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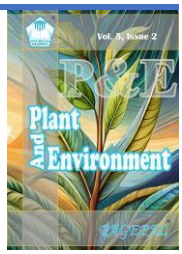

Unveiling the bioherbicidal potential of weed-suppressive bacteria to control growth of *Echinocloa crusgalli* in maize crop

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Abstract

Excessive use of tillage and chemical herbicides in intensive agricultural systems has produced many issues related to environment, human health, food quality and sustainability of natural resources. Additional non-conventional tools for weed management must be explored and/or strengthened in order to minimize the crop losses caused by weeds and produce healthier food. Present work has explored rhizosphere of barnyard grass (Echinochloa crusgalli) (BYG) and maize to find out weed suppressive bacteria (WSB) and demonstrate their bioherbicidal potential. A large collection of rhizobacteria from maize and barnyard grass was characterized for production of cyanide, inhibition of Escherichia coli, release of siderophores and indole-3-acetic acid, activities of chitinase, catalase and oxidase, and effects on germination and seedling growth of BYG and maize. Six promising strains were evaluated for control of BYG and implications on growth of maize under axenic conditions of growth room. We found that all strains were suppressive to germination, shoot length (-59%), root length (-63%), fresh biomass (-64%) and dry matter (-63%) of BYG with respect to control. Three strains did not significantly affect germination, growth and chlorophyll content of maize, while other three strains significantly improved these parameters with respect to control. Therefore, these strains may be used as bioherbicides to selectively suppress growth of BYG in maize crop.

1. Introduction

Weeds are the plant species which compete with crop plants for limited resources in the field. They cause more losses to crop than other pests (Oerke, 2006). Weed invasion in maize causes losses worth Rs. 6.3 annually in Pakistan (Khan *et al.*, 2016). Generally, the yield loss due to weeds in the maize crop is 30-70% (Rusu *et al.*, 2010). Major weeds that invade in the maize crop are barn yard grass (*Echinochloa crusgalli*), purple nutsedge (*Cynodon dactylon* (L.) Pers.), horse purslane (*Trianthema portulacastrum*), slender amaranth (*Amaran thusviridis* L) and jungle rice (*Echinocloa colona*). Growth of barnyard grass in maize can reduce crop yield from 50 to 80% as described by (Spitters *et al.*, 1989). Recent studies showed that this weed can consume 60-80% of the nitrogen from the soil (Abbas *et al.*, 2018).

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Conventional weed control methods include physical and chemical approaches. Physical methods involve tillage, hoeing, hand weeding, sickling, mowing and many others (Abbas *et al.*, 2018). This approach is the oldest one and is now considered to contribute towards loss of soil fertility, soil erosion, damages to roots and eutrophication (Birkás *et al.*, 2004). Physical methods are also labor-intensive (Scarabel *et al.*, 2010). Chemical herbicides are widely adopted due to convenience, quick response and efficient control. They have improved crop production worldwide (Sun *et al.*, 2012). However, limitations of herbicides were often ignored in order to get short-term benefits of higher yield. They cause damage to biodiversity by killing sensitive species of plants and animals. Various human health issues like cancer, nervous disorders, respiratory disorders, and many other chronic diseases have been related to exposure to herbicides (Collotta *et al.*, 2013).

The above scenario has increased the relevance of biological weed control in crop production. Advantages of biological control, involving safer and healthier food, conservation of natural resources, and environmental benefits, are appealing for scientists and society. It uses living organisms and their products to suppress harmful effects of weeds on crops (Harding & Raizada, 2015). Four basic strategies can be used in biological control (i) Introduction: a classical technique whereby an exotic helpful organism is introduced into a new region and become fully established. This strategy is usually used against introduced pests that have no indigenous antagonists. (ii) Augmentation: the laboratory-bred individuals can be released to compensate for the inefficiency of present biocontrol agents. The inadequate level of control can be driven by the low number of native natural enemies. (iii) Inoculation: when an indigenous antagonist is not present or an introduced one cannot survive permanently, an inoculative release is made at the beginning of planting season. This process may need to be repeated for each following crop. (iv) Inundation: the mass culture of a biocontrol agent is carried out for urgent use at critical periods when rapid suppression of pest population is necessary (Li & Kremer, 2006).

