Beneficial interaction of allelopathic bacteria with chemical herbicides for sustainable wheat (*Triticum aestivum* L.) production under wild oat (*Avena fatua* L.) infestation

Muhammad Tauseef Jaffar,[1,2] Zahir Ahmad Zahir,[2] Jianguo Zhang,[1] Abubakar Dar,[3] Muhaimen Ayyub,[2] Hafiz Naeem Asghar[2]

[1]College of Natural Resources and Environment, Northwest A&F University, Yangling, China; [2]Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan; [3]Department of Soil Science, Islamia University of Bahawalpur, Pakistan

Highlights

- *Allelopathic rhizobacteria reduced the seed germination, growth, and yield attributes of wild oat.*
- *Allelopathic rhizobacterial inoculation significantly improved the seed germination, growth, and yield of wheat.*
- *Allelopathic rhizobacteria depicted promising outcomes with 50% recommended dose of Axial.*

Correspondence: Jianguo Zhang, College of Natural Resources and Environment, Northwest A&F University, Yangling, China.
E-mail: zhangjianguo21@nwafu.edu.cn

Key words: allelopathic bacteria, chemical control, integrated weed management, weed inhibition, wheat growth.

Contributions: all the authors made a substantial intellectual contribution, read and approved the final version of the manuscript, and agreed to be accountable for all aspects of the work.

Conflict of interest: the authors declare that they have no competing interests, and all authors confirm accuracy.

Funding: this study was supported by the Higher Education Commission (HEC), Pakistan (project No. TDF-011), the Key Scientific and Technological Project of Shaanxi Province (2022ZDLNY02-03), and the National Science Foundation of China (41877541).

Availability of data and materials: data and materials are available from the corresponding author upon request.

Received: 23 January 2023.
Accepted: 21 June 2023.
Early view: 7 August 2023.

Italian Journal of Agronomy 2023; 18:2193
doi:10.4081/ija.2023.2193

Abstract

Weeds are one of the major limiting factors for wheat production. So, a study was conducted to integrate allelopathic bacteria with a reduced dose of chemical herbicide for sustainable wheat production in wild oat infestation. Cyanide-producing *Pseudomonas* strains were applied in 4 combinations with and without 2 chemical herbicides (Axial and Atlantis) at the 25% and 50% recommended doses under axenic conditions. Results showed that the C4 bacterial combination (combination of B11×T19×T24×T75 bacterial strains) significantly reduced the growth and development of wild oat under 50% Axial while increasing wheat growth. Subsequently, C4 combination and Axial herbicide were selected for field evaluation, where they reduced the weed density (82.1%), soil plant analysis development (SPAD) value (26.0%), grain yield (88.2%) under 75% Axial, relative wild oat density (70.9%), photosynthetic rate (26.6%), and transpiration rate (25.6%) under 50% Axial in wild oat. While the C4 combination improved SPAD value (26.9%), shoot length (10.1%), tillering (33.3%), biological yield (32.7%), straw yield (24.4%), grain yield (46.8%), transpiration rate (9.6%), and stomatal conductance (14.7%) in wheat under 75% Axial. The increase in growth and yield of wheat was found to be similar to C4 under 50% and 75% Axial. Thus, it is concluded that allelopathic bacteria could be used with 50% Axial for sustainable wheat production under wild oat.

Introduction

Wheat is the most indispensable food grain for many countries in the world. It represents a major part of the diet and occupies a central position in agriculture policies (Azam and Shafique, 2017). Improper weed control practices, reduced seed rate, lack of irrigation water with poor management practices, and imbalanced use of fertilizers are the major causes of the production gap among common and progressive farmers (Iqbal *et al.*, 2017). Increasing crop yield in existing farmland to meet food requirements is a crucial concern (Foley *et al.*, 2011). However, weed is one of the most vital biotic factors that affect the yield of wheat crops (Jabran *et al.*, 2017; Ab Rahman *et al.*, 2018; Scavo and Mauromicale, 2020). These plant species, which compete for resources to diminish the intensification, production, and value of crop plants, are known as weeds (Ashiq and Aslam, 2014). The economic loss through yield reduction by weeds was estimated at millions of dollars (Razzaq *et al.*, 2012). Wild oat is one of the most noxious weeds, widely distributed in more than 55 countries and causing huge yield losses in more than 20 different crops with its prolific nature of seed production (Mahajan and Chauhan, 2021). It is very difficult to control because it produces 480 seeds per plant (Sahil *et al.*, 2020) and reduces the crop yield by up to 70% (Beckie *et al.*, 2012). However, the extent of loss in crop yield depends on the environmental conditions, density of weed plants, and crop species (Korres *et al.*, 2019). Therefore, it is very important to control the wild oat infestation for sustainable crop production.

