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Field Performance of Allelopathic Bacteria for Biological Weed Control in Wheat: Innovative, Sustainable and Eco-Friendly Approach for Enhanced Crop Production

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Abstract: Application of allelopathic bacteria (AB) for weed suppression may be helpful to solve various environmental challenges posed by conventional weed control techniques. In our earlier studies, around 400 strains of rhizobacteria of five weeds and wheat were isolated, screened for production of phytotoxic substances, and tested for phytotoxic activity on wild oat and little seed canary grass, and possible effects on wheat under laboratory conditions. We obtained 13 strains inhibitory to wild oat (*Avena fatua* L.) and 11 to little seed canary grass (*Phalaris minor* Retz.). Five of these (13 and 11) strains also suppressed wheat (*Triticum aestivum* L.) while others either stimulated or remained ineffective on wheat in separate bioassays. The success of any weed biocontrol technique, however, depends on its response under field conditions. Therefore, the present study was conducted to investigate biological weed control of the five most efficient strains of AB under natural conditions in pot and field trials. Wheat was artificially invaded with wild oat in the pot trial through seeding. Wheat of the field trial was artificially invaded with wild oat and little seed canary through seeding. The selected strains belonged to pseudomonads (*Pseudomonas putida*, *P. fluorescence*, *P. aeruginosa*, and *P. alcaligenes*) and their inocula were prepared using sterilized peat. The inoculated seeds of wild oat and wheat were sown together in a pot trial. The inoculated seeds of wild oat, little seed canary grass, and wheat were sown together in the field experiment. The field was selected based on chronic infestation of these weeds. However, weed invasion was ensured by adding seeds of weeds (inoculated with the respective strains of AB, according to treatment plan). A severe invasion of wild oat was observed in the pot trial, which reduced the grain yield of infested wheat up to 60.8%. The effectiveness of applied strains controlled 22.0–76.3% loss of grain yield of infested wheat. Weed invasion in the field trial reduced the grain yield of the crop up to 56.3% and effectiveness of the applied strains controlled 29.0–60.7% loss of grain yield of infested wheat. The study of other agronomic, physiological, and chemical parameters of the crop and weeds supported these findings. Harnessing the potential of these strains exhibited in our studies may be helpful to introduce an innovative, sustainable, and eco-friendly weed control technique for production of wheat.

Keywords: allelopathic bacteria; weed control; wild oat; little seed canary grass; wheat; phytotoxic metabolites

1. Introduction

Weed growth in crops hampers crop production worldwide. The losses due to weeds continue even after an increase in intensity of control practices. Conventional control relied on human labor, mechanical operations, and chemical herbicides. The shift of labor to industry, businesses, and services has increased the cost of labor and reduced its availability for weed control [1]. Furthermore, using human labor also increases the cost of crop production. Mechanical control, on the other hand, is limited to few crops. Tillage-oriented mechanical control aggravates deterioration of natural soil resources and environmental problems like soil compaction, erosion, eutrophication, and disturbance in useful soil biological processes [1]. Chemical control has gained popularity due to its quick response, lower cost, and easy application. Many devastating effects of herbicides on weed ecology, human health, and environment have appeared over time. They have induced intraspecific and interspecific selection in weed flora, leading to herbicide resistance and shift of weed flora, respectively [2]. Contamination of the environment has exposed the susceptible and non-target species to herbicides, leading to loss of biodiversity [3]. Accumulation of herbicides and their residues in the environment and edible parts of plants expose human beings to these substances. Apart from causing poisoning of body, these substances are also known to cause several chronic diseases and disorders, leading to death. Hence, chemical weed control is not considered a sustainable practice. It further loses its usefulness when the costs of chemical herbicide development, remediation of the environment, treatment of human diseases, and efforts to save precious lives are included in the cost of chemical weed control. The yield losses to crops due to weeds are extensive. Therefore, alternative weed control techniques are needed, which should effectively control weeds in a sustainable and eco-friendly manner. These will help to reduce the negative impacts of current control practices on man, biodiversity, agriculture, and environment [4].

Although work on biological weed control has been carried out in the past, focusing on phytophagous insect biocontrol agents [5], pathogens of weeds [6], and plant allelochemicals [7], the potential of rhizosphere-inhabiting bacteria for biological weed control has been largely ignored. It counts as the major reason for the low success rate of biological weed control. The phytotoxic activities of rhizobacteria are caused by production of phytotoxic substances and their absorption by plants. This results in growth inhibition, loss of vigor, loss of reproductive potential and impact of weeds on crops. This mechanism of weed suppression (i.e., the independent existence of the individuals interacting with each other through the release of chemical substances) and the substances involved resemble plant allelopathy. Therefore, the group of rhizobacteria responsible for these activities may be more conveniently called allelopathic bacteria (AB) [4,8,9]. The salient characteristics of AB offer wide prospects for development of successful biological weed control. These characteristics include survival in the rhizosphere for longer periods, continuous release of secondary metabolites into the rhizosphere and host specificity created by differential toxicity, differential availability of substrates, and diverse mechanisms or compounds produced by AB [8,10–13]. These bacteria commonly belong to pseudomonads and produce cyanide, membrane-degrading enzymes, extracellular polysaccharides, high amounts of plant growth regulators, germination inhibitor substances, and antibiotics [14,15]. These characteristics of allelopathic bacteria and their mechanisms offer opportunities for development of innovative, more sustainable, and eco-friendly biological weed control techniques.

We isolated 393 isolates of rhizobacteria from five weeds and wheat. These isolates underwent a comprehensive screening process based on the production of different phytotoxic metabolites, growth inhibition of sensitive indicator plants and bacteria, inhibition of weeds, and impacts on wheat [16]. We got 13 strains inhibitory to wild oat and 11 to little seed canary grass, of which five strains suppressed the growth of wheat while others either stimulated or remained ineffective on the growth of infested wheat. These strains needed to be tested under natural conditions upon which the success of such biocontrol agents is dependent. Therefore, the present study was conducted to test the potential of the five most efficient strains of AB under natural conditions in pot and field trials.

