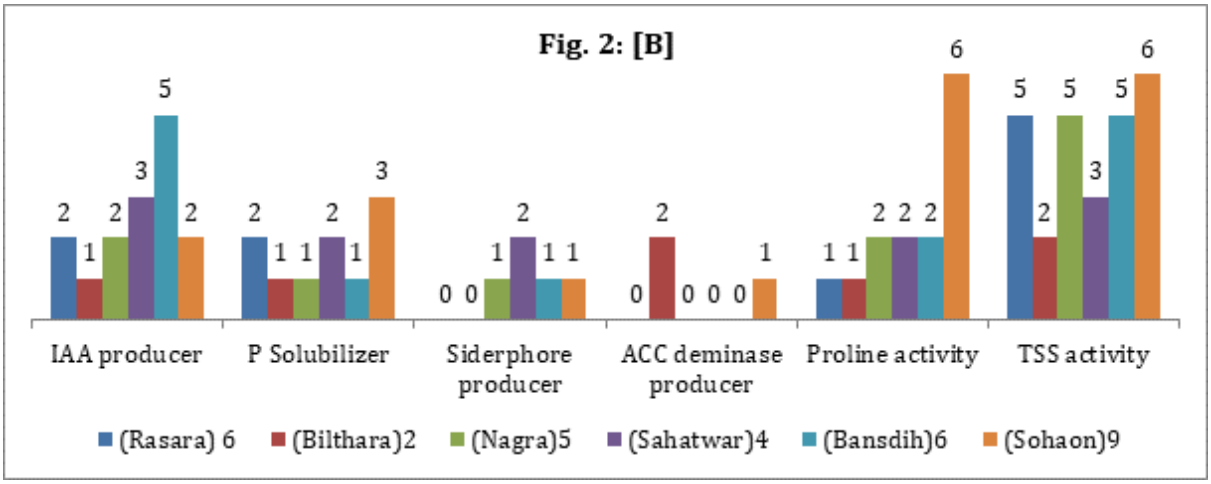
3) RESULTS AND DISCUSSION

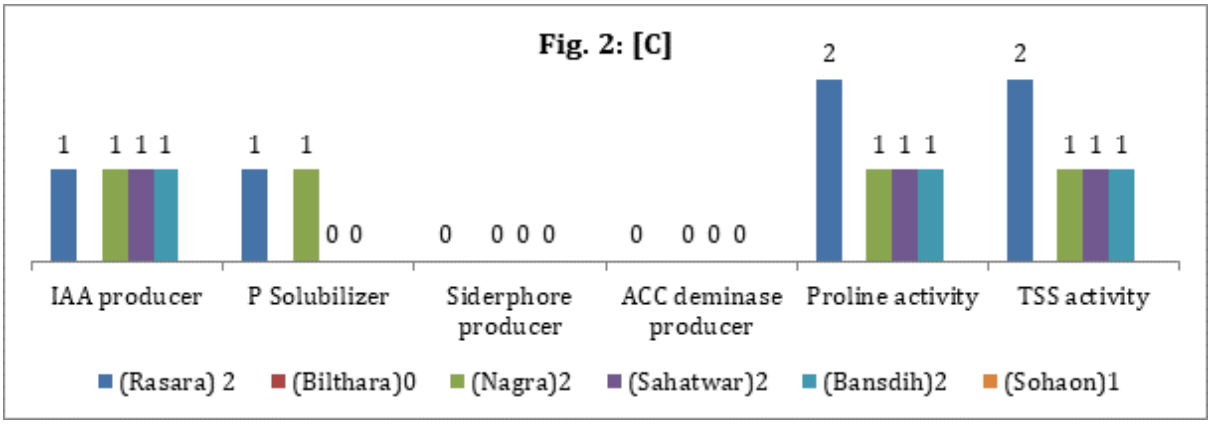
In the present study, the selection of six agricultural sites was based on our previous study [5], and soil samples (>30n of each site) of all sites exhibit arsenic concentration with a range from 8 to 26 ppb shown in table 1. In the present study, rhizobacterial isolates, isolated from rhizospheric soil of vegetable grown in aforesaid selected sites of district Ballia. The cultural method was applied to evaluate the rhizobacterial population among all soil samples by using different media such as Nutrient Agar (NA), King's-B, TSA, and SEA respectively. The maximum rhizobacterial population was observed in NA (average; Cfu=135×10^6) followed by King's-B (average; Cfu=57×10^6), SEA (average; Cfu=11×10^6), and TSA (average; Cfu=9×10^6) (Table 1). A similar approach was applied earlier by Upadhyay et al., [27] for the isolation of rhizobacterial isolates by using different media. Screening of arsenic tolerant rhizobacterial isolate revealed that about 1% of the bacterial isolate was from Nutrient Agar and King's-B tolerate at 20ppb arsenic concentration, while 0.8% and 0.7% survived from TSA and SEA media respectively (Table 1). The tolerance behavior of rhizobacterial isolates was earlier described and reported [28]. The presence of arsenic tolerant rhizobacteria indicates the coping behaviors of bacteria against arsenic stress, mediates several mechanisms such as immobilization, mobilization, or transformation of metals rendering them inactive to tolerate the uptake of toxic metal [17,18]. Further screening at 50ppb concentration of arsenic revealed that more survivability was observed in rhizobacterial isolates that isolated from King's-B media followed by NA, TSA, and SEA respectively. Several reports suggested that reduction of arsenic concentration in soil was carried out by several microbes through various mechanisms such as sorption, biomethylation, complex action, and redox reaction [9]. 