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Full Length Article

Biological Weeds Control in Rice (*Oryza sativa*) using Beneficial Plant Growth Promoting Rhizobacteria

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Abstract

Weeds are termed as pests owing to their harmful impact on agroecosystems. Although the herbicidal weed control is the best strategy to control weeds but over and non-judicious use of herbicides resulted many negative impacts on human health and crop ecology. Weeds control through biological approach is considered less destructive. In this study, three efficient bacterial strains *i.e.*, *Pseudomonas putida, P. aeruginosa* and *P. alcaligenes*, isolated during preliminary studies and their consortium were applied to *Cyperus rotundus* and *Echinochloa colonum* co-seeded with rice grown in pots. Effect of allelopathic bacteria on the infested rice and weeds were studied at tillering, booting and harvesting stage of rice. Results indicated that the applied strains significantly improved rice growth and yield and hindered the weeds germination to variable extent. The weeds infestation also decreased plant height, 1000-grain weight, spikelet length and nutrient concentration under control condition. However, the *P. putida* recovered the maximum losses in shoot dry weight and grain yield of rice infested with *C. rotundus* (42 and 78%, respectively) and *E. colonum* (78 and 69%, respectively). Nonetheless, allelopathic bacteria application also improved the gas exchange traits and nutrient contents of infested rice. In conclusion, inoculation with bacterial strains reduced the biomass of weeds and promoted the growth, yield and photosynthetic parameters of infested rice. Therefore, allelopathic bacteria application seemed a viable strategy to minimize the competition between rice crop and weeds; and these strains, *P. putida* in particular, can serve as biological control agents of rice weeds *i.e.*, *E. colonum*, *C. rotundus*.

Keywords: Allelochemicals; Biocontrol; Rhizobacteria; Rice; Weeds; Photosynthetic apparatus

Introduction

Abiotic and biotic stresses are the major constraints of crop production and global food security. Among them only biotic stresses including pathogens, insects and weed cause significance reduction in crop yield (Rahman *et al.* 2018). Weeds are a major biotic stress faced by crop plants which lead to an annual loss in the yield up to 34% (Oerke 2006), increase the crop protection costs by increasing chemical and mechanical expenditures and add to the already existing problems in the crop ecosystem as they are a habitat for various plant pests (Islam and Kato-Noguchi 2013). The control of weeds is possible by using different approaches including physical, mechanical, biological and chemical ones (Ashiq and Aslam 2014; Farooq *et al.* 2011; 2017). Chemical weeds control (herbicides) gains central position in weed control strategy due to a significant reduction in yield losses (Mustafa *et al.* 2019). Herbicides application is time and labor saving as well as it could be applied through multiple methods (Pacanoski 2007). However, the commercial herbicides contaminate the air, soils and ground waters, adversely affecting human health, microflora and fauna, and increase herbicide resistance among weeds (Geiger *et al.* 2010; Tabaglio *et al.* 2013). The farmers mainly depend upon herbicides for controlling weeds, but weedicides fail to reduce their infestation, as intensity increases with the passage of time. According to Prado *et al.* (2004), a continuous use of herbicides results in the expansion of resistant biotype weeds. Several weeds gain resistance against many herbicides over the time.

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In case of rice (*Oryza sativa*), recent findings have revealed that 39 resistance weeds species have evolved, and 300 herbicide resistance weeds have been reported against 15 families of chemical herbicides (Heap 2015). Acetolactate synthase (ALS) inhibitor shows a predominant form of resistance in rice weeds especially *E. colonum* (Heap 2014). The herbicide application in general leads to a destruction of natural predators, parasites of pathogens and wildlife; leading to the development of herbicide resistance in weeds (Pimentel 2005). Herbicide residues not only reduce yield of sensitive crops but chemicals also enter into the food chain (Crone *et al.* 2009), and prove injurious to poultry and livestock (Hakansson 2003). Nearly one million people are reported to suffer from chronic diseases annually due to herbicide exposure (Blair *et al.* 2015). Weedicides result in hypertension, heart, liver and kidney disorders, itching, and paralysis of nervous system and disturb digestive system in human beings (Ashiq and Aslam 2014). Guyton *et al.* (2015) have recently reported that popular herbicides of the world (including atrazine, glyphosate and hexazinone) have carcinogenic effects as well.