Nature of biocontrol agents employed in the past included herbivorous/phyto-phagous insects, pathogens of weeds and plant allelopathy. However, the interest of scientists in these biocontrol agents has reduced over time owing to multiple factors. Insect biocontrol agents developed in the past obtained very exhaustive and lengthy research work. Wider host range, chances of change of host in the environment and emergence of new pests also reduce reliance on these biocontrol agents (Boyette & Hoagland, 2013). Success of pathogens of weeds as biocontrol agents depends on conducive environmental conditions in the absence of which they rarely cause infections on weeds to suppress their population and harmful effects on crops (Mitchell & Power, 2003). An alternative and attractive approach for enhancing scope of biological weed control is the use of rhizobacteria with deleterious activity towards growth of weeds (Abbas *et al.*, 2018). These may be called weed suppressive bacteria (WSB). Previously rhizobacteria have been shown to inhibit growth of downy brome (*Bromus tectorum L.),* Japanese brome (*B. japonicum Thumb. ex Murr*.), and jointed goat grass (*Aegilops cylindrica* Host.) in wheat fields (Bhowmik, 2003). They produce phytotoxic substances, suppress growth of target plants (may be directed towards weeds), exhibit selectivity and are environmentally friendly. Their metabolites are biodegradable and if not utilized do not persist in the environment (Fox *et al.*, 2011). They are known to produce cyanide, higher amounts of phytohormones and many other phytotoxic metabolites (Kremer & Souissi, 2001b). Kremer and Souissi (2001b) reported that BYG was sensitive to cyanide produced by rhizobacteria. Therefore, cyanide producing strains of rhizobacteria can be used to selectively suppress growth of BYG in crops. However, success of weed control depends on competitive root colonization, adequate population and availability of substrates to produce phytotoxic metabolites (Kremer & Kennedy, 1996; Kremer & Souissi, 2001a; Sivasakthi *et al.*, 2014). Present work has explored rhizobacterial strains possessing the ability to produce cyanide and other phytotoxic metabolites; investigated their weed suppressive effects on BYG; and explained their implications on growth of maize.

2. Materials and Methods

2.1. Isolation of rhizobacterial strains

Bacterial strains were isolated from the rhizosphere of BYG and maize. Intact plant seedlings were collected using a trowel or a probe, placed in Ziploc bags, and transported to the laboratory in an icebox. Rhizobacteria were isolated from these samples using serial dilution plating technique (Dworkin and Foster (1958). Serial dilutions (10^{-2} - 10^{-7}) of rhizosphere were spread on Petri plates containing King's B agar media (K_2HPO_4 at 1.5 g L^{-1}, proteose peptone at 20 g L^{-1}, $MgSO_4.7H_2O$ at 1.5 g L^{-1}, glycerol at 10 mL L^{-1} and agar at 20 g L^{-1}) (King *et al.*, 1954). This media was sterilized in an autoclave at temperature of 121° C and pressure of 15 psi for 20 minutes before poured into Petri plates. These plates containing serial dilutions were incubated at 28±1°C for 48 hours. Fast-growing colonies were then streak-cultured on separate Petri plates and purified.

2.2. Screening of strains by HCN assay

Rhizobacterial strains were tested for cyanide production using a hydrogen cyanide assay (Schippers et al., 1987). Filter papers were cut and soaked overnight in 1% picric acid solution, then dried under a laminar flow hood. Glycine-

amended King's B media was prepared and poured onto Petri plates. The dried filter papers were sprayed with 10% Na_2CO_3 and attached to the lids of the Petri plates. Strains were streaked onto the glycine amended king's B media and covered with the alkaline picrate filter paper-fitted lids. Parafilm was wrapped around each Petri plate twice to seal them. These Petri plates were incubated at 28 ± 1 °C for 4-5 days. The appearance of dark yellow to reddish-brown color on the filter paper indicated cyanide production, with darker color qualitatively indicated greater amount of hydrogen cyanide produced by the strains.

2.3. Characterization of cyanogenic rhizobacterial isolates

Non-pathogenic *E. coli* strain K12 is sensitive to toxic secondary metabolites of rhizobacteria and hence, can be used as an indicator for the production of potential phytotoxic metabolites. Stock culture of sensitive *E. coli* strain K12 was obtained from the Centre for Agricultural Biochemistry and Biotechnology, University of Agriculture, Faisalabad. It was sub-cultured on Luria Bertani (LB) agar media at 28°C for 48 hours. For assay, fresh LB agar Petri plates were prepared. One hundred microliter broth culture of *E. coli* was spread on these plates. Each rhizobacterial isolate was spotted with bacteriological loop aseptically on four points on plates containing layer of *E. coli.* Killing of *E. coli* is exhibited by zones of clearing around growth of rhizobacterial isolates.