50ppb arsenic tolerant rhizobacteria were screened for plant growth-promoting activity such as IAA, Phosphate solubilization, Siderophore production, ACC deaminase activity shown in figure 2. Rhizobacterial isolates including several microbes can promote plant growth under varied environmental conditions/stress [15,16,21,29,30,31]. Maximum IAA activity was observed in rhizobacterial isolates, isolated from different media (Fig: 2A, B, C, and D). P-solubilizer, Siderophore producer, ACC deaminase, proline, and TSS activities were observed in the isolates of NA media followed by King's-B media, while isolates from TSA media had not shown Siderophore and ACC deaminase activities (Fig: 2C), but proline and TSS activities are more in TSA isolate as compared to IAA and P- solubilizer. In general NA media support to the growth of *Bacillus* genera, King's-B support to *Pseudomonas* genera, TSA support *Azotobacter* genera, and SEA support to *Arthobacter* genera. Singh et al., [32] earlier reported that *Arthobacter* induces plant growth. 50ppb tolerant best suitable PGP traits producing isolates were inoculated to observe carrot plant growth in the pot experiment (Fig: 3). A similar result was observed in the case of salt tolerant PGPR induces wheat plant growth [15,16]. Interesting and significant ($p<0.05$) results were observed in King's-B media producer isolates, which induces plant length, chlorophyll-a, and chlorophyll-b content of the plant after 60 days followed by 30 days. The rest of the rhizobacterial isolates from NA media, TSA, and SEA media revealed good growth performance but not attain more as King's-B isolates. The results indicate that the *pseudomonas* family was able to tolerate 50ppb arsenic and induces plant growth performance of the carrot plant.

Table 1: Isolation of rhizobacterial isolates at different media from arsenic infested zone of district Ballia.