Keeping all these negative impacts in view, biological control of weeds decreases the dependence on chemical herbicides (Mustafa *et al.* 2019). Moreover, it is environment friendly approach largely focusing on target and reduces the development cost as compared to synthetic herbicides (Bailey *et al.* 2010). Biological control is termed as use of living organisms for inhibition of pests under natural conditions. For control of weeds, weed pathogen and insects have been used during the past decades (Charudattan and Dinoor 2000). Insects control weeds slowly, possessing broad host variety but may emerge as new pests (Ghorbani *et al.* 2005). Biological control of weeds is least dependent-chemical herbicide method that reduces the cost of chemical based weedicides (Hershenhorn *et al.* 2016).

As early as 1973, Daniel *et al.* (1973) have shown that high dose of mycoherbicide application at specific growth stage of plant can also inhibit various weeds. In view of the difficulties faced in the mass production and their specific requirement for action, mycoherbicides are not accepted as economical (Heraux *et al.* 2005). Plant growth promoting rhizobacteria (PGPR) for a control of weeds is the least investigated area. The PGPR release certain chemicals in the rhizosphere, which inhibit germination and growth in host specific manner (Sturz and Christie 2003).

In previous studies, bacterial strains were used for controlling weeds in wheat (*Triticum aestivum*) crop (Abbas 2017; Mustafa *et al.* 2019). In present study it is hypothesized that application of pre-isolated rhizobacterial strain from wheat may be more effective when applied as single spp. than consortium application due to difference rhizosphere characteristics of wheat and rice crop. In the current study, three bacterial strains *Pseudomonas putida* KT2440, *P. aeruginosa* PAO1 and *P. alcaligenes* NBRIC14159 were used against rice co-seeded with (*C. rotundus* and *E. colonum*). These rhizobacterial strains were also evaluated for enhancing growth and yield of rice under weedy check and weed free conditions.

Materials and Methods

Collection and screening of allelopathic bacterial strains

Several hundred strains of presumed allelopathic bacteria were previously isolated from rhizosphere of wheat and associated weeds (Abbas *et al.* 2017a). Four bacterial strains (*P. putida*, *P. aeruginosa* and *P. alcaligenes* and *P. fluorescens*) were collected from Soil Microbiology and Biochemistry Laboratory, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. Among these bacterial strains, three were selected for further evaluation. These strains were tested against rice associated weeds (*C. rotundus* and *E. colonum*) in order to improve rice growth and yield under weedy check and weed free conditions. *E. colonum* seed and *C. rotundus* rhizomes were surface sterilized by using ~95% ethanol and then 0.2% $HgCl_2$ for 3 min and subsequently washed with sterilized water. Weed seeds and rhizomes were soaked in inoculum for five min. Autoclaved water agar (1:100) was poured into petri plates and 16 seeds of *E. colonum* were added in petri plates for weed inhibition assay. Similarly, 7 surface sterilized rhizomes were placed in petri plates having autoclaved water agar. The experiment was conducted for 25 days in controlled chamber at 25 ± 1°C set at a 16 h photoperiod, and the light intensity was adjusted to 350 mmoL m^2s^1. Experimental apparatus was randomly arranged with three replicates and data regarding germination percentage recorded. Inoculation of weed seeds and rhizomes with presumed allelopathic bacteria significantly reduced the germination of *E. colonum* and *C. rotundus*. All the bacterial strains significantly reduced the germination of both weeds. Maximum inhibition of germination of *E. colonum* was caused by inoculation with three allelopathic bacterial strains (*P. putida, P. aeruginosa* and *P. alcaligenes*) over uninoculated control. Similarly, minimum rhizomes germinated in the case of *C. rotundus* with same three bacterial strains over uninoculated control. Therefore, these three strains were further used in this study.