2. Materials and Methods

2.1. Selection of Strains of Allelopathic Bacteria

Samples of five weeds and wheat (growing under biotic stress of weeds) were taken from the wheat fields facing chronic weed invasions across the District of Faisalabad in Pakistan. The samples were collected along with the earth ball. The rhizosphere soil of these samples was obtained by gently removing non-rhizospheric soil. The rhizosphere soil was processed for isolation of rhizobacterial isolates with the help of a dilution plating technique. Three hundred and ninety-three strains were isolated, which required a comprehensive screening process based on the release of plant inhibitory metabolites, inhibition of sensitive indicator plants and bacteria, and effects on wild oat, little seed canary grass, and wheat. The screening tests included production of hydrogen cyanide (HCN), antibiosis against sensitive *Escherichia coli*, and antibiosis against the indicator plant, lettuce. The screening gave us 19 strains, which were then tested on wild oat, little seed canary grass, and wheat in agar bioassays. Thirteen strains were found inhibitory to wild oat and 11 to little seed canary grass. Among these strains, five strains inhibited the germination and/or growth of wheat, six strains stimulated wheat, and the remaining two strains remained ineffective on wheat. The strains were further characterized for biochemical characteristics such as production of siderophores, chitinase, catalases, oxidases, exopolysaccharides, and indole 3-acetic acid. The most efficient strains from these studies (i.e., strains L9, T42, W9, $O_0$10, and 7O_0) were identified through 16 s rDNA sequencing as pseudomonads. They were *Pseudomonas fluorescens*, *P. putida*, *P. alcaligenes*, *P. aeruginosa*, and *P. fluorescens*, respectively. The characteristics of these strains indicated their potential for biocontrol of wild oat and little seed canary grass invading wheat, but they needed investigation under natural conditions. Therefore, these five strains were selected for this study for weed control in pot and field trials.

2.2. Preparation of Culture Suspension

King's B medium was prepared using 1.5 g K_2HPO_4, 20 g proteose peptone, 10 mL glycerol, and 1.5 g $MgSO_4.7H_2O$ in 1 L distilled water [17]. The contents were sterilized at 121 °C and 15 psi pressure for 20 min in five Erlenmeyer flasks in an autoclave. These flasks were inoculated with the strains in the laminar flow hood using a bacteriological loop aseptically. The flasks were placed in a shaking incubator set at 28 ± 1 °C and 100 rpm. Population of bacterial cells in the culture around 10^7–10^8 per milliliter was achieved by taking optical density at 600 nm. Culture suspension of each strain was then prepared by centrifugation and collecting the supernatant in the 0.01 M $MgSO_4$ solution.

2.3. Inoculum Preparation

Sterilized peat was used as carrier material to aseptically transfer the selected strains of AB from laboratory to natural conditions. It ensured application of a high population of AB to the seeds and the soil. Peat was sterilized at 121 °C and 15 psi for 20 min in an autoclave. The process was repeated thrice to ensure that the original microflora and microfauna in the peat was killed. The culture suspension of AB strains was mixed with sterilized peat in the ratio of 1.25:1. To prepare control treatments, the sterilized peat was treated with sterilized buffer (0.01 M $MgSO_4$). This treated peat was incubated at 28 ± 1 °C for one day. It helped the bacterial population to establish in the peat, which was tested through a dilution plating technique on the following day.

2.4. Pot Trial

The pot trial was conducted in the warehouse of the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad Pakistan (31.438976° N and 73.069029° E). The general climate of the area is semi-arid where irrigation with canal water supports crop production. It is suitable for getting higher production of wheat. However, the invasions of wild oat and little seed canary grass limit the crop yields. Pots of uniform size having the dimensions of 30 cm upper diameter and 45 cm height were used to conduct the trial. Soil was collected from the topsoil (upper 6" soil)

of one of the fertile fields of the area. It was processed by air-drying, grinding, and sieving before filling the pots. A soil sample from this processed soil was taken before filling the pots with soil at the rate of 8 kg per pot and analyzed for its properties. The size of the soil sample was 1 kg. Its texture was determined to be sandy clay loam. It contained 0.89% organic matter contents, 30.2% saturation percentage, 1.44 dS m^{-1} electrical conductivity (EC), 7.5 pH, 0.04% total nitrogen contents, 7.8 mg kg^{-1} available phosphorus, and 132 mg kg^{-1} extractable potassium contents. The taxonomic class of the soil is Typic Haplocambids. Urea, diammonium phosphate (DAP), and muriate of potash (MoP) were added according to the nutrient requirement of the crop, i.e., 120–90–60 kg N–P–K ha^{-1}. Germination tests of the seeds of wild oat and wheat were performed.

2.5. Seed Inoculation

The inoculated peat of each strain was treated with sterilized sucrose solution (10%) to make slurry of the inoculum. The seeds of wild oat and wheat were coated with this slurry containing a specific strain, as per the treatment plan. The buffer-treated and incubated peat was mixed with the same sucrose solution to make slurry. Seeds of wild oat and wheat were coated with this slurry to make the weedy control. Seeds of wheat alone were treated with this slurry to make the weed-free control. A similar method for making slurry of inoculated peat and 10% sucrose solution was used, and coating seeds of crops with this slurry was reported by Qureshi et al. [18].

2.6. Treatments

There were seven treatments in the experiment, which included the weedy control, weed-free control, and five strains. The control where wild oat was grown with wheat but no strain applied was termed as the weedy control. The growth and yield of weed and crop in this treatment indicated the extent of losses to wheat caused by the weed in the existing conditions, when it was compared with the weed-free control. The control where weed was not allowed to grow with the crop was termed the weed-free control. It also indicated how much growth and yield of the crop could be maximally obtained under the existing conditions. Both control treatments were compared with other treatments where strains were applied to determine how much of the crop loss was recovered by the application of AB strains. All the treatments were replicated thrice in order to get statistically valid data. Hence, there were 21 experimental units. Three sets of these 21 experimental units were arranged in the experiment: the first set to be harvested at the tillering stage, second set at the booting stage, and third set at the physiological maturity of the crop. The layout of experiment is given in Table 1.

Table 1. Layout of pot trial.

Set 1 (for Harvesting at Tillering Stage)			**Set 2 (for Harvesting at Booting Stage)**			**Set 3 (for Harvesting at Physiological Maturity)**		
T4	T3	T2	T4	T3	T2	T4	T3	T2
T6	T7	T1	T6	T7	T1	T6	T7	T1
T1	T2	T5	T1	T2	T5	T1	T2	T5
T5	T6	T3	T5	T6	T3	T5	T6	T3
T7	T4	T7	T7	T4	T7	T7	T4	T7
T3	T1	T6	T3	T1	T6	T3	T1	T6
T2	T5	T4	T2	T5	T4	T2	T5	T4

Where T1 = weed-free control (only wheat grown), T2 = weedy control (wheat + wild oat), T3 = Strain L9 applied to wheat + wild oat, T4 = Strain T42 applied to wheat + wild oat, T5 = Strain $7O_0$ applied to wheat + wild oat, T6 = Strain O_010 applied to wheat + wild oat, and T7 = Strain W9 applied to wheat + wild oat.