Traditionally, weeds are eradicated by chemical, mechanical, and manual approaches, which give rise to several restrictions (Farooq *et al.*, 2011; Chauvel *et al.*, 2012; Farooq *et al.*, 2017). However, continuous use of chemical herbicides results in herbicide resistance in weeds (De Prado *et al.*, 2004; Pimentel, 2005), dreadful effects on human health, and environmental degradation (Blair *et al.*, 2015). Numerous investigators have recommended that chemical herbicides be replaced with other innovative techniques with different mechanisms of action to overcome these problems (Kao-Kniffin *et al.*, 2013).

Biological approaches may be adopted to control weeds to decrease the dependence on chemical herbicides (Mustafa *et al.*, 2019). In addition, it is an eco-friendly approach, largely focusing on the target and reducing the development cost as compared to synthetic herbicides (Bailey *et al.*, 2010). Biological techniques involve the application of living organisms and their products to control weeds by decreasing their impact through restricted weed development and reducing their numbers and multiplication (Charudattan, 2005). Therefore, various rhizospheric bacteria have been investigated to suppress weeds through the production of various phytotoxic secondary metabolites (Kremer, 2006). These are termed allelopathic bacteria (AB), which reside in the plant rhizosphere and release toxic chemicals to reduce plant development (Kremer, 2013).

AB have the property to suppress the weeds in the wheat crop (Abbas *et al.*, 2017a). They release allelochemicals in the rhizosphere of certain plants to inhibit their germination, seedling growth, and development (Sturz and Christie, 2003). They can secrete metabolites that are phytotoxic and reduce the growth of weeds by imparting continuous stress (Kremer, 2006). Cyanide production by AB is responsible for reducing weed germination rate and growth (Kremer and Souissi, 2001; Zeller *et al.*, 2007). Cyanide production inhibits various enzymes that are involved in different metabolic processes such as carbohydrate metabolism, respiration, and assimilation of nitrate and CO_2 (Grossmann, 1996). Antibiotics such as 2,4-diacetylphloroglucinol and phenazine (Bender *et al.*, 1999), cell wall and cell membrane degrading enzymes, and lytic agents released from AB suppress weed growth and may also be considered important mechanisms of action (Kremer *et al.*, 2006).

However, the use of only microbial populations is not sufficient to suppress weeds due to their slower mode of action and vulnerability to adverse environmental conditions (Lacey and Shapiro-Ilan, 2008). Hence, the use of AB may be more beneficial for weed inhibition when applied with reduced doses of chemical herbicides (Sargent, 1986). Peng and Byer (2005) applied *Pyricularia setariae* with chemical herbicide at reduced levels to counter the growth of green foxtail. Moreover, there was a significant decrease in the growth of weed (Canada thistle) with the integrated use of *pseudomonas syringae* pv. targetis with low doses of herbicides (Bailey *et al.*, 2000). Integrated approaches to control weeds for sustainable crop production are gaining more attention nowadays. Therefore, the use of AB with chemical herbicides should be encouraged to get optimum yield (Culliney, 2005). To our knowledge, this is the first-time cyanide-producing bacteria have been integrated with a reduced dose of chemical herbicide to suppress wild oat in wheat. Keeping in view the above facts, a study was conducted to evaluate the effectiveness of allelopathic rhizobacteria as bioherbicides for the suppression of wild oat with reduced doses of chemical herbicides such as Axial (for narrow leaves) and Atlantis (for narrow and broad leaves) and to assess their potential for increasing wheat growth.

Materials and Methods

Minimum inhibition concentration test

A total of 4 pre-isolated and pre-characterized cyanide-producing allelopathic *Pseudomonas* strains B11 (*Pseudomonas fluorescence* MK203827), T19 (*Pseudomonas fulva* MK203816), T24 (*Pseudomonas thivervalensis* MK203821), and T75 (*Pseudomonas fulva* MK203826) were taken from the culture collection of the Soil Microbiology and Biochemistry Laboratory (SMBL), Institute of Soil and Environmental Sciences (ISES), University of Agriculture, Faisalabad (Dar *et al.*, 2020; Dar *et al.*, 2023). AB were assessed for their abilities to endure and grow at various concentrations of chemical herbicides. Therefore, King's B agar media was prepared, autoclaved, and placed to cool down at 37□. Then filter sterilized chemical herbicides (Axial and Atlantis) were added to the media at a rate of 25%, 50%, 75%, and 100% of the recommended dose, and after pouring this media into Petri plates, the bacterial strains were streaked on them and incubated for 48 hours at 28°C.