Culture of allelopathic bacteria

Culture of bacterial strain was prepared in sterile King's B broth in Erlenmeyer flasks (King *et al.* 1954; Abbas *et al.* 2017b). All the strains were transferred to the flasks by using bacteriological loop aseptic technique and further incubated at 100 rpm for 48 h at 28 ± 1°C using a shaking incubator (Firstek Scientific, Tokyo, Japan). The optical density of the culture was measured at wavelength 600 nm using a Nicolet Evolution 300 LC (Cambridge, U.K.) and adjusted to 0.5 to obtain a uniform bacterial population (10^8-10^9 cfu mL^{-1}) (Naveed *et al.* 2014).

Experimental details

Pot trial: Based upon germination inhibition activity observed in weed inhibition water agar bioassay under axenic conditions with three most promising allelopathic bacterial strains (*P. putida*, *P. aeruginosa* and *P. alcaligenes*) along with their consortium were carefully selected as PGPR and further tested for pot experiment in the wire house of Institute of Soil and Environmental sciences, University of Agriculture, Faisalabad Pakistan. The suppression of rice associated weeds in addition to increase the growth and yield was thoroughly investigated. Pots having diameter 30 cm were filled with air dried and sieved soil at 8 kg per pot. The composite soil sample was analyzed for various physical and chemical parameters. The texture of soil was sandy-clay-loam (Typic Haplocambid), pH 7.5, extract electrical conductivity (ECe) 1.5 dS m^{-1} (Abbas *et al.* 2017b), saturation percentage 30.2%, organic matter 0.88%, total N 0.036%, available P 7.8 mg kg^{-1}, and extractable K 158 mg kg^{-1}. Three rice seedlings, fifteen seeds of *E. colonum* and five rhizomes of *C. rotundus* were sown in each pot. Experimental units were placed in the wire house under ambient light and temperature; however, without inoculating bacterial strains three controls were setup as; weed free rice, *E. colonum* and *C. rotundus*. Experiment was laid out following completely randomized design (CRD) under factorial arrangements and replicated three times. Chemical fertilizers *i.e.*, N, P and K were applied at the rate of 46-31-29 mg kg^{-1} of soil before sowing as urea, diammonium phosphate and single super phosphate respectively, and canal water was applied as irrigation water whenever needed. Growth and yield parameters; plant height, plant weight, 1000-grain weight, root length/weight, number of tillers and spikelet length of both weeds and rice were recorded. Shoot and root length were recorded at the time of harvesting and uprooting the plant. Grain yield was measured after crop harvesting (Nadeem *et al.* 2007).

Inoculation: Inoculum of each strain was mixed with sterilized peat (autoclaved thrice at 121°C and 15 psi) at the ratio of 1.25:1 and incubated at 28 ± 1°C for 24 h. Inoculated peat was mixed with sterilized sugar solution and used (Abbas *et al.* 2017a) for seed and rhizome coating of *E. colonum* and *C. rotundus* respectively. Rice seedlings were dipped in inoculum. For weed free control, only rice seedlings were dipped in agar broth. Inoculated fifteen viable seeds of *E. colonum*, five rhizomes of *C. rotundus* were co-seeded below soil surface under pot condition. Later, 25 mL fresh culture of selected strains was added onto soil surface of respective pots sown with rice and weeds followed by a thin surface layer of sand. For weed free control treatments, 25 mL of King's B broth (Abbas *et al.* 2017b) was applied on surface of soil and thereafter covered with sand.

Plant physiological parameters

Gaseous-exchange measurements *i.e.*, [photosynthetic rate (net-rate of CO_2 assimilation at light saturation) (Asat)], stomatal conductance (gs), substomatal conductance (Ci), transpiration rate (E) and vapor pressure deficit (VpdL) were measured with a CIRAS-3 portable photosynthesis system (PP system, U.S.A.) during 9:00 to 12:00 a.m. (1200–1400 μmol m^{-2} photon flux density). Upper 3rd leaf was selected to monitor physiology of each plant at the ambient light intensity. Chlorophyll contents of both weeds and rice were measured 75 days after sowing with chlorophyll meter (Konica-Minolta, Japan) and values were representing as SPAD value (Coste *et al.* 2010).