2.7. Cultural Practices

Thirty prepared seeds of wild oat were sown together with eight prepared seeds of wheat in each pot, except where the weed-free control was to be applied. Eight prepared seeds of wheat were

sown alone in pots to make the weed-free control treatment. The pots were placed in the warehouse of the Institute in order to avoid any damage from animals and wildlife. The crop was irrigated with good quality water having an EC of 0.78 dS m^{-1}, residual sodium carbonate (RSC) of 1.37 m L^{-1}, and sodium adsorption ratio (SAR) of 4.56 (mmol $L^{-1})^{1/2}$. The gravimetric method was used to maintain the moisture level in the pots at field capacity and avoid the hypoxic or anoxic conditions. A healthy crop growth was achieved with no symptoms of any insect or disease attack. Therefore, the crop was not treated with any pesticide in its life cycle. However, a severe invasion of wild oat was observed in the pots where it was sown. Hand weeding was performed several times to restrict the growth of weeds in the weed-free control throughout the cropping season. The first set of pots was harvested at the tillering stage. Data of the growth parameters of the weed and infested wheat, like number of tillers, biomass, and lengths of roots and shoots, were taken. The second set of pots was harvested at the booting stage and data of similar parameters of wild oat and wheat were taken. Physiological measurements of chlorophyll contents, CO_2 assimilation rate, and stomatal conductance of wheat were taken at 60 days after sowing. The third set of pots was harvested at physiological maturity of the crop. Data of growth and yield parameters of wild oat and wheat were taken. Chemical parameters of the crop were analyzed from grain and straw samples of the mature crop. The data were analyzed statistically to determine the impact of AB strains on the biological control of wild oat and to relieve the biotic stress of the weed on the crop.

2.8. Field Trial

2.8.1. Site Selection

The experimental site was selected based on its history of chronic weed invasions in the past few years. Such an experimental site could ensure the invasion of wild oat and little seed canary grass in the upcoming experimental crop. It was located at the Research Area of the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan (31.438738° N and 73.070080° E). The climate of the area is semi-arid, i.e., rainfall is insufficient to support crop production and water requirements of the plants are met through additional water supplies from canals or tube wells. The climate is suitable for production of wheat. However, invasions of wild oat and little seed canary grass cause huge economic losses to the crop. Historically, invasions of these weeds were the most notorious at the site of experiment. A representative sample of soil was taken from the field by mixing sub-samples, which, in turn, were collected from multiple locations within the field. The soil sample was analyzed for determination of various physico-chemical properties. Its texture was determined to be sandy clay loam with 0.85% organic matter contents, 30.7% saturation percentage, 1.76 dS m^{-1} EC, 7.5 pH, 0.05% total nitrogen contents, 7.5 mg kg^{-1} available phosphorus, and 138 mg kg^{-1} extractable potassium contents. The taxonomic class of the soil was Typic Haplocambids. These characteristics indicated the suitability of the land for experimental use.

2.8.2. Seed Inoculation

An inoculum of selected strains was prepared as described in above section. The inoculated peat as well as the buffer-treated peat were taken from the incubator on the next day. These were treated with sterilized sucrose solution (10%) to make slurry. The slurry made from inoculated peat was used for coating the seeds of wild oat, little seed canary grass, and wheat. The slurry made from buffer-treated peat was used for seed coating of the wild oat, little seed canary grass, and wheat to make the weedy control treatment. Seeds of wheat alone were treated with this slurry to make the weed-free control. A similar method for seed coating of inoculum of rhizobacteria was used by Qureshi et al. [18].

2.8.3. Treatments

The experiment was comprised of seven treatments: the five strains of AB formed the five treatments, and the remaining two treatments were formed by the weedy control and the weed-free

control. The layout is given in Table 2. Seeds of the weeds and wheat were coated with the slurry of inoculum-treated peat to form the five treatments where the selected strains had to be applied. The seeds of the weeds and wheat were treated with the buffer-treated peat, which formed the weedy control. However, only the wheat seeds were coated with the buffer-treated peat to form the weed-free control. The weedy control depicted how much crop loss could be caused under the given level of weed invasion. The weed-free control depicted how much crop yield could be obtained if there was no weed invasion. The data from other treatments indicated the extent to which crop loss recovered when strains of AB were applied. The field area was divided into three equal blocks, where each treatment was replicated. The treatments were allocated in each block according to the randomized complete block design (RCBD). There were 21 experimental units (5 × 4 m each) in the field experiment.

2.8.4. Cultural Practices

The seedbed was prepared when the moisture level was at field capacity. The soil was fertilized with the recommended doses, i.e., 120–90–60 kg N–P–K ha^{-1} using urea, diammonium phosphate, and muriate of potash fertilizers. The layout of the field was drawn according to the treatment plan. The prepared wheat seeds were sown together with the prepared seeds of wild oat and little seed canary grass in all the plots except where the weed-free control was to be applied. Prepared wheat seeds were sown alone in plots allocated to the weed-free control treatment. The seeds were sown in lines 22 cm apart. The optimum germination rate of crop and weeds was observed. Good quality irrigation water from the canal (EC of 0.78 dS m^{-1}, RSC of 1.37 m L^{-1} and SAR of 4.56 (mmol $L^{-1})^{1/2}$) was used to fulfill the water requirement of the crop. The crop was irrigated five times for the whole season. There was no incident of insect or disease attack on the crop throughout its life cycle. The crop growth was uniform. A severe attack of wild oat and little seed canary grass on the crop was observed. Hand weeding was performed several times to restrict the growth of weeds in the weed-free control throughout the cropping season. The data regarding agronomic parameters, like density/plant population, biomass, and plant height of crop and weeds, were collected at the tillering and booting stages of the crop as these are considered most critical periods of weed–crop competition [19]. Physiological measurements of chlorophyll contents, CO_2 assimilation rate, and stomatal conductance of wheat were taken at 60 days after sowing. At physiological maturity, data of growth and yield parameters of wheat and weeds were taken. Grain and straw samples were taken from the mature crop to analyze for chemical parameters.

2.9. Study of Physiology of Wheat

Different physiological parameters of the crop were measured to determine the effect of the biotic stress of weeds on these parameters. A Soil Plant Analysis Development chlorophyll meter (SPAD-502 of Konica Minolta, Japan) was used for the measurement of leaf chlorophyll contents. The photometric measurement gives a coefficient, called the SPAD value, that is proportional to the chlorophyll contents and leaf N concentration. A portable photosynthesis system (CIRAS-III of PP Systems, Amesbury, MA, USA) was used to measure the assimilation rate and stomatal conductance of the flag leaf of the crop plants. These parameters were measured at the photon flux density of 1200–1400 m^{-2} s^{-1}.

Table 2. Layout of field trial.