Siderophores production assay was performed according to Smith *et al.* (1985). Petri plates containing chrome azurole S (CAS) agar media were used for this assay. Culture of each strain was poured into wells made on media and incubated at 28 ± 1 ºC for 48 hours. Change in color of media from blue to orange indicated production of siderophores.

Strains were inoculated at 4 different points on Petri plates containing RCV-glucose agar media and incubated at 28±1 ºC for four days. Mucoid growth of colonies in these plates indicated production of exopolysaccharides (EPS) (Ashraf *et al.*, 2004).

Phosphate solubilizing assay was conducted according to Mehta and Nautiyal (2001). Strains were spot inoculated on NBRI bromophenol media and incubated at 28 ± 1 ºC. Appearance of clearing zone around growth of strains indicated solubilization of rock phosphate.

Strains were inoculated on chitin, amended King's B media and incubated at 28 ± 1 ºC for four days. Clearing zone around point of inoculation indicated chitinase activity by the strains (Chernin *et al.* (1998).

The catalase test was simply conducted on the microscopic slides. Loopful culture of each strain was placed on microscopic slides and hydrogen peroxide added. Bubbling from culture indicated catalase activity (MacFaddin, 1980).

Filter papers of small size were soaked in 1% Kovács oxidase reagent. Fresh culture of each strain was rubbed on the dry filter paper. The change in color to dark purple indicated oxidase activity.

Mobility of bacterial strains was checked by using the Kings B agar (0.4% agar) media having 1% Triphenyl tetrazolium chloride, (TTC) in it. Loopful culture of each strain was stab inoculated in these plates and incubated at 28±1ºC. appearance of red color around point of inoculation indicated motility of the strain (Tasawar Abbas *et al.*, 2017).

2.4. Agar bioassay to test germination inhibition of BYG by weed suppressive bacteria

Strains screened in the above steps were used to conduct this test. Water agar (100:1) containing 1% tryptone was prepared and sterilized in autoclave (121ºC and 15 psi pressure for 20 minutes). It was poured in large sized (15 cm diameter) Petri plates. Uniform seeds of BYG and maize were taken. Surface of seeds was sterilized using ethanol (70%), NaOCl (5%) and rinsing with sterilized distilled water as explained by Abd-Alla and El-Enany (2012). Seeds were dipped in fresh culture of selected strains for 10 minutes and placed on the surface of water agar. These plates were placed on the laboratory shelf for 5 days. Finally, the germination count of seeds was taken.

2.5. Evaluation of bioherbicidal activity of weed suppressive bacteria under axenic conditions

Six efficient strains collected after screening in the above steps were used to evaluate their effects on growth of BYG and maize under axenic conditions. The trial was conducted in the growth room of Institute of Soil and Environmental Sciences.

Each strain was cultured in King's B broth media. Bacterial cells were harvested from culture through centrifugation at 4000*g*. Cells were suspended in 0.01 M $MgSO_4$ buffer to get population of 10^8 cells per milliliter by measuring optical density at 600 nm (Zahir *et al.*, 2018).

Sand was filled in pots (diameter of 6 cm and height of 13 cm) and sterilized in autoclave at 121ºC and 15 psi pressure for 20 minutes for three times. Outer surface of seeds of BYG and maize was sterilized with 70% ethanol and 5% NaOCl followed by rinsing as described by (Abd-Alla & El-Enany, 2012). Seeds were separately dipped in inoculant of each strain for 20 minutes followed by sowing in pots containing sand. These inoculated seeds were sown in the pots. BYG seeds were sown at 10 seeds per pot and those of maize at 4 seeds per pot. One milliliter inoculum was added onto sand surface in each pot followed by covering sand with thin layer of sterilized sand. For control treatment, seeds dipped in sterilized buffer (0.01 M $MgSO_4$) and one milliliter buffer added into these pots. Hence, there were seven treatments in the experiment: six for 6 strains and one for control. Each treatment was replicated thrice, and pots were placed according to completely randomized design (CRD).

The experiment was conducted in the growth room provided with fluorescent and incandescent bulbs. They

provided a light intensity of 350 µmol m^{-2} s^{-1}. The temperature of growth room was set at 25±1ºC. A light period of 16 hours and a dark period of 8 hours were adjusted. Hoagland's nutrient solution was applied to provide nutrients and moisture to seedlings of maize and BYG (Tahir Abbas *et al.*, 2017). Plants of weed and maize were harvested after 22 days to collect data of agronomic parameters.