Plant analysis

At physiological maturity stage, shoot and grain samples of crop were collected to determine nitrogen, extractable phosphorus and potassium. Collected samples were grounded and digested (Wolf 1982; Naveed *et al.* 2014). Total nitrogen was determined by using Kjeldhal ammonium distillation apparatus. Phosphorus was measured by adding 10 mL Barton reagent in 5 mL sample by spectrophotometer (T80 UV/VIS Spectrometer PG Instruments Ltd.). Actual concentration of phosphorus was determined by comparing with standard curve (Naveed *et al.* 2014). Potassium was measured by flame photometer (Jenway PFP-7, England) and its concentration was calculated by calibration curve (Naveed *et al.* 2014).

Statistical analysis

Statements of statistical significance among growth, physiological and chemical parameters were tested and defined as $P \leq 0.05$. Variance analyses (Two-way ANOVA) was performed by S.P.S.S. software v. 19 (IBM SPSS Statistics 19, U.S.A.) while least significant difference test (LSD) was used to compute statistically different mean values (Steel *et al.* 1997).

Results

Growth and yield parameters

Interaction among weeds and inoculation of allelopathic bacteria had significant effect on plant height, root and shoot dry weight, number of tillers, spikelet length and 1000-grain weight of rice (Table 1). Rice plants observed maximum plant height, number of tillers and shoot and root dry weight per pot in weeds free pots inoculated with *P. putida* strain. However, it was at par only for plant height with rice plant grown in *C. rotundus* infested pots inoculated with bacterial strain *P. putida*. Whereas rice plant observed minimum plant height and shoot and root dry weight in *E. colonum* infested pots without inoculated with allelopathic bacteria (Table 1). The spikelet length and 1000-grain weight were maximum with the inoculation of strain *P. alcaligenes*

Table 1: Effect of allelopathic bacteria on plant height, shoot dry weight, root dry weight, number of tillers, 1000-grain weight and spikelet length of weeds infested rice

Treatments	Plant height (cm)			Shoot dry weight (g pot^{-1})			Root dry weight (g pot^{-1})		
	Weed Free	*C. rotundas*	*E. colonum*	Weed Free	*C. rotundas*	*E. colonum*	Weed Free	*C. rotundas*	*E. colonum*
Control	80.30 cd	65.30 d	32.20 f	35.33 fg	22.55 e-g	18.30 h	80.47 fg	47.23 e-g	25.10 h
T_{42}	115.3 a	100.00ab	90.66 cd	52.31 a	32.04 cd	32.66 cd	122.71 a	90.98 cd	41.27 fg
T_{19}	85.66 bc	77.50 cd	80.03 cd	41.81 bc	27.65 d-f	28.03 g	84.18 bc	68.40 d-f	32.86 g
T_{70}	95.33 b	87.00 bc	82.73 bc	44.70 b	29.92 de	29.73 d-g	91.78 b	83.16 de	38.37 e-g
Consortia	84.00 bc	95.33bc	77.66 de	41.31 bc	23.12 d-g	26.66 g	88.72 bc	53.15 g	35.15 g
LSD value at 5%		7.03			9.02			15.49	
Treatment	Number of tillers (pot^{-1})			1000-grain weight (g)			Spikelet length (cm)		
Control	15 fg	10 i	10 i	12.93 e-g	11.96 fg	11.13 gh	8.06 e-g	6.76 gh	5.26 i
T_{42}	24 a	21 b	18 c-e	19.16 a-c	20.13 ab	18.86 a-d	8.56 ba	8.26 ab	8.00b
T_{19}	19 b-d	17 f-h	12 d-f	14.93 d-f	15.10 d-f	15.76 c-e	8.44 a-d	7.01 e-g	6.23 h
T_{70}	21 b	19 bc	14 gh	21.46 a	17.40 b-d	17.26 b-e	9.97 a	7.56 e-g	7.12 e-g
Consortia	16 d-f	17 ef	11 hi	17.76 b-d	17.60 cd	16.06 d	8.18 a-c	7.36 ef	6.83 f-h
LSD value at 5%		2.82			4.14			0.91	