	Block 1			**Block 2**			**Block 3**			
	Non-experimental area			Non-experimental area			Non-experimental area			
	Strain L9 applied to wheat + weeds	Weed-free control (only wheat)	Strain $7O_0$ applied to wheat + weeds	Strain T42 applied to wheat + weeds	Strain $O_0$10 applied to wheat + weeds	Strain W9 applied to wheat + weeds	Weed-free control (only wheat)	Weedy control (wheat + weeds)	Strain $O_0$10 applied to wheat + weeds	
Non-experimental area	Weedy control (wheat + weeds)	Strain T42 applied to wheat + weeds		Weed-free control (only wheat)	Strain $7O_0$ applied to wheat + weeds		Strain $7O_0$ applied to wheat + weeds	Strain L9 applied to wheat + weeds		Non-experimental area
	Strain $O_0$10 applied to wheat + weeds	Strain W9 applied to wheat + weeds		Strain L9 applied to wheat + weeds	Weedy control (wheat + weeds)		Strain W9 applied to wheat + weeds	Strain T42 applied to wheat + weeds		
	Non-experimental area									

2.10. Study of Chemical Parameters of Wheat

Grain and straw samples of the infested wheat were taken at the time of harvesting of the crop at physiological maturity. These samples were digested for determination of contents of nitrogen, potassium, and phosphorus, following [20]. The nitrogen contents were determined through distillation, using Kjeldahl's apparatus. The distillate was back titrated with boric acid to determine the nitrogen contents in the plant samples. For determination of the phosphorus contents in the plant samples, diluted concentrations of the digest were mixed with Barton's reagent and placed for half an hour to allow for color development. The absorbance of light of 410 nm through this colored solution was measured using a spectrophotometer (Hitachi Ratio Beam spectrophotometer U-5100). This revealed the phosphorus contents in the plant samples. The potassium contents in the digest of plant samples were determined with the help of a flame photometer (Jenway-PFP7). The dilution of digest was fed to the meter, along with standard solutions of potassium chloride. An atomized solution/sample, when passed from above the flame of the flame photometer, excited the potassium ions. The excited ions turned to ground state, emitting specific radiations. The radiations emitted by potassium were separated from the bulk with the help of a potassium filter. The signal strength was correlated to the concentration of potassium in the known and unknown solutions [21].

2.11. Data Collection and Statistical Analysis

The data of density, biomass, and other related parameters of the weeds were measured at multiple growth stages, i.e., at the time of tillering, at the time of booting, and at crop maturity. The data of agronomic parameters of the crop were also taken at these growth stages to determine how much crop loss was caused by the weeds at each stage and how the bacteria helped to reduce this loss, if any. The data of physiological parameters of the crop were collected at 60 days after sowing of the crop when the weed–crop competition was at a critical stage. The chemical parameters of the crop were analyzed after harvesting of the crop. All these data were analyzed statistically using the standard procedures [22]. ANOVA test was performed using a linear model before comparison of treatments for significant differences. The significant differences among the means of the weedy control, weed-free control, and other treatments were determined by applying the least significant difference test (LSD) with all pairwise comparisons. We used the software Statistix 8.1 to conduct these statistical analyses.

3. Results

3.1. Weed Suppression Effects of AB Strains on Wild Oat and Wheat in Potted Soil at Different Growth Stages

3.1.1. Tillering Stage

The tillers, shoot length, shoot dry weight, and the root dry weight of the crop, when compared with the weed-free control, were significantly reduced up to 60.3, 22, 64.9 and 67.9%, respectively, without inoculation (Table 3). All the strains caused significant inhibition in root dry weight and tillers of the weed from 18.1 to 59.5% and 11.9 to 42.2%, respectively, due to the variable effects of individual strains. However, the reduction in shoot dry weight of the weed was caused by four strains (L9, W9, $7O_0$, and T42) from 22.2 to 49.3%. Effectiveness of weed control using these four strains controlled 13.2 to 65.8%, 21.9 to 84.1%, 13.7 to 65.3%, and 16.7 to 62.8% loss of tillers, shoot length, shoot dry weight, and root dry weight of the infested crop, respectively.

3.1.2. Booting Stage

Weed invasion caused a reduction in tillers, shoot length, shoot dry weight, and root dry weight of wheat up to 67.2, 19.8, 65.2, and 71.1%, respectively, when the weedy control was compared with the weed-free control (Table 4). The tillers, shoot dry weight, and root dry weight of wild oat were significantly inhibited from 14.4 to 41.7%, 14.6 to 51.4%, and 19.7 to 64%, respectively, by these strains, compared with these parameters of the weed in the weedy control. This reflected the variable potential

of each strain to suppress the weed. Loss of tillers, shoot length, shoot dry weight, and root dry weight of infested wheat were controlled to variable extents from 15.6 to 53.3%, 27 to 82.6%, 13.4 to 62.5% and 18.3 to 62.4%, respectively, owing to the effectiveness of AB. A pictorial view of the effects of strain L9 on control of wild oat and improvement in the growth of wheat, as a consequence, is available in Figure 1.