Seed coating with allelopathic bacteria

Liquid culture of King's B medium was prepared in 4 Erlenmeyer flasks of 250 mL for the inoculation of 4 bacterial strains separately. These flasks containing broth media were autoclaved and inoculated with AB strains after cooling. Inoculated broth media was incubated at 100 rpm and 28°C for 48 hours. Bacterial cells were harvested in pallet form after 48 hours through centrifugation at 9000 *g* and 4°C. These pallets were resuspended in a solution of saline buffer to get an identical bacterial population of 10^7-10^8 cfu mL^{-1} by optical density measurements at 600 nm (Zahir *et al.*, 2018). Moreover, the compatible bacterial combinations C1 (combination of B11×T75 bacterial strains), C2 (combination of T19×T24 bacterial strains), C3 (combination of B11×T24×T75 bacterial strains), and C4 (combination of B11×T19×T24×T75 bacterial strains) were formed by taking an equal volume of inoculated broth cultures. The surfaces of the wild oat and wheat seeds were disinfected by the procedure described by Abd-Alla *et al.* (2012). The disinfected seed was used for coating with AB. For this purpose, a slurry was made with bacterial broth culture, sterilized press mud, and 10% sugar solution with a ratio of 4:5:1, respectively. While sterilized broth medium was used for uninoculated control treatments (Zahir *et al.*, 2018).

Pot experiment under axenic conditions

Plastic pots (15 cm in height and 8 cm in diameter) were filled with 400 g of 2 mm sieved sand and autoclaved 3 times at 121°C and 15 psi for 20 minutes. Afterward, the trial was carried out in the growth room of SMBL, University of Agriculture Faisalabad, to evaluate the allelopathic bacterial potential along with reduced doses of chemical herbicides for growth suppression of wild oat. Therefore, 4 different allelopathic bacterial combinations C1, C2, C3, and C4 were applied with 25% and 50% recommended doses of 2 chemical herbicides: Axial (50g L^{-1} Pinoxaden at the rate of 815 mL ha^{-1}) (Syngenta, Karachi, Pakistan) and Atlantis (Mesosulfuron-methyl 3% + Iodosulfuron-methyl sodium at the rate of 247 g ha^{-1}) (Bayer, Karachi, Pakistan). To check their capability to inhibit wild oat, bacterial combinations were applied through seed coating with press mud, and herbicides were applied through foliar application at the 2-leaf stage of wild oat. The experiment was arranged in a completely randomized design under factorial settings with 3 replications. Side by side, these 4 combinations were also applied to wheat to assess their effects on its growth. The experiment was conducted in a completely randomized design with 3 replications. Fluorescent lamps were used to maintain the light intensity of 275 µmol m^{-2} s^{-1} in the air-conditioned growth room. Dark (14 hours) and light (10 hours) cycles were maintained at 16 and 21°C, respectively. Hoagland nutrient solution of half strength was added in sand pots to fulfill the requirement of water and nutrients. Data regarding seed germination percentage, fresh biomass, chlorophyll a, chlorophyll b, carotenoid contents, and protein contents were collected after 30 days for both wheat and wild oat.

Selection of chemical herbicide and allelopathic bacterial combinations

Keeping in view the results of the growth room experiment, a combination of bacterial strains (C4) and chemical herbicide (Axial) was selected based on the growth reduction of wild oat and the growth promotion of wheat to conduct the field experiment.