Means sharing different letters, within a column or row, differ significantly from each other at 5% level of probability
Here T_{42} = *Pseudomonas putida*; T_{19} = *Pseudomonas aeruginosa*; T_{70} = *Pseudomonas alcaligenes*)

Table 2: Effect of allelopathic bacteria on root length, stomatal conductance, vapor pressure deficit, transpiration rate, photosynthesis rate and substomatal conductance of weed infested rice

Treatment	Root length (cm)			gs (μmol CO_2 m^{-2} s^{-1})			VpdL (KPa)		
	Weed Free	*C. rotundus*	*E. colonum*	Weed Free	*C. rotundus*	*E. colonum*	Weed Free	*C. rotundus*	*E. colonum*
Control	31.40 f-h	22.80 i	20.2 j	61.33 g	41.66 hi	34.66 i	3.63 d	3.21 ef	2.92 f
T_{42}	44.76 a	40.03 ab	37.3 b-d	101.0 a	78.42 b	42.66 g	5.07 a	4.68 ab	4.44 bc
T_{19}	40.00 bc	30.31 gh	30.60 d-f	69.33 cd	57.32 f	40.00 gh	3.64 d	4.33 bc	3.63 d
T_{70}	37.30 b-d	35.46 b-d	32.20 e-h	76.00 bc	62.33 ef	62.66 ef	4.32 bc	4.54 b	4.40 b
Consortia	35.6 c-f	32.36 de-h	27.16 hi	71.33 bc	66.00 de	54.66 f	3.92 cd	4.34 b	3.61 df
LSD value		1.8074			8.5425			0.4101	
Treatment	E (μmol CO_2 m^{-2} s^{-1})			A (μmol CO_2 m^{-2} s^{-1})			Ci (μmol CO_2 m^{-2} s^{-1})		
Control	1.99 d-f	1.80 f	1.80 f	7.10 f-h	6.66 gi	3.93 i	125.66 d-f	116.00 ef	111.00 f
T_{42}	3.47 a	3.40 ab	3.20 a-c	12.13 a	9.9 bc	6.9 c-f	212.33 a	197.00 ab	173.00 a-c
T_{19}	2.35 d	2.43 cd	1.53 ef	8.10 b-f	8.46 b-e	4.83 hi	101.00 b-d	130.33 c-f	140.33 c-e
T_{70}	2.89 bc	2.89 bc	2.72 bc	9.06 b-d	10.3 b	6.16 e-h	172.66 a-c	160.00 a-d	163.00 bc
Consortia	2.5 bc	2.82 bc	2.27 c-e	8.43 b-e	7.36 d-g	4.46 gh	149.00 b-d	142.33 c-e	142.33 c-e
LSD value		0.8117			2.3175			51.530	

Means sharing different letters, within a column or row, differ significantly from each other at 5% level of probability
Here T_{42} = *Pseudomonas putida*; T_{19} = *Pseudomonas aeruginosa*; T_{70} = *Pseudomonas alcaligenes*; gs = stomatal conductance; VpdL = vapor pressure deficit; E = transpiration rate; A = carbon dioxide assimilation rate; Ci = sub-stomatal conductance

which was at a par to strain *P. putida* in weed free pots; in the case of weeds condition *P. putida* showed maximum grain weight and spikelet length as compared to their weedy control. Similarly, the data regarding root length (Table 2) of rice plants has shown that allelopathic bacterial strains increased the root length under weeds free and weedy condition as compared to respective control. Maximum root length increased with the application of strain *P. putida* under weeds free pots and also remained dominant in *C. rotundus* infested pots (Table 2).