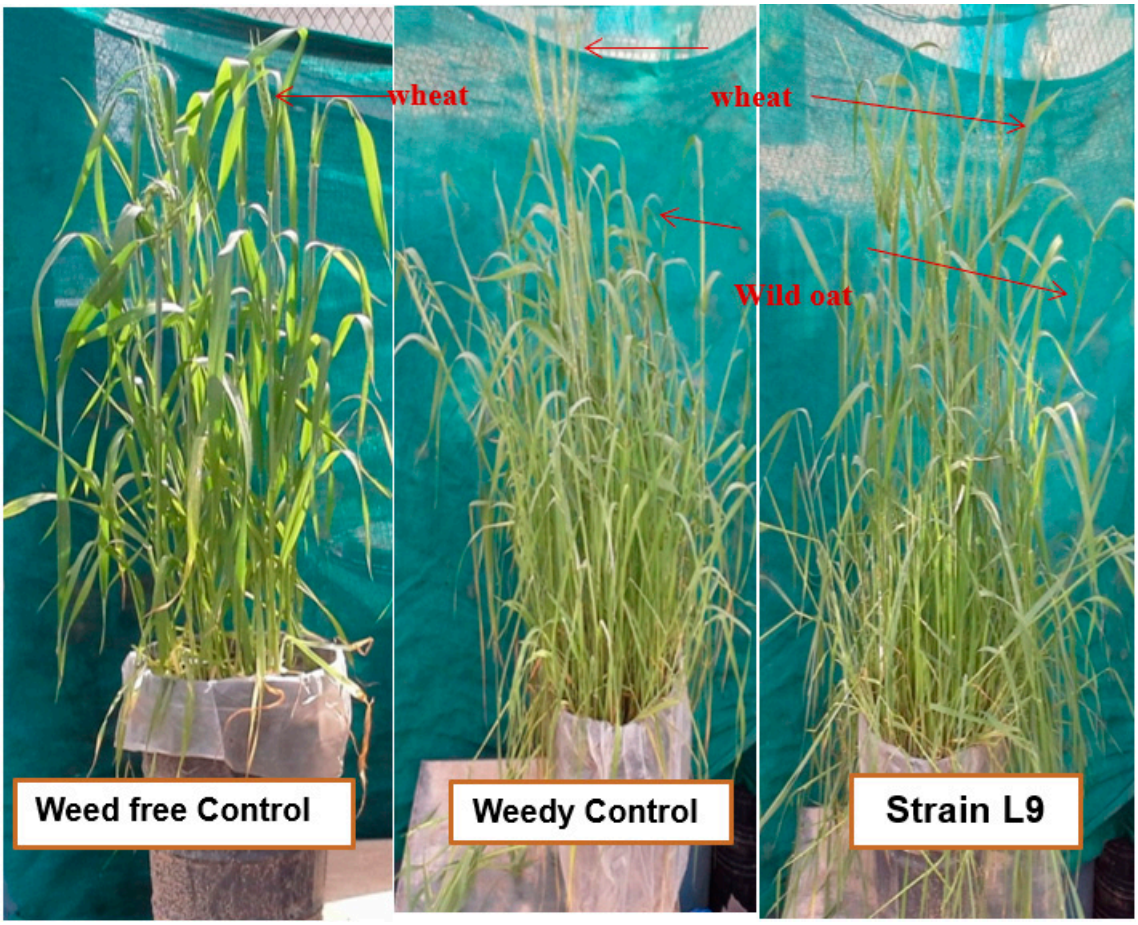

Figure 1. Pictorial view of biocontrol activity of allelopathic bacteria on wild oat grown in wheat in potted soil.

Table 3. Weed control of allelopathic bacteria on wild oat and improvement of wheat at tillering stage in potted soil.

Treatments	Wild Oat			Wheat			
	No. of Tillers Pot^{-1}	Root Dry Weight (g pot^{-1})	Shoot Dry Weight (g pot^{-1})	No. of Tillers Pot^{-1}	Shoot Length (cm)	Root Dry Weight (g pot^{-1})	Shoot Dry Weight (g pot^{-1})
Weed-free control	-	-	-	21.0 a	34.5 a	17.0 a	21.0 a
Weedy Control	45.0 a	11.0 a	12.0 a	8.3 f	26.9 d	5.5 e	7.4 d
T42	31.3 d	6.1 d	7.4 de	15.3 bc	32.0 a–c	11.5 bc	14.7 bc
L9	26.0 e	4.5 e	6.1 e	16.7 b	33.3 ab	12.7 b	16.3 b
$7O_0$	37.3 bc	7.7 bc	9.4 bc	12.3 de	30.3 b-d	9.5 cd	12.2 c
O_010	39.7 b	9.0 b	10.5 ab	10.0 ef	28.6 cd	7.4 de	9.2 d
W9	35.0 cd	6.6 cd	8.8 cd	13.7 cd	31.3 a–c	10.9 bc	13.9 bc
SE	1.856	0.718	0.794	1.333	1.907	1.071	1.258
LSD	4.04	1.57	1.73	2.86	4.09	2.30	2.70

Values sharing same letter(s) in a column are statistically non-significant with each other at $p < 0.05$. SE indicates the standard error of comparison.

Table 4. Weed control of allelopathic bacteria on wild oat and improvement of wheat at the booting stage in potted soil.

Treatments	Wild Oat			Wheat			
	No. of Tillers Pot^{-1}	Root Dry Weight (g pot^{-1})	Shoot Dry Weight (g pot^{-1})	No. of Tillers Pot^{-1}	Shoot Length (cm)	Root Dry Weight (g pot^{-1})	Shoot Dry Weight (g pot^{-1})
Weed-Free Control	-	-	-	22.3 a	49.1 a	26.6 a	31.9 a
Control	44.0 a	15.7 a	33.7 a	7.3 e	39.4 e	7.7 f	11.1 e
T42	31.0 c	7.4 cd	20.9 c	14.3 bc	46.0 a–c	16.6 c	22.0 bc
L9	25.7 d	5.7 d	16.4 d	15.0 b	47.4 ab	19.5 b	24.1 b
$7O_0$	34.3 bc	10.0 bc	26.1 b	11.7 cd	43.8 cd	13.3 de	17.9 d
O_010	37.7 b	12.6 b	28.8 b	9.7 de	42.0 de	11.1 e	13.9 e
W9	35.7 b	9.0 c	25.4 bc	13.3 bc	44.9 b-d	15.4 cd	20.4 cd
SE	1.710	1.238	2.061	1.357	1.643	1.262	1.589
LSD	3.73	2.70	4.49	2.91	3.52	2.71	3.40

Values sharing same letter(s) in a column are statistically non-significant with each other at $p < 0.05$. SE indicates the standard error of comparison.

3.1.3. Harvesting Stage

Without inoculation, the weed invasion caused loss of grain yield, straw yield, tillers, root dry weight, and shoot length up to 60.8, 66.1, 67.1, 66.8 and 19.2%, respectively, when the weedy control was compared with the weed-free control (Table 5). The application of AB strains caused reduction of seed production, straw yield, tillers, and root dry weight of wild oat from 22.4 to 60.0%, 14.1 to 47.3%, 17.0 to 45.0% and 19.0 to 54.3%, respectively. This showed the variable potential of individual strains to suppress the weed. The losses of grain yield, straw yield, tillers, shoot length, and root dry weight of the infested crop were controlled from 22.0 to 73.6%, 18.0 to 67.7%, 17.0 to 51.1%, 36.9 to 87.2%, and 23.2 to 72.1%, respectively.

3.1.4. Influence of AB Strains on the Physiology of Wheat

Without inoculation, the weed invasion caused a reduction in assimilation rate, chlorophyll contents, and stomatal conductance of wheat up to 28.4, 22.3, and 27.6%, respectively, when the weedy control was compared with the weed-free control (Table 6). The AB strains relieved the crop from the biotic stress of weeds and controlled loss of these parameters from 32.1 to 96.0%, 29.9 to 91.4%, and 25.5 to 100.0%, respectively. This indicated the variable effects of individual strains on the amelioration of the physiology of crop plants.

3.1.5. Influence of AB Strains on N–P–K Contents of Wheat

The weed invasion reduced the N, P and K contents in the grains of wheat up to 32.8, 40.5, and 40.1%, respectively (Table 6). The suppression of the weed by AB strains improved the grain N–P–K contents of the crop. It helped the crop to control its loss of these contents from 22.5 to 86.3%, 30.1 to 83.3%, and 18.1 to 96.2%, respectively. It showed the variable effects of each strain on weed suppression and the improvement in grain N–P–K contents of wheat.

3.2. Effects of AB Strains on Weed Control in Wheat under Field Conditions at Different Growth Stages

3.2.1. Tillering Stage

The weed attack on the crop caused reduction in tillers, dry matter, and shoot length of wheat up to 51.9, 58.5, and 26.9%, respectively (Table 7). Three strains ($7O_0$, L9, and O_010) significantly reduced the dry matter (37.9–51.2%) and density (30.6–45.4%) of little seed canary over the weedy control. The other two strains (W9 and T42) remained ineffective to suppress the growth of little seed canary grass. The strains $7O_0$, T42, L9, and O_010 suppressed the dry matter and density of wild oat from 22.0 to 52.8% and 18.0 to 46.1%, respectively. The effectiveness of each strain was reflected in the improvement in growth of the crop. The loss of tillers, shoot length, and dry matter of infested wheat was controlled from 27.9 to 78.1%, 42.1 to 95.2%, and 32.1 to 68.5%, respectively. The strain L9 showed maximum weed control in these conditions, which was followed by strains T42, $7O_0$, W9, and O_010.