Field trial

After the pot trial, a field experiment was conducted to confirm the efficacy of the integrated application of allelopathic bacterial combination C4 with a reduced dose of chemical herbicide (Axial). The field experiment was conducted in the field of ISES at the University of Agriculture Faisalabad, Pakistan, under a randomized complete block design (RCBD) with 3 replications. 2 control treatments were used: one as a negative control (weed-free control achieved by manual weeding) and the other as a positive control (weedy control). Wild oat (150 seeds m^{-2}) and wheat (Galaxy-2013 variety at a rate of 100 kg ha^{-1}) were sown through a hand drill machine with an area of 2.0 m^2 for each plot. The suggested dose of chemical fertilizers (N: P: K; 120:90:60 kg ha^{-1}) and 100 mm D of good-quality canal water per hectare were applied. Moreover, phosphorous and potassium were applied at sowing time, while nitrogen was applied in 3 splits at seedlings, tillering, and stem extension. The data regarding the growth and physiological parameters of both weed and wheat were recorded at physiological maturity, while parameters regarding yield were collected after harvesting. The soil plant analysis development (SPAD) value was calculated using the SPAD meter (SPAD-502 Plus, Konica Minolta, Tokyo, Japan), while weed density was calculated by counting the plants of wild oat per m^2 and the relative density of wild oat was measured by the following formula (1):

$$\text{Relative wild oat density (\%)} = \frac{\text{Density of wild oat}}{\text{Total density of weeds}} \times 100 \qquad (1)$$

Photosynthetic rate, transpiration rate, and stomatal conductance were measured by the Portable Photosynthesis System CIRAS-3 (PP Systems, Amesbury, MA, USA) between 10:00 a.m. and 13:00 p.m. at photons with a flux density of 1200-1400 µmol m^{-2} s^{-1}. 1000 grain weight, biological yield, straw yield, and grain yield were measured through an electric balance.

Data analysis

The data were analyzed using a completely randomized design (CRD) with the factorial arrangement and a CRD with 3 replications for wild oat and wheat crops under axenic conditions, respectively. While the field trial data were statistically analyzed using RCBD (Steel *et al.*, 1997). Treatment means were compared using the honestly significant difference test at the 5% significance level (Montgomery, 2017).

Results

Pot experiment

All the allelopathic bacterial strains were evaluated for the minimal inhibitory concentration test towards chemical herbicides (Axial and Atlantis) before their application in the pot experiment. It has been found that all the allelopathic bacterial strains showed their ability to grow on all levels (25%, 50%, 75%, and 100%) of chemical herbicides.

Deleterious effects on seed germination of wild oat

The application of AB significantly reduced the germination percentage of wild oat (Figure 1). The statistics regarding the germination reduction of wild oat revealed that the application of the

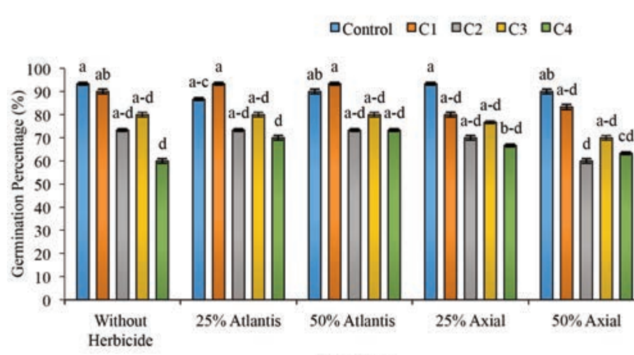

Figure 1. Influence of allelopathic bacterial combinations integrated with chemical herbicides on germination percentage in wild oat. Statistically, Tukey's honestly significant difference test showed no significant difference where the means of 3 replications sharing the same letters were at p≤0.05. Control, without bacterial strain; C1, combination of B11 and T75 bacterial strains; C2, combination of T19 and T24 bacterial strains; C3, combination of B11, T24 and T75 bacterial strains; C4, combination of B11, T19, T24 and T75 bacterial strains.

C4 combination without chemical herbicide significantly reduced the germination percentage of wild oat by up to 35.7% as compared to the control. However, a 29.6% reduction in germination was observed under the C4 combination when applied with 50% Axial as compared with the uninoculated treatment. Whereas the C2 combination with 50% Axial reduced the germination percentage by 33.3%, it was statistically at par with the decrease under the C4+50% Axial combination.

Detrimental effects on height and fresh biomass of wild oat

Plant height and fresh biomass of wild oats were significantly reduced under the application of AB (Figure 2). The data related to plant height indicated that a significant decline (30.5%) in plant height was found under the C4 combination when a 50% dose of Axial was applied, followed by 26.6%, 11.8%, 10.5%, and 8.7% decreases under 25% Axial, 25% Atlantis, without herbicide, and 50% Atlantis treatments, respectively, as compared to their uninoculated controls (Figure 2A). Similarly, a maximum reduction of 49.7% in fresh biomass was found under C4+50% Axial treatment, followed by a 44.8% decrease in biomass under C4+25% Axial as compared to their respective controls (Figure 2B).