The antagonistic bacterial strains for weeds had significant effect on the reduction of growth parameters like plant height, root length, plant dry weight, root dry weight and No. of tiller per plant of *C. rotundus* and *E. colonum* (Table 4). The maximum reduction in growth attributes were observed with inoculation of *P. putida* strain in both weeds.

Plant physiological parameters

The physiological parameters were improved significantly by inoculation with allelopathic bacterial strains compared to the control under both weedy and weeds free conditions (Table 2). The maximum CO_2 assimilation, vapor pressure deficit, transpiration rate and stomatal and substomatal conductance was recorded by rice plants inoculated with bacterial strains under weeds free condition. However, in case of transpiration rate and substomatal conductance *P. putida* inoculation showed similar results in *C. rotundus* and *E. colonum* infested pots. The minimum response in all physiological parameters ware recorded in *E. colonum* infested pots without inoculated with allelopathic bacteria (Table 2).

Inoculation of allelopathic bacteria led to significant decrease in the chlorophyll contents of *C. rotundus* and *E. colonum*. The maximum decrease in chlorophyll content was recorded in pots inoculated with *P. putida* strain in *C. rotundus* and *E. colonum* infested pots. (Table 4). While, in case of rice plant the antagonistic bacterial strains for weeds improved chlorophyll contents of rice under weeds infested conditions. Among all the treatments maximum response was observed by inoculation of *P. putida* under weeds free

Table 3: Effect of allelopathic bacteria on percentage of nitrogen, phosphorus and potassium in grain and straw of weed infested rice

Treatment	Weed Free	*C. rotundus*	*E. colonum*	Weed Free	*C. rotundus*	*E. colonum*	Weed Free	*C. rotundus*	*E. colonum*
	Grain nitrogen content (%)			Straw nitrogen content (%)			Grain phosphorus content (%)		
Control	1.54 d-g	1.41 e-g	1.25 g	1.03 c-e	0.96 e-g	0.89 gh	0.093 e-h	0.090 f-h	0.083 gh
T_{42}	2.53 a	1.72 b-d	1.85 bc	1.18 a	1.06 cd	0.98 ef	0.153 a	0.133 b	0.113 cd
T_{19}	1.57c-f	1.51 d-g	1.32 fg	1.07 b-d	0.88 gh	0.77 ij	0.100 c-f	0.087 gh	0.084 gh
T_{70}	1.96 b	1.80 b-d	1.69 b-e	1.14 ab	0.82 hi	0.71 jk	0.116 bc	0.110 c-e	0.106 c-f
Consortia	1.70 bcd	1.55 def	1.34 fg	1.10 bc	0.75 ij	0.67 k	0.096 efg	0.086 gh	0.079 h
LSD value	0.2960			0.0832			0.0190		
Treatment	Straw phosphorus content (%)			Grain potassium content (%)			Straw potassium content (%)		
Control	1.04 f-h	0.97 ij	0.93 j	1.23 ef	1.19 fg	1.10 g	1.87 g	1.78 h	1.70 h
T_{42}	1.24 a	1.20 ab	1.16 bc	1.52 a	1.48 ab	1.42 a-c	2.18 a	2.02 b-d	1.97 ef
T_{19}	1.09 d-f	1.07 e-h	1.02 hi	1.33 cd	1.31 de	1.27 d-f	1.96 ef	1.86 g	1.74 h
T_{70}	1.18 b	1.15 bc	1.13 cd	1.41 bc	1.37 cd	1.31 de	2.10 a-c	2.12 ab	2.07 b-d
Consortia	1.12 c-e	1.08 d-g	1.03 gh	1.37 cd	1.34 cd	1.29 de	2.02 c-e	1.99 de	1.91 fg
LSD value	0.0521			0.0980			0.0784		