2.6. Statistical analysis

Data regarding the agronomic parameters was recorded and analyzed statistically by using a completely randomized design CRD and comparisons were made by using LSD test. Software XLSTAT was used in the mean comparison (Elliott & Woodward, 2007). Principal component analysis was done using Minitab ® Software 2017.

3. Results

3.1. Microbial Characterization of rhizobacteria for bioherbicidal activity

Bacterial strains were isolated from the rhizosphere of maize and barnyard grass. Seventy strains were isolated from maize and sixty from BYG. These strains were subjected to multiple screening tests to find out potentially weed suppressive bacteria. Results of these tests are given in table 1. The isolated strains were first evaluated for ability to produce hydrogen cyanide. We have six strains to be capable of producing HCN. Cyanide producing strains were tested in *E. coli* antimetabolite assay. Hallo zones were visualized around the point of stabbing of rhizobacterial isolates. Strain BN2 killed *E. coli* around the spot of its inoculation while others indicated positive relationship with *E. coli.* In phosphate solubilization assay, all strains showed ability to solubilize inorganic phosphate. Siderophores production assay, conducted on CAS media, indicated that all strains were capable of producing siderophores.

Auxins production assay was conducted with and without L-tryptophan. With L-tryptophan, indole-3-acetic acid production of 0.85, 2.49, 2.35, 2.04, 3.18 and 3.27 µmol L^{-1} were observed for strain BN1, BN2, BN3, BN4, BN5 and BN6, respectively. Without L-tryptophan, indole-3-acetic acid production of 0.09, 0.65, 1.05, 0.66, 1.36 and 1.05 µmol L^{-1} were observed for strain BN1, BN2, BN3, BN4, BN5 and BN6, respectively.

All strains exhibited chitinase activity in chitinase assay. All strains produced mucoid growth on RCV-glucose media indicating their capability to produce exopolysaccharides. Bubbling from culture upon treatment with hydrogen peroxide indicated that all strains possessed catalases. However, individual strains varied in exhibiting catalase activity. Strain BN1, BN3, BN4 and BN5 indicated high catalase activity while strain BN2 and BN6 indicated low catalase activity. Oxidase activity was measured with 1% Kovacs reagent which indicated that all strains possessed oxidases. Motility of strains was tested on TTC containing media which indicated that all these strains were motile. Gram reaction of all our strains was negative.

3.2. Evaluation of weed suppressive bacteria for their ability to suppress growth of BYG

Results of effects of six strains on germination of BYG in agar bioassay are shown in Figure 1. It indicates that all strains significantly reduced germination of BYG as compared to control. The maximum reduction in germination was caused by strain BN3 and BN5 (49 and 50% lesser than control, respectively) which was also significantly lesser than

Table 1. Microbial characteristics of weed suppressive rhizobacterial isolates

Microbial characteristic	BN1	BN2	BN3	BN4	BN5	BN6
Gram reaction	–	–	–	–	–	–
Extra-cellular polysaccharides	+	+	+	+	+	+
E. Coli anti-metabolite assay	+	–	+	+	+	+
Phosphate Solubilization Assay	+	+	+	+	+	+
Siderophores production assay	+	+	+	+	+	+
Chitinase activity	+	+	+	+	+	+
Catalase	+++	+	+++	+++	+++	+
Oxidase	+	+	+	+	+	+
Motility	+	+	+	+	+	+
Indole-3-acetic acid (IAA) production (With L-Tryptophan)	0.85	2.49	2.35	2.04	3.18	3.27
Indole-3-acetic acid (IAA) production (Without L-Tryptophan)	0.09	0.65	1.05	0.66	1.36	1.05

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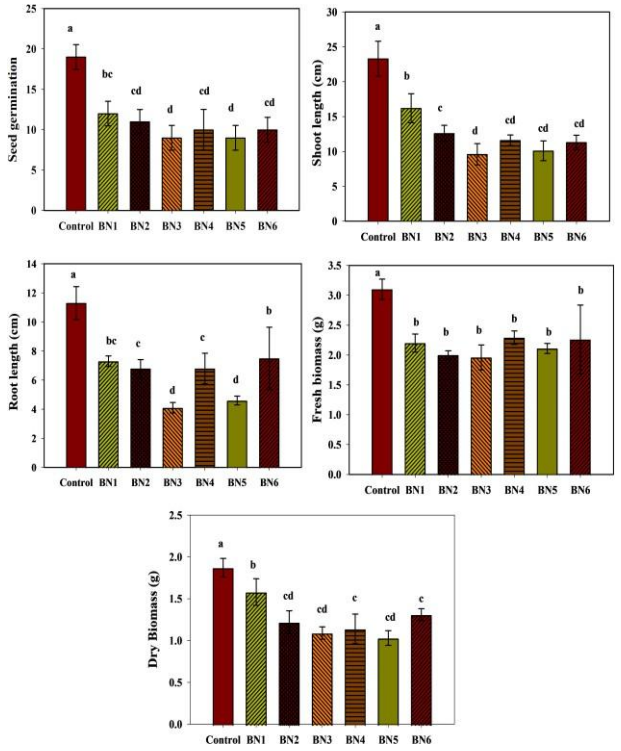