Place and number of sampling sites (N)	Latitude	Longitude	Arsenic concentration in Soil (ppb)	Nutrient Agar (NA)		King's-B		Trypticase soy agar (TSA)		Soil extract agar (SEA)	
				(Cfu=B X 10^6)	Nos of bacteria Survived at 20ppb	(Cfu=B X 10^6)	Nos of bacteria Survived at 20ppb	(Cfu=B X 10^6)	Nos of bacteria Survived at 20ppb	(Cfu=B X 10^6)	Nos of bacteria Survived at 20ppb
Rasara (N=32)	25.8564	83.8636	12±2	156±22	14±2	35±4	6±1	12±4	2±1	10±2	2±1
Bilthara (N=34)	25.8623	83.8952	08±2	122±14	15±2	52±3	2±1	6±1	0	12±2	1±1
Nagra (N=42)	25.8734	83.86	26±5	109±15	10±4	46±2	5±1	11±4	2±1	15±2	6±2
Sahatwar(N=30)	25.8344	84.3127	19±2	110±6	12±2	88±6	4±2	14±2	2±1	10±2	2±1
Bansdih (N=30)	25.8774	84.3912	22±7	185±11	18±7	42±7	6±1	5±4	2±1	8±2	5±1
Sohaon (N=41)	25.8572	83.8498	10±2	126±17	10±1	82±3	9±1	9±1	1±1	11±2	4±1







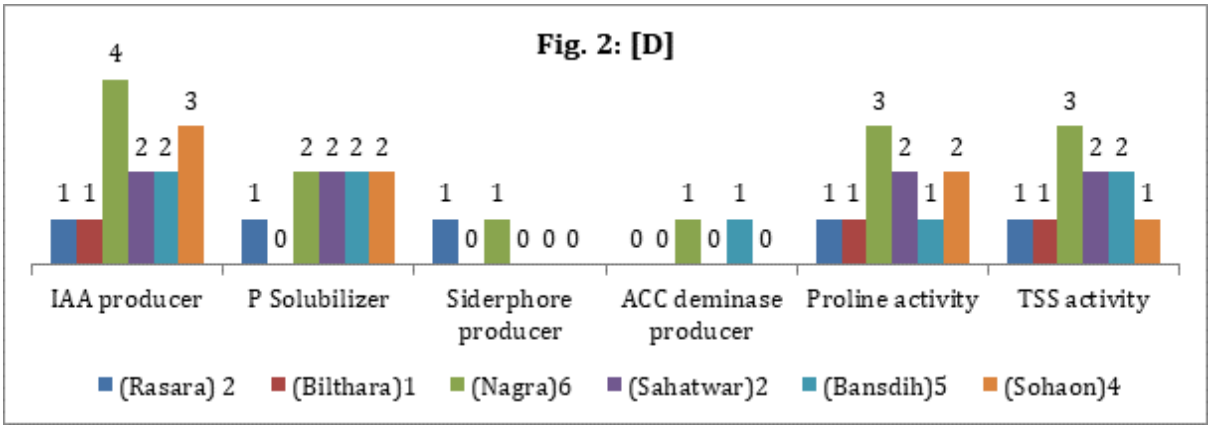


Figure 2: Arsenic tolerant plant growth-promoting traits of rhizobacteria, isolated by using different media such as Nutrient agar [fig. A], King's b [fig. B], Trypticase soya agar [fig. C], and Soil Extract media [fig. D], from different places at district Ballia .