3.2.2. Booting Stage

The weedy control gave out 48.3, 30.9, and 49.6% fewer tillers, lesser shoot length, and dry matter of wheat, respectively, when it was compared with the weed-free control (Table 8). This loss of crop showed the severity of weed attack. Three strains (O_010, $7O_0$, and L9) reduced the dry matter and density of little seed canary grass from 37.5 to 54.6% and 32.6 to 48.4% over the weedy control, respectively. The strains L9, T42, W9, and $7O_0$ reduced the dry matter and density of wild oat from 24.1 to 55.0% and 20.3 to 48.4%, respectively. The response of each strain for weed control was reflected in the improvement of growth of the crop. Losses in tillers, shoot length, and dry matter of the infested crop were controlled from 34.4 to 94.4%, 41.2 to 90.2%, and 48.4 to 96.6%, respectively. The strain L9 was the most effective in weed control, followed by strains T42, $7O_0$, W9, and O_010.

3.2.3. Harvesting Stage

The weedy control reported a reduction in the growth of wheat owing to weed invasion. The grain yield, straw yield, tillers, and shoot length of wheat in the weedy control were reduced up to 56.3, 57.0, 48.9 and 30.4% more than the weed-free control, respectively (Table 9). Three strains of AB ($O_0$10, 7O_0, and L9) significantly inhibited the seed production, density, and straw yield of little seed canary grass from 23.4 to 34.1%, 34.1 to 50.5%, and 44.5 to 58.6%, respectively, indicating the variable potential of each strain to suppress the weed. The straw yield and density of wild oat were significantly reduced by four strains (7O_0, L9, W9, and T42) from 28.1 to 62.2% and 21.3 to 50.1%, respectively. Seed production of wild oat was significantly reduced by three strains (L9, 7O_0, and T42) from 21.3 to 43.9%. Loss of grain yield, straw yield, tillers, and shoot length of infested wheat were controlled from 29.0 to 60.7%, 36.6 to 61.0%, 34.8 to 75.0% and 41.8 to 75.2%, respectively. The strain L9 caused maximum weed control, followed by strains 7O_0, T42, W9, and $O_0$10.

3.2.4. Influence of AB Strains on Physiology of Wheat

The biotic stress of weeds caused a reduction in assimilation rate, chlorophyll contents, and stomatal conductance of wheat up to 21.0, 17.1 and 31.2%, respectively (Table 10). The potential of AB strains to suppress weeds was reflected in the improvement of physiology of the crop. Control of losses in these parameters of infested crop varied from 44.5 to 90.3%, 46.9 to 98.9%, and 28.8 to 87.8%, respectively. The strain L9 showed maximum improvement of physiology of wheat, followed by strains 7O_0, T42, W9, and $O_0$10.

3.2.5. Influence of AB Strains on Chemical Parameters of Wheat

Without inoculation, the invasion of weeds reduced the grain N, P and K contents of wheat up to 32.9, 28.8 and 36.3%, respectively, when these parameters of wheat of the weedy control were compared with the weed-free control (Table 10). The effectiveness of the strains of allelopathic bacteria that controlled the loss of infested crop in these parameters varied from 41.6 to 97.8%, 46.6 to 90.1%, and 37.6 to 91.9%, respectively. The strain L9 caused maximum improvement in the chemical parameters of the crop, followed by strains 7O_0, T42, W9, and $O_0$10.

Table 5. Weed control of allelopathic bacteria on wild oat and improvement of wheat at the harvesting stage in potted soil.

Treatments	Wild Oat				Wheat				
	No. of Tillers Pot^{-1}	Root Dry Weight (g pot^{-1})	Grain Yield (g pot^{-1})	Straw Yield (g pot^{-1})	No. of Tillers Pot^{-1}	Root Dry Weight (g pot^{-1})	Shoot Length (cm)	Grain Yield (g pot^{-1})	Straw Yield (g pot^{-1})
Weed-Free Control	-	-	-	-	23.3 a	19.8 a	54.3 a	21.1 a	31.6 a
Weedy Control	43.0 a	18.5 a	9.2 a	32.3 a	7.6 f	6.1 f	43.9 d	8.3 f	10.7 f
T42	29.0 c	9.5 cd	4.9 d	19.2 d	16.6 bc	12.9 c	51.1 a–c	15.9 bc	21.9 bc
L9	23.7 d	8.5 d	3.7 e	15.5 e	17.7 b	15.1 b	53.0 ab	17.7 b	24.8 b
$7O_0$	32.7 bc	13.3 b	6.5 c	26.1 bc	13.3 de	9.9 de	48.8 c	12.8 de	16.9 de
$O_0$10	35.7 b	15.0 b	7.1 b	27.7 b	11.0 e	9.0 e	47.8 c	11.1 e	14.4 e
W9	33.3 bc	11.1 c	6.4 c	24.4 c	14.7 cd	11.3 cd	49.6 bc	14.6 cd	20.1 cd
SE	2.117	0.891	0.232	1.451	1.309	1.045	1.740	1.208	1.688
LSD	4.61	1.94	0.50	3.16	2.81	2.24	3.73	2.59	3.62

Values sharing same letter(s) in a column are statistically non-significant with each other at $p < 0.05$. SE indicates the standard error of comparison.

Table 6. Influence of allelopathic bacteria on physiological and chemical parameters of wheat under biotic stress of the weed in potted soil.

Treatments	Chlorophyll Contents (Soil Plant Analysis Development (SPAD) Value)	Assimilation Rate (μmol CO_2 m^{-2} s^{-1})	Stomatal Conductance (mmol m^{-2} s^{-1})	Grain Nitrogen Contents (%)	Grain Phosphorus Contents (%)	Grain Potassium Contents (%)
Weed-free Control	44.9 a	12.0 a	246.0 a	2.41 a	0.14 a	1.63 a
Weedy Control	34.9 d	8.6 e	178.0 d	1.62 e	0.08 d	0.98 f
T42	42.8 ab	11.4 ab	232.3 ab	2.25 b	0.12 a–c	1.42 bc
L9	44.0 a	11.9 a	246.0 a	2.38 ab	0.14 ab	1.52 ab
$7O_0$	39.0 c	10.3 cd	201.3 c	1.95 cd	0.11 b-d	1.20 de
$O_0$10	37.9 cd	9.7 d	195.3 c	1.86 d	0.1 cd	1.11 ef
W9	40.8 bc	11.0 bc	224.3 b	2.07 c	0.12 a–c	1.28 cd
SE	1.457	0.391	7.815	0.072	0.014	0.072
LSD	3.12	0.84	16.76	0.155	0.03	0.154

Values sharing same letter(s) in a column are statistically non-significant with each other at $p < 0.05$. SE indicates the standard error of comparison.