Means sharing different letters, within a column or row, differ significantly from each other at 5% level of probability
Here T_{42} = *Pseudomonas putida*; T_{19} = *Pseudomonas aeruginosa*; T_{70} = *Pseudomonas alcaligenes*

Table 4: Effect of allelopathic bacteria on *Cyperus rotundas* and *Echinochloa colonum* dry root weight, plant weight, chlorophyll contents (SPADE), root length and plant length

Treatment	*C. rotundus*	*E. colonum*	*C. rotundus*	*E. colonum*	*C. rotundus*	*E. colonum*
	Chlorophyll contents (SPAD value)		Dry root weight (pot^{-1})		Root length (cm)	
Control	35.26 ab	36.40 a	27.50 b	37.50 a	17.23 d	34.63 a
T_{42}	25.60 f	26.16 ef	9.70 fg	8.63 g	7.33 g	12.05 ef
T_{19}	32.26 c	33.60 bc	24.20 c	22.64 c	16.53 d	29.56 b
T_{70}	27.45 d-f	28.20 de	12.85 e	11.48 ef	10.72 f	15.86 d
Consortia	29.46 d	29.33 d	19.89 d	18.41 d	13.09 e	23.15 c
LSD value	2.0246		2.7167		2.2746	
Treatment	Plant height (cm)		Plant dry weight (pot^{-1})		Number of tillers (pot^{-1})	
Control	91.33 b	109.00 a	40.56 b	47.75 a	25.66 ab	28.00 a
T_{42}	61.66 h	49.16 h	8.90 g	11.03 fg	12.00 fg	10.23 g
T_{19}	88.83 bc	92.66 b	34.40 c	29.33 d	21.33 cd	23.67 bc
T_{70}	75.12 e	68.34 f	13.94 ef	16.56 e	13.66 ef	15.00 e
Consortia	83.00 cd	82.47 d	29.52 d	37.10 c	15.71 e	19.20 d
LSD value	4.2450		4.3295		2.9170	

Means sharing different letters, within a column or row, differ significantly from each other at 5% level of probability
Here T_{42} = *P. putida*; T_{19} = *P. aeruginosa*; T_{70} = *P. alcaligenes*

and weedy condition (Fig. 1).

Plant chemical parameters

There was a significant effect of interaction between weeds and allelopathic bacteria inoculation on nutrient uptake of rice plant (Table 3). The maximum increase in grain and straw nitrogen, phosphorus and potassium content were observed with *P. putida* in weeds free pots. However, it was nonsignificant for straw P and grain K content in rice plant infested with *C. rotundus* inoculated with *P. putida* strain. In weeds free rice plant consortium inoculation had nonsignificant effect on grain and straw N content. However, the minimum nutrient uptake was observed in *C. rotundus* and *E. colonum* infested pots without inoculation with allelopathic bacteria (Table 3).

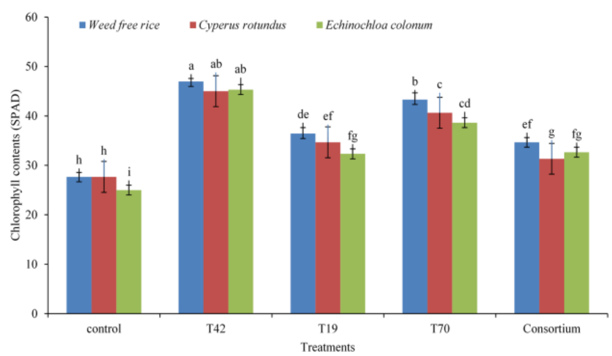

Fig. 1: Effect of allelopathic bacteria on chlorophyll contents of rice under weed free and weed infested conditions
Here T_{42} = *P. putida*; T_{19} = *P. aeruginosa*; T_{70} = *P. alcaligenes*