Figure 1: Impact of weed suppressive bacteria on growth of barnyard grass (error bars indicate the standard error of the mean of the treatments)

reduction in germination caused by strain BN1. Strain BN1 reduced germination up to 35% than control. This strain caused non-significant difference from strain BN2, BN4 and BN6 in affecting seed germination. Strain BN2, BN4 and BN6 reduced germination of BYG. The rest of the strains showed the suppression of the seed germination.

Shoot length of barnyard grass, grown in growth room, was affected by the applied strains (Figure 1). All strains significantly reduced the shoot length of BYG as compared to control. The maximum shoot length reduction was caused by strain BN3 which was 59.1% less than control. It was also significantly less than the reduction caused by strain BN1 and BN2. Strain BN4, BN5 and BN6 caused reduction in shoot length up to 50, 56 and 51% than control, respectively. These strains also significantly reduced shoot length as compared to strain BN1. Strain BN2 reduced shoot length up to 45.9% than control which was also significantly less than strain BN1. Strain BN1 caused the least reduction in shoot length which was 30.1% lesser than control.

All strains significantly reduced the root length of BYG as compared to control. Strain BN3 and BN5 caused maximum reduction of 63.7 and 59.2% as compared to control. This reduction was also significantly lesser than that caused by other strains. Strain BN2 and BN4 reduced root length up to 39.5 and 52.8% than control, respectively. These strains also performed significantly better than strain BN6 in affecting root length of BYG. Strain BN1 and BN6 caused least reduction in root length, which was 35.1 and 42% lesser than control, respectively (Figure 1).

Applied strains significantly reduced fresh biomass of BYG as compared to control (Figure 1). However, these strains caused non-significant differences from each other in affecting fresh biomass of BYG. This parameter was reduced up to 29.7, 36.0, 64.6, 60.1, 57.5 and 59.0% than control by strain BN1, BN2, BN3, BN4, BN5 and BN6, respectively.

All strains significantly reduced the dry weight of BYG as compared to control (Figure 1). Strain BN2, BN3 and BN5 more effectively reduced dry weight of BYG than control and other strains. They reduced dry weight up to 34, 63.8 and 54% than control, respectively. This reduction was also significantly lesser than strain BN1. BN4 and BN6 reduced dry weight up to 62 and 56% than control which was also significantly lesser than strain BN1.

3.3. Evaluation of maize under axenic conditions

The shoot length of the strains BN3, BN5 and BN6 were significantly increased as compared to the control. The increases in shoot length by these strains were 48, 49 and 52% respectively. But BN4 suppresses the growth of the maize crop to some extent. The reduction in shoot length that was caused by strain BN8 was 3%. Figure 2 shows that the remaining strains were equal in shoot length as compared to control while the AB strains BN1 showed a very small increase in the shoot length which is almost same as control. The increase by these two isolates was 17 and 6% only. Root length of the maize crop was measured under axenic conditions and the results shown in Figure 2 demonstrated that three strains performed best as compared to the rest of the applied strains. The strains BN3, BN5 and BN6 acted as a PGPR and increased the root length of the maize crop under axenic conditions. The increase due to application of the AB strains was 53, 54 and 60%, respectively. BN4 suppresses the root length of the maize crop and reduction was noted up-to 8%. BN1 and BN2 applications did not make too much difference than the control. Their differences were noted only 14 and 12% only as compared to control.