Table 7. Effects of allelopathic bacteria on weed control and improvement of wheat at the tillering stage under field conditions.

Treatments	Little Seed Canary Grass		Wild Oat		Wheat		
	Density (No. of Tillers m^{-2})	Dry Matter Yield (t ha^{-1})	Density (no. of Tillers m^{-2})	Dry Matter Yield (t ha^{-1})	No. of Tillers m^{-2}	Shoot Length (cm)	Dry Matter Yield (t ha^{-1})
Weed-free control	-	-	-	-	320.0 a	40.9 a	2.82 a
Weedy control	65.3 a	0.28 a	140.3 a	0.51 a	154.0 e	29.9 e	1.17 e
T42	58.7 ab	0.24 a	92.3 c	0.31 c	235.7 c	37.9 bc	1.97 c
L9	42.3 bc	0.17 b	75.7 c	0.24 c	283.7 b	40.3 ab	2.3 b
$7O_0$	35.7 c	0.14 b	115.0 b	0.39 b	234.3 c	36.9 cd	1.94 cd
O_010	45.3 bc	0.18 b	138.7 a	0.52 a	200.3 d	34.5 d	1.7 d
W9	67.0 a	0.29 a	113.7 b	0.39 b	209.7 d	35.3 cd	1.78 cd
SE	7.784	0.025	9.314	0.036	8.983	1.336	0.115
LSD	17.34	5.54	20.75	8.08	19.57	2.91	0.25

Values sharing same letter(s) in a column are statistically non-significant with each other at $p < 0.05$. SE indicates the standard error of comparison.

Table 8. Effects of allelopathic bacteria on weed control and improvement of wheat at the booting stage under field conditions.

Treatments	Little Seed Canary Grass		Wild Oat		Wheat		
	Density (No. of Tillers m^{-2})	Dry Matter Yield (t ha^{-1})	Density (No. of Tillers m^{-2})	Dry Matter Yield (t ha^{-1})	Density (No. of Tillers m^{-2})	Shoot Length (cm)	Dry Matter Yield (t ha^{-1})
Weed-Free Control	-	-	-	-	334.7 a	80.1 a	5.71 a
Weedy Control	71.7 a	0.62 a	134.3 a	1.42 a	173.0 d	55.3 d	2.88 d
T42	64.0 a	0.55 a	86.0 bc	0.84 c	268.3 b	71.1 bc	4.79 b
L9	45.0 b	0.35 b	69.3 c	0.64 d	325.7 a	77.7 ab	5.61 a
$7O_0$	37.0 b	0.28 b	106.0 b	1.06 b	270.3 b	71.4 bc	4.95 b
O_010	48.3 b	0.39 b	132.0 a	1.43 a	228.7 c	65.5 c	4.25 c
W9	74.0 a	0.61 a	107.0 b	1.08 b	239.0 c	67.0 c	4.44 c
SE	6.910	0.067	9.924	0.078	10.924	3.511	0.122
LSD	15.4	15.03	22.11	17.42	23.8	7.65	0.266

Values sharing same letter(s) in a column are statistically non-significant with each other at $p < 0.05$. SE indicates the standard error of comparison.

Table 9. Effects of allelopathic bacteria on weed control and improvement of wheat at the harvesting stage under field conditions.

Treatments	Little Seed Canary Grass			Wild Oat			Wheat			
	Density (No. of Tillers m^{-2})	Grain Yield (t ha^{-1})	Straw Yield (t ha^{-1})	Density (No. of Tillers m^{-2})	Grain Yield (t ha^{-1})	Straw Yield (t ha^{-1})	Number of Tillers m^{-2}	Shoot Length (cm)	Grain Yield (t ha^{-1})	Straw Yield (t ha^{-1})
Weed-free control	-	-	-	-	-	-	365.0 a	112.1 a	3.78 a	6.00 a
Weedy control	69 a	0.35 a	0.76 a	148 a	0.91 a	2.36 a	186.7 e	78.0 d	1.65 e	2.58 d
T42	62 a	0.33 a	0.65 a	92 c	0.64 bc	1.30 c	291.7 c	100.0 bc	2.67 c	4.32 b
L9	42 b	0.25 bc	0.39 b	74 c	0.51 c	0.89 d	320.3 b	103.7 ab	2.94 b	4.66 b
$7O_0$	34 b	0.23 c	0.31 b	114 b	0.71 b	1.61 b	295.3 c	100.1 bc	2.79 bc	4.50 b
$O_0 10$	46 b	0.27 b	0.42 b	147 a	0.91 a	2.33 a	248.7 d	92.3 c	2.27 d	3.86 c
W9	71 a	0.35 a	0.74 a	117 b	0.75 ab	1.70 b	256.7 d	95.2 bc	2.43 d	3.83 c
SE	6.66	0.01	0.057	9.77	0.077	0.102	10.77	4.04	0.088	0.157
LSD	14.85	0.022	0.128	21.8	0.172	0.227	23.48	8.80	0.193	0.343

Values sharing same letter(s) in a column are statistically non-significant with each other at $p < 0.05$. SE indicates the standard error of comparison.

Table 10. Influence of allelopathic bacteria on physiological and chemical parameters of wheat under the biotic stress of weeds under field conditions.

Treatments	Chlorophyll Contents (SPAD Value)	Assimilation Rate (μmol CO_2 m^{-2} s^{-1})	Stomatal Conductance (mmol m^{-2} s^{-1})	Grain Nitrogen Contents (%)	Grain Phosphorus Contents (%)	Grain Potassium Contents (%)
Weed-free control	54.1 a	11.4 a	308.0 a	1.87 a	0.186 a	1.81 a
Weedy control	44.9 d	9.0 d	212.0 e	1.26 e	0.132 d	1.15 e
T42	52.7 ab	10.8 a–c	276.3 c	1.69 bc	0.173 ab	1.62 c
L9	54.0 a	11.2 ab	296.3 ab	1.86 a	0.181 a	1.76 ab
$7O_0$	53.0 ab	11.0 a–c	281.7 bc	1.71 b	0.175 ab	1.66 bc
$O_0 10$	49.2 c	10.1 c	239.7 d	1.51 d	0.157 c	1.4 d
W9	50.3 bc	10.3 bc	246.0 d	1.59 cd	0.164 bc	1.49 d
SE	1.58	0.482	7.58	0.053	0.007	0.056
LSD	3.45	1.05	16.5	0.116	0.015	0.123

Values sharing same letter(s) in a column are statistically non-significant with each other at $p < 0.05$. SE indicates the standard error of comparison.