Discussion

The present study explored the efficacy of allelopathic bacterial strains for the suppression of rice associated weeds. Allelopathic bacteria produce cyanide that inhibits growth of weeds by blocking many enzymes, involved in the normal metabolic pathway (Abbas *et al.* 2017a). Potential of these strains (*P. putida*, *P. aeruginosa* and *P. alcaligenes*) may be dependent on their phytotoxic secondary metabolites (such as siderophore), antibiotics, phenolic compounds and cyanide production (Vyas and Gulati 2009; Ali *et al.* 2017). These biomolecules are derived from microorganisms and they boost up the molecular process in soil, which eventually leads to inhibition of weeds growth (Ali *et al.* 2017). Different antibiotic classes such as blasticidin, thaxtomin, hydantocidin and methoxyhygromycin, could inhibit weed growth at pre-and post-emergence stages of weeds (Kremer and Souissi 2001). The bacteria secrete antibiotics, siderophore, toxin, antimicrobial volatiles compounds and wall hydrolytic enzymes that act antagonistically with phytopathogens (Sheoran *et al.* 2015). Previous studies reported *P. fluorescens* has the dramatic ability to reduce germination of goatgrass and downy brome (Vyas and Gulati 2009). This study confirmed that inoculation of bacterial strains had comparatively lesser weed suppression under pot condition than controlled condition, as previously studied by Abbas *et al*. (2017a, b). This might be due to competition among introduced bacteria, indigenous microbial communities and severe environmental stress influencing their efficacy in rhizosphere of weeds (Horwath *et al.* 1998).

In the case of consortium, the decline in suppression of *C. rotundus* and *E. colonum* by might be due to survival competition among strains. Similar to previous findings, rice growth also improved with the inoculation of bacterial strains in weed free condition than rice infested with weeds. This might be due to the depletion of nutrients in rhizosphere and production of allelochemicals by weeds, which suppress the growth of rice. Interestingly, in weedy conditions, rice growth increased which might be possible due to degradation or detoxification of allelochemicals produced by weeds or the competitive abilities of crop against weeds were strengthened. Moreover, Mejri *et al*. (2010) also reported that weed antagonistic bacteria produce growth promoters in the rhizosphere of infested wheat that improve potential of plants against weed that also suppresses the weed growth (Ali *et al.* 2017). It has been reported that *Actinetobacter calcoaceticus* converts BOA to 2, 2-oxo-l, l-azobenzene (AZOB), and this newly transformed compound causes more inhibition of weeds. Mustafa *et al*. (2019) recently reported that *Pseudomonas* ssp. has the potential to suppress the weeds growth and biomass that support our findings. Application of plant growth promoting rhizobacteria had increased all the nutrients in rice compared control condition in our study. This might be due to nitrogen fixation ability (Thaweenut *et al.* 2011), potassium and phosphorus solubilization by applied bacteria in soil (Qin *et al*. 2011). Our findings are supported Chen *et al*. (2014) because they confirmed that bacteria produce different acidifying agents including salicylate and benzene acetic that produce acidic environment near root, resulting in solubilization of nutrients especially phosphorus.

In weedy condition, weeds chlorophyll content reduced as compared to uninoculated control, which might be due to iron (Fe^{+3}), which is basic component of chlorophyll and considered. It has been reported that allelopathic bacteria produce siderophore compounds that bind Fe^{+3} and transport it to the plant cells (Yang *et al.* 2009). It might be possible that this Fe^{2+} is not available to those weeds where bacterial strains were not inoculated. Under weedy control condition chlorophyll content is more because no siderophore is produced by allelopathic bacteria. It has been reported by Zdor *et al*. (2005) that efficacy of strains increase exponentially, when applied by using a suitable carrier, in the present study strains were applied by using peat as carrier which might have helped in their survival and efficiency.

Conclusion

Inoculation with bacterial strains significantly reduced the biomass of weeds and promoted the growth, yield and photosynthetic parameters of infested rice. Results revealed that strain *P. putida* possess potential for inhibition of weeds in rice crop than consortium application. Thus, single rhizobacterial strains maybe more effective against weeds of rice than consortium application. However, apart from growth and yield parameters, future work on further exploration and mechanistic studies through introducing more kinds of weeds is needed.

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