Maize crop shoot weight was measured that was grown under axenic conditions. Results are shown in Figure 2. The results demonstrated that three strains increased the shoot weight of the maize crop. The two AB strains, BN1 and BN2 performed nearly same as control with showing only small increase in its weight that was 2.7 and 4.9%. The BN4 AB strains reduced the shoot weight, and that reduction was noted as 9.68%. BN3, BN5 and BN6 strain increased the shoot weight as these acts as PGPR and their increments were observed up-to 55.4, 51.82 and 54.22% respectively. The root weight of maize crop was measured that was grown under axenic conditions. Results are shown in Figure 2. The results

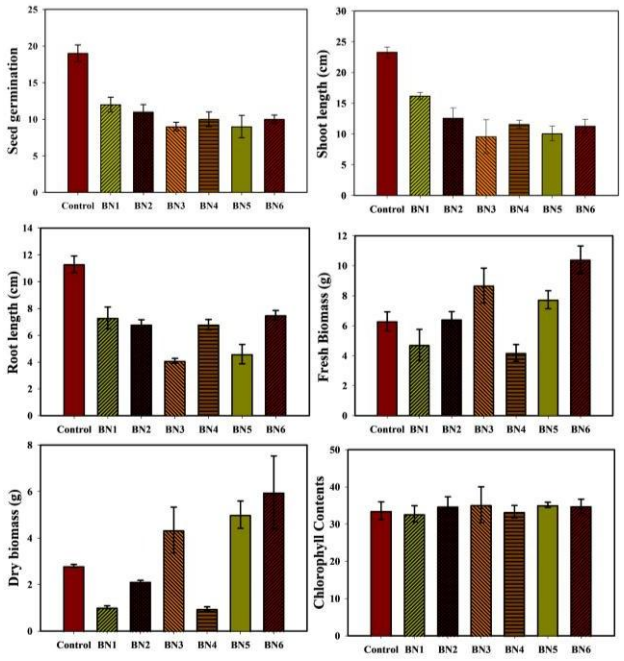


Figure 2: Impact of weed suppressive bacteria on growth of maize crop under ambient conditions (error bars indicate the standard errors in the means of the treatments)

demonstrated that three strains increased the root weight of the maize crop. The two AB strains, BN1 and BN2 performed nearly same as control with showing only small increase in its weight that was 1.91% and 1.09%. The BN4 AB strains reduced the root weight, and that reduction was noted as 8.50%. BN3, BN5 and BN6 strain increase the root weight as these acts as PGPR and its increments were observed up to 45.86, 41.96 and 44.46% respectively.

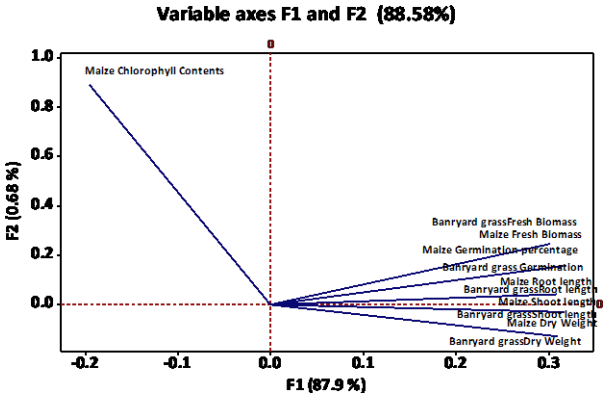
Fresh biomass of maize crop is given in Figure 2. Maize biomass was increased by the application of three AB strains BN3, BN5 and BN6. The increase percentage was calculated as 44, 47 and47% respectively. Reduction in the biomass of the maize crop was measured as 9.07% due to the application of the AB isolate BN4. The two applied AB strains remained near to the control as these only increased the fresh biomass only up-to a certain limit. The BN1 and BN2 AB strains increase the fresh biomass 3.7 and 5.56% respectively. Dry weight of maize crop is given in Figure 2. Maize biomass was increased by the application of three AB strains BN3, BN5 and BN6. The increase percentage was calculated as 54.16, 62.917 and 62.5% respectively. The BN4 isolate decreased the dry weight up-to 14.58% as compared to the control value. The two applied AB strains remained near to the control as these only increase the dry weight only up-to a certain limit. The BN1 and BN2 AB strains increased dry weight up-to 3.75 and 5%, respectively.

Dry weight was increased by the application of three AB strains BN3, BN5 and BN6. The increased percentage was calculated as 21.71, 22 and 21% respectively. Minimum increase was observed in strain BN4 that was 2.7% only. Two strains performed moderately as these increased the shoot dry weight 17 and 12% by the strains BN1 and BN2 respectively. Dry root weight of the maize crop is given in the Figure 2. Dry root weight of the maize crop was increased due to the application of the AB strains. The increase in the root dry weight was observed maximum in the AB strain BN3, BN5 and BN6 that was observed as 27, 30 and 25% respectively. BN1 and BN2 increased the root dry weight of maize crop 8 and 10% respectively. Minimum growth was observed in the strain BN4 that was considered near as control value that is only 3%. The value of the chlorophyll was taken by the chlorophyll meter. The strain BN3, BN5 and BN6 showed that chlorophyll content was maximized in these two treatments.