4. Discussion

The performance of allelopathic bacteria under field conditions would ultimately decide the fate of their application for control of weeds in an environmentally friendly manner. The present study investigated the feasibility of use of allelopathic bacteria for control of weeds under field conditions after their successful testing under laboratory conditions [23]. The present study investigated the potential of the five most efficient strains of allelopathic bacteria, which were encoded as L9, W9, $7O_0$, T42, and O_010. The seeds of wild oat were sown together with wheat in potted soil to cause weed invasion in the crop artificially. This practice was adopted to artificially cause weed invasion in crops for experimental purposes by Flores-Vargas and O'Hara [24] and Kennedy et al. [25]. Weedy and weed-free controls were established to compare with the pots where strains were applied. This determined how many losses were caused to wheat by weed; how much growth of the weed was suppressed by these strains; and how much loss of the crop was recovered owing to weed control of AB. In the field trial, the wheat crop was invaded by little seed canary grass and wild oat. These are considered the most notorious weeds of wheat fields in semi-arid regions of the subcontinent [26,27]. The strains of allelopathic bacteria exhibited control of these weeds similar to the control achieved in our laboratory studies. However, the scale of control was less in the natural conditions than in the laboratory conditions. The strains L9 and $7O_0$ were more suppressive to weeds than the strains O_010, W9, and T42. This agreed with Kremer and Kennedy [28] and Zdor et al. [29]. They also reported lesser control of weeds in natural than in laboratory conditions. They reported that environmental conditions like soil moisture dynamics, nature and composition of root exudates of weeds and crops, temperature, soil type, and soil organic matter affected the survival, colonization, and multiplication of rhizobacteria weed control agents. Li et al. [30] also reported the effect of soil properties on the success of inoculation of weed biocontrol agents. Harris and Stahlman [31] identified 162 strains inhibitory to downy brome, 202 strains inhibitory to Japanese brome, and 129 strains inhibitory to jointed goatgrass in laboratory bioassays. Only nine of their strains could inhibit the growth of downy brome in the potted soil experiment and only two strains inhibited this weed in the field experiment. They linked the adverse conditions of high temperature and low moisture to the lesser response of weed biocontrol agents in the field. The competition of the applied strains with the native and established soil microorganisms also caused a lesser degree of weed control in their study. A recent study by Scavo et al. [32] indicated that physico-chemical conditions of soils influence the production of phytotoxic secondary metabolites of rhizobacteria and their phytotoxicity on plants. The influence of tillage and other soil and crop management practices on the biological weed control activities of rhizobacteria was reported by Kennedy and Stubbs [33]. Optimum levels of moisture, soil aeration, nutrition, and substrates are required for production of phytotoxic secondary metabolites by AB. The inherent soil processes of transformation, translocation, retention, biodegradation, and other soil reactions may affect the bioavailability as well as phytotoxicity of substances to target plants [34]. Hence, the greater decrease in the scale of weed control by the AB strains in the present study than in the laboratory studies might be due to similar reasons.

The potential of each strain to control the weeds and, hence, the resultant improvement of crop was different. The strain L9 caused the highest weed control in the pot trial, which was followed by strains T42, W9, $7O_0$, and O_010. The weed control in field conditions was maximally exhibited by strain L9, followed by strain $7O_0$, T42, W9, and O_010. The improvement in the physiological and chemical parameters of the crop also indicated the weed biocontrol potential of these strains. Hence, this study suggests the use of more effective strains of AB for control of wild oat and little seed canary grass under field conditions. It reflects that these strains might have sufficiently established themselves in the rhizosphere of target plants and produced the desired metabolites. This resulted in weed suppression and improved growth of crop. This behavior of the weed control rhizobacteria was also reported by Gurusiddaiah et al. [35]. They reported the production of growth inhibitory substances by these bacteria and their release into the rhizosphere of weeds, which resulted in weed suppression. Our strains of AB were highly cyanogenic in nature. Therefore, cyanide production in the rhizosphere

of weeds by these strains might have reduced their growth, leading to biological weed control. These strains also produced antibiotics against sensitive bacteria in a laboratory assay. Hence, these strains might be inhibitors of symbionts of weeds, as reported by Omer et al. [36].

The weed control rhizobacteria are more susceptible to environmental stresses when they are applied without any carrier material or formulation. This leads to loss of their survival, establishment, and desired weed control activities [37]. Hence, the weed control agents should be formulated before application to achieve the desired objectives. The strains of AB used in our study were formulated in sterilized peat for their efficient delivery to the field. It might be one of the factors accounting for the good response of AB strains for weed control in the present study.

The strains L9 and $7O_0$ exhibited more weed control potential than others in this study. These strains also had the capability of promoting the growth of wheat, as evidenced from our previous study [23]. The growth promotion ability of the strains for wheat might have strengthened the crop to outgrow/out-compete the weeds, thereby resulting in the high scale of weed control by these strains [37]. This study also derives support from Boyetchko [38], who reported a competitive advantage to the crop when the green foxtail-inhibiting bacteria of his study also stimulated the growth of the crop. The strains L9 and $7O_0$, used in this study, possessed several of the characteristics reported by Zahir et al. [39] for the promotion of plant growth. We reported these in Abbas et al. [23]. Any increment in the growth of the crop may result in reduced growth of weeds. Li and Kremer [40] suggested that the weed control by rhizobacteria might be more successful if these control agents also possessed the characteristics for the promotion of growth of the crop. Hence, the dual characteristics of the two strains of this study made them more successful weed biocontrol agents under the field conditions. The reasons may be traced to the host specificity of rhizobacteria weed control agents, as described by Owen and Zdor [11] and Zeller et al. [13]. Further exploration of the opportunities provided by AB for weed control and augmentation of their functions may help to devise an environmentally friendly and sustainable weed control technique.

5. Conclusions

Five strains of allelopathic bacteria, inhibitory to wild oat and little seed canary grass but non-inhibitory (either stimulatory or neutral) to wheat, were applied to these weeds and wheat in a peat formulation in pot and field trials. Invasion of wild oat in the pot trial reduced the grain yield of the crop up to 60.7%, and the AB strains helped the crop to recover 22.0 to 73.6% of this loss. The invasion of wild oat and little seed canary grass in the field trial reduced the grain yield of the crop up to 56.3%, and the AB strains helped the crop to recover 29.0 to 60.7% of this loss. The effects of weed control and the crop improvement, as a consequence, were also indicated by data of other agronomic, physiological, and chemical parameters of the weeds and the crop. Each strain showed variable potential to control weeds and crop losses. We further reported that the strains of AB stimulatory to wheat, in lab studies, exhibited more weed control and a healthier crop than other strains. The scale of weed control was, however, smaller than in our laboratory studies, indicating the effects of natural conditions. Increasing the response of these strains through the use of augmentation techniques and co-inoculation may improve the scale of biological weed control by allelopathic bacteria.

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