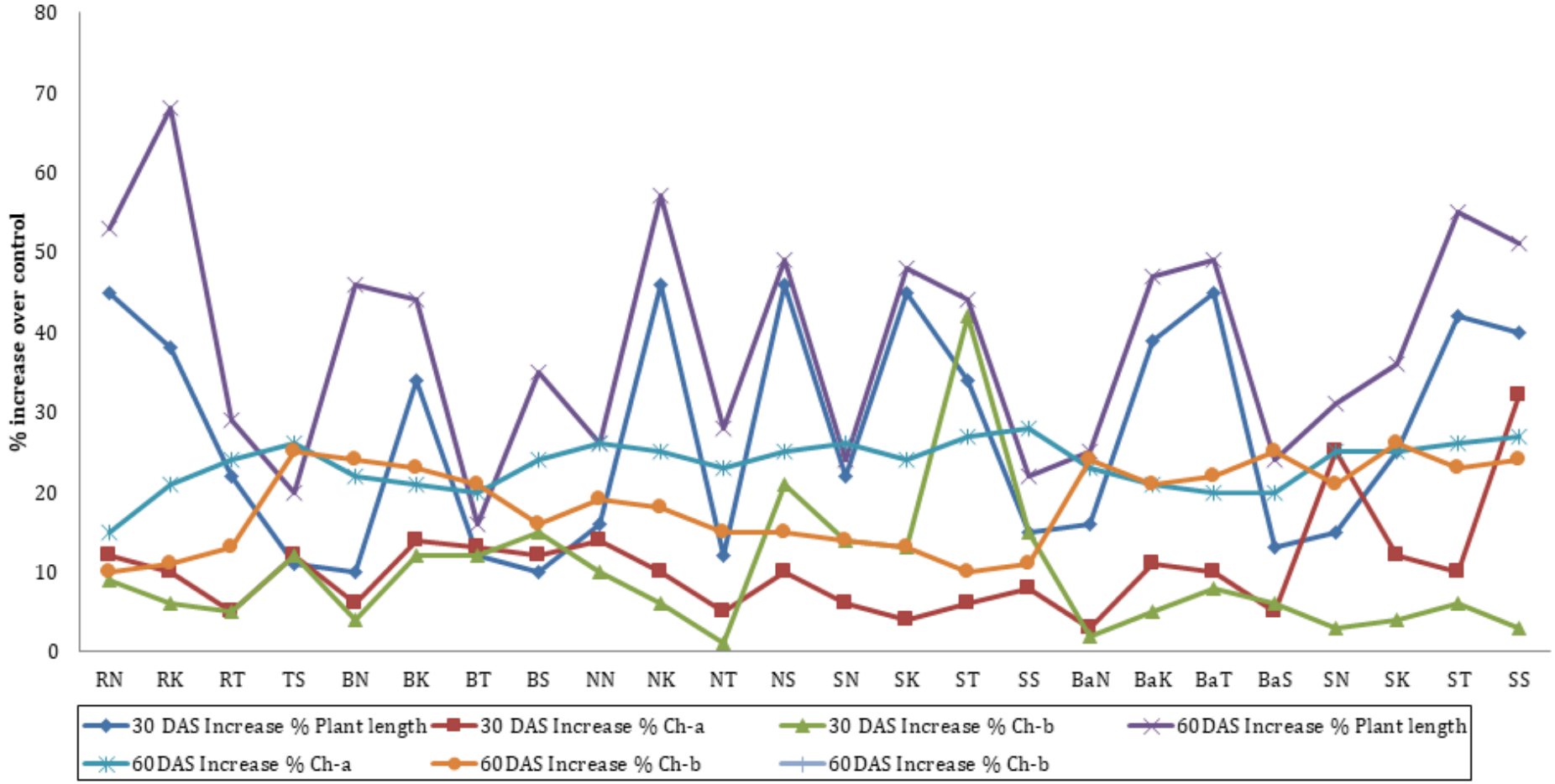


Figure 3: Growth performance of carrot plant (after 30 and 60 days respectively) with Inoculation of arsenic tolerant best PGP producer isolate, isolated from different media. R=Rasara, B= Bilthara,N= Nagra, S= Sahatwar,Ba= Bansdih, S= Sohaon and N=Nutrient agar, K=Kings's B media, T=Tryticose soya agar, S=Soil extract Agar.

4) CONCLUSION

Arsenic tolerant plant growth- promoting rhizobacteria could be an effective eco-friendly tool to mitigate the arsenic problem in agricultural field. The present study demonstrated that *pseudomonas* family was able to tolerate high level of arsenic concentration and induces plant growth performance of the carrot plant. The finding suggested that the potent arsenic tolerant plant growth-promoting rhizobacteria could be used as a bio-inoculant under arsenic infested agricultural zone.

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