Results of PC Analysis

Results of PCA analysis demonstrated that all the competing factors lies in the same group showing a positive correlation with the application of hydrogen cyanide producing bacterial action on the plant growth. The PCA fraction of F1 showed 87.9% proportion fall in the competing factors plot. This also confirmed by the other researchers that application of rhizobacteria can also enhance the morphological attributes of the maize crop (Figure 3). As in a study by Abbas et al 2017 already illustrated the application of the AB which produces the hydrogen cyanide reduce the herbicides growth by improving the corps health and agronomic growth.

Figure 3: Principal component analysis for the agronomic



attributes of the barnyard grass and maize crop along with the chlorophyll contents. F1:87.9% and F2: 0.68% proportion of variation.

Pearson correlation data also confirmed the positive correlation with all attributes except with the chlorophyll contents that could be possible due to varying factors. The correlation matrix presents relationships between various growth parameters of barnyard grass and maize. Strong positive correlations ($r > 0.90$) are observed among traits within the same species, such as germination, shoot length, root length, fresh biomass, and dry weight. This indicates that an increase in one growth trait is associated with an increase in others. Notably, maize chlorophyll content exhibits negative correlations with all other traits, especially shoot length (-0.61) and dry weight (-0.62), suggesting a trade-off between biomass accumulation and chlorophyll synthesis. The high correlation values between barnyard grass and maize traits ($r \approx 0.90$) suggest that growth conditions similarly affect both species. However, minor inconsistencies in correlation values (e.g., 0.912, 0.892) suggest a need for data verification (Table 2). Overall, this matrix highlights strong interdependencies among plant growth traits, providing valuable insights for understanding species interactions and their responses to environmental conditions or treatments.

Table 2: Pearson correlation matrix of the growth factors attributed to allelopathic bacterial strains application

	BYG GR	BYG SL	BYG RL	BYG FW	BYG DW	Maize GR	Maize SL	Maize RL	Maize FW	Maize DW	Maize *Chl*
BYG GR	1										
BYG SL	0.98	1									
BYG RL	0.92	0.91	1								
BYG FW	0.93	0.89	0.90	1							
BYG DW	0.93	0.96	0.91	0.83	1						
Maize GR	1.00	0.98	0.92	0.93	0.93	1					
Maize SL	0.98	1.00	0.91	0.89	0.96	0.98	1				
Maize RL	0.92	0.91	1.00	0.90	0.91	0.92	0.91	1			
Maize FW	0.93	0.89	0.90	1.00	0.83	0.93	0.89	0.90	1		
Maize DW	0.93	0.96	0.91	0.83	1.00	0.93	0.96	0.91	0.83	1	
Maize Chl	-0.49	-0.61	-0.56	-0.46	-0.62	-0.49	-0.60	-0.56	-0.46	-0.62	1

* GR: Germination rate;
SL: Shoot length
RL: Root length
FW: Fresh weight
DW: Dry weight
Chl: chlorophyll content

4. Discussion

Rhizosphere of all plant species is known to contain diverse type of microorganisms with diverse characteristics. Some quickly colonize and establish in the rhizosphere than others. Growth and activities of these microorganisms influences growth and development of plants. Exact mechanisms of competitive colonization and plant responses are still unknown (Hayat *et al.*, 2010; Weller, 1988). Some of the rhizosphere inhabiting bacteria facilitate growth of plants while others influence plants negatively. Plant growth promoting characteristics of rhizobacteria have been widely exploited for development of biofertilizers. However, the potential of plant antagonistic bacteria for the development of bioherbicides has been largely ignored by scientists. They produce secondary metabolites including cyanide, antibiotics, exopolysaccharides, siderophores plant cell degrading enzymes and some other substances which suppress the growth of host plants (Haas & Défago, 2005; Nehl *et al.*, 1997). The present study was conducted to explore such rhizobacteria with potential for suppression of plant growth. Further, if these rhizobacteria selectively suppressed growth of weeds, they could be used for development of bioherbicides. Microbial characterization of isolated rhizobacteria of our study showed that they had capability to produce cyanide, siderophores, exo-polysaccharides, chitinases, oxidases, catalases, indole-3-acetic acid and motility. These strains killed growth of sensitive *E. coli* indicating potential for phyto-toxicity of these strains. These findings are in agreement with (Tasawar Abbas *et al.*, 2017; Príncipe *et al.*, 2007a). They have reported similar characteristics for rhizobacteria strains which suppressed growth of weeds.