Adverse effects on soil plant analysis development value, chlorophyll a, and chlorophyll b of wild oat

Data indicated that different bacterial combinations, when integrated with reduced doses of chemical herbicides, reduced the SPAD value, chlorophyll a and chlorophyll b in wild oat (Figure 3).

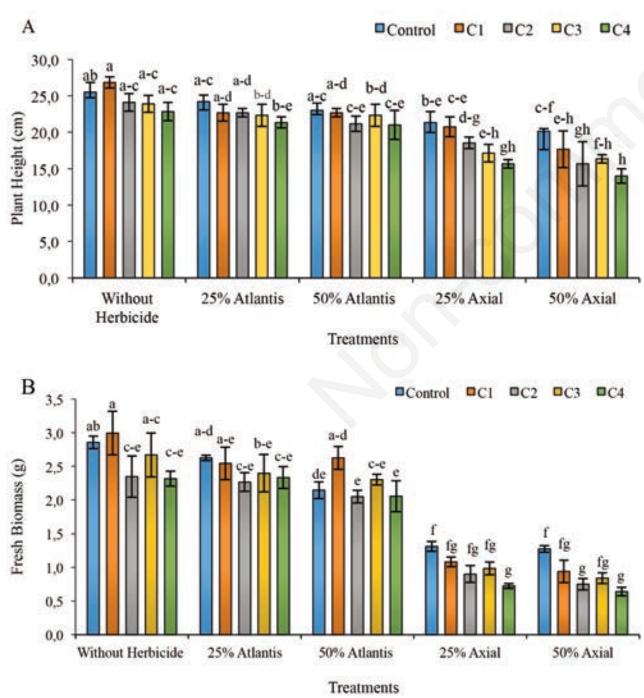

Figure 2. Influence of allelopathic bacterial combinations integrated with chemical herbicides on plant height (**A**) and fresh biomass (**B**) reduction in wild oat. Statistically, Tukey's honestly significant difference test showed no significant difference where the means of 3 replications sharing the same letters were at p≤0.05. Control, without bacterial strain; C1, combination of B11 and T75 bacterial strains; C2, combination of T19 and T24 bacterial strains; C3, combination of B11, T24 and T75 bacterial strains; C4, combination of B11, T19, T24 and T75 bacterial strains.

The combination with all bacterial strains (C4) showed a significant decrease in SPAD value (49.2%) under a 50% dose of Axial, followed by 34.4%, 13.1%, and 1.0% reductions in cases of 25% Axial, no herbicide, and 50% Atlantis, respectively, when compared with their respective controls (Figure 3A). The reduction in chlorophyll a was maximum under the C4 combination of AB, which reduced the chlorophyll a content by 54.1% and 47.9% under 25 and 50% doses of Axial, respectively, when compared with their respective controls (Figure 3B). The data regarding chlorophyll b contents showed that in the case of the C4 bacterial combination, the maximum decrease in chlorophyll b (50.7%) was found under 25% Axial, followed by a 36.4% reduction under 50% Axial when compared with their relative control treatments (Figure 3C).

Harmful effects on carotenoid and protein contents of wild oat

Similarly, the inoculation of allelopathic bacterial combinations with a reduced dose of herbicide resulted in a reduction of

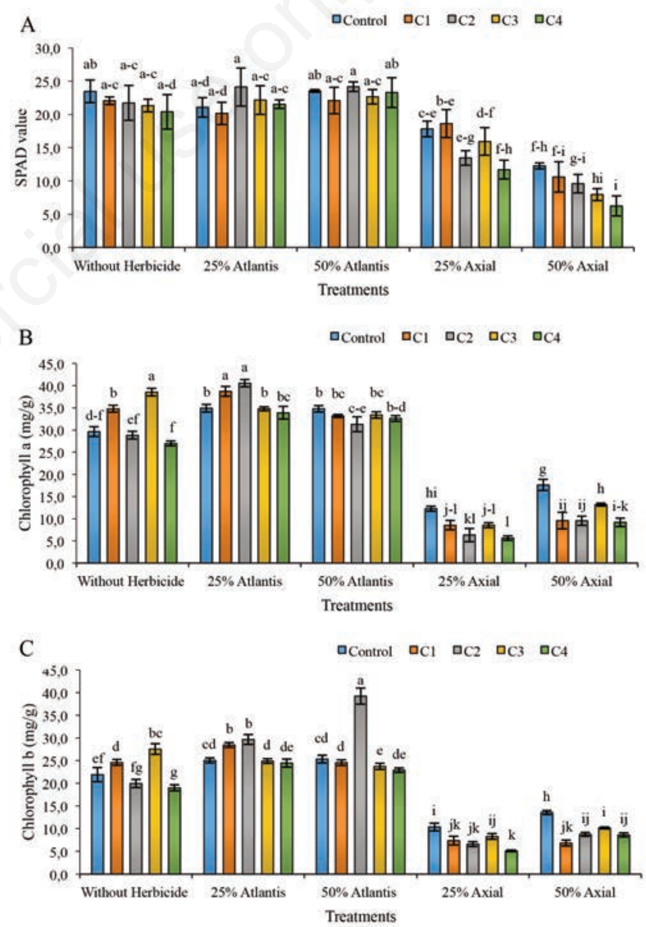

Figure 3. Influence of allelopathic bacterial combinations integrated with chemical herbicides on soil plant analysis development value (**A**), chlorophyll a (**B**) and chlorophyll b (**C**) reduction in wild oat. Statistically, Tukey's honestly significant difference test showed no significant difference was found where the means of 3 replications sharing the same letters were at p≤0.05. Control, without bacterial strain; C1, combination of B11 and T75 bacterial strains; C2, combination of T19 and T24 bacterial strains; C3, combination of B11, T24 and T75 bacterial strains; C4, combination of B11, T19, T24 and T75 bacterial strains.

carotenoid and protein contents (Figure 4). However, the C4 combination caused a significant reduction in carotenoid contents by 36.7% under 25% Axial application when compared with the control (Figure 4A). The data depicted that the maximum reduction in protein contents (65.3%) of wild oat was found under C4 inoculation with a 25% Axial treatment as compared to the control treatment (Figure 4B).

Influence on seed germination, plant height, root length, and fresh biomass of wheat

The results revealed that the allelopathic bacterial combinations enhanced the germination percentage, plant height, root length, and fresh biomass of wheat (Table 1). The highest increase (27.8%) in seed germination percentage was observed when wheat seeds were inoculated with C4 and C1 bacterial combinations, followed by 16.7% and 11.1% increases in seed germination under C3 and C2 combinations, as compared to uninoculated control treatments. The statistics revealed that the highest increase of 23% in plant height was found under the inoculation of the C4 combination as compared to the control treatment, followed by a 14.4% rise in plant height in the case of both C1 and C3 combinations. However, a 0.2% reduction in height was noted in the case of the C2 combination when compared to their control. The data illustrated that root length considerably increased by 35.2% under the C4 allelopathic bacterial combination, while the increase in root length was found to be 16.9% and 9.9% more under the C1 and C3 inoculations, respectively, as compared with their uninoculated control. Whereas, a 1.4% reduction in root length was found under C2 inoculation. The results showed that there was a significant increase of 26.7% in fresh biomass of wheat when inoculated with the C4 combination, followed by a 21.4% and 13% increase in biomass under the C1 and C3 combinations, respectively, and a 2.6% reduction in biomass was noted in terms of C2 bacterial combination inoculation over uninoculated control.

Beneficial impacts on soil plant analysis development value, chlorophyll a, and chlorophyll b of wheat

The findings showed that the allelopathic bacterial combinations improved the SPAD value and chlorophyll (a and b) contents of wheat (Table 2). The data regarding the SPAD value showed that a maximum increase of 27.8% was observed in the case of the C4 combination, followed by a 15.5% increase in the SPAD value under the C3 treatment, while a 2.1% and 0.5% reduction in the SPAD value were noted in the case of the C1 and C2 combination as compared with the control uninoculated treatment. However, the results showed a 29.1% and 20% increase in the contents of chlorophyll a under C1 and C4 inoculations, respectively, when compared with the control treatment. Whereas, the C4 bacterial combination caused the highest increase in chlorophyll b contents (31.1%) and carotenoid contents (52.4%) in wheat as compared to their respective controls.

Positive impacts on carotenoid and protein contents of wheat

The results depicted that the allelopathic bacterial combinations enhanced the carotenoid and protein contents of wheat (Table 2). The data regarding carotenoids showed that there was a noteworthy increase in carotenoid contents. Application of C4, C1, and C3 combinations significantly increased the carotenoid contents by 52.4%, 47%, and 42%, respectively, when compared with the uninoculated control. The C2 combination enhanced the carotenoid contents by 20.5% over the control treatment.