Anwar *et al.* (2021) have also reported reduction in growth of BYG upon application of rhizobacteria capable of

producing hydrogen cyanide. Kennedy and Stubbs (2007) have reported that rhizobacteria reduced density of weeds. Cheng and Cheng (2015) have established that cyanide produced by rhizobacteria in the rhizosphere interferes with root cell metabolism. It restricted root growth of some weeds. The same strains when applied to maize did not restrict the growth of maize.

Present study has reported reduction in length of root length and shoot of BYG up to 63 and 59% over control, respectively, by application of six cyanogenic strains of rhizobacteria. These strains have reduced fresh biomass and dry weight of BYG up to 64 and 63% over control, respectively. These results are in agreement with Khan *et al.* (2016). Kremer and Souissi (2001b) have related growth inhibition of BYG seedlings by rhizobacteria with some metabolites of these rhizobacteria. They suggested that HCN produced by applied rhizobacteria was responsible for growth inhibition of BYG. Abbas *et al.* (2018) have reported reduction in root length from 17 to 93.5% and shoot length from 28 to 93% over control by application of multiple cyanogenic strains of rhizobacteria.

Some studies have reported improvement in growth of different crops upon application of weed suppressive bacteria. Such strains provide dual benefits of weed suppression and growth stimulation of crop. It improves competitive ability of crop against weeds. It increases the scale of weed control using WSB (Kremer & Souissi, 2001a). The present study has reported a significant increase in root length, shoot length, fresh biomass and dry weight of maize plants due to application of five strains of BYG suppressive bacteria under axenic conditions. Growth promotion of crops by weed suppressive bacteria has been in several past studies (Kremer & Ben-Hammouda, 2009; Saharan & Nehra, 2011)

Contrarily, one of our strains has significantly reduced root length, shoot length, fresh biomass and dry weight of maize plants under axenic conditions. It gets support from Li and Kremer (2006) and (Zahir *et al.*, 2011). They have also reported the growth inhibition of crop plants by few of their strains of weed suppressive bacteria. These results can be attributed to dominance of weed suppressive mechanisms by these strains in the rhizosphere of maize plants.

Our strains have the capability to produce auxins, siderophores, catalases, chitinase and phosphorus solubilization. These characteristics of rhizobacteria have been reported to be responsible for growth promotion of crop plants in several studies. Our strains might have stimulated growth of maize plants owing to these characteristics. Príncipe *et al.* (2007b) has related improvement in root growth, plant growth, and nutrient uptake of plants to auxins production by rhizobacteria. Solubilization of phosphorus increases the nutrient content of plants which results in better growth and yield (Majeed *et al.*, 2018). Production of siderophores improves iron nutrition of plants and also suppresses population and activities of pathogens in rhizosphere. It results in better growth and yield of crops. Our strains also possess chitinase activity. It degrades cell wall of fungi thereby controlling population of pathogenic fungi in the rhizosphere and promoting plant growth (Wang *et al.*, 2022). The possession of catalase activity increases the resilience of bacteria against environmental stresses (Hassan *et al.*, 2016; Rathaur *et al.*, 2012). Hence, the potential of our strains exhibited in this study can be utilized for development of bioherbicide for biological control of BYG.

5. Conclusion

This study concluded that with the application of PGPRs which produced secondary metabolite i.e., hydrogen cyanide can effectively reduce the growth of barnyard grass with substantial improvement in the maize growth. The results also showed that significant production of the siderophores, and other microbial assisted enzymes contributes to the growth of maize crop. However, more research is still warranted to explore the impact of the PGPR-assisted rhizobacterial strain under field conditions and in large scale experiments in different crops.

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8. Authors' Contribution

Tasawar Abbas: conducted the experiment and prepared the initial form of the manuscript; Sana Abbas: data analysis and revision of the manuscript; Uswa Abbas: data arrangement, revision, and proofreading of the manuscript Hamna Bashir: Proofreading and writing reviews; Ayesha Sonia: Proofreading; Zahir Ahmad Zahir: Supervisor and proofreading of the manuscript; Muhammad Naveed: Conceptualize, writing reviews.

9. Conflict of Interest

The authors declare no conflict of interest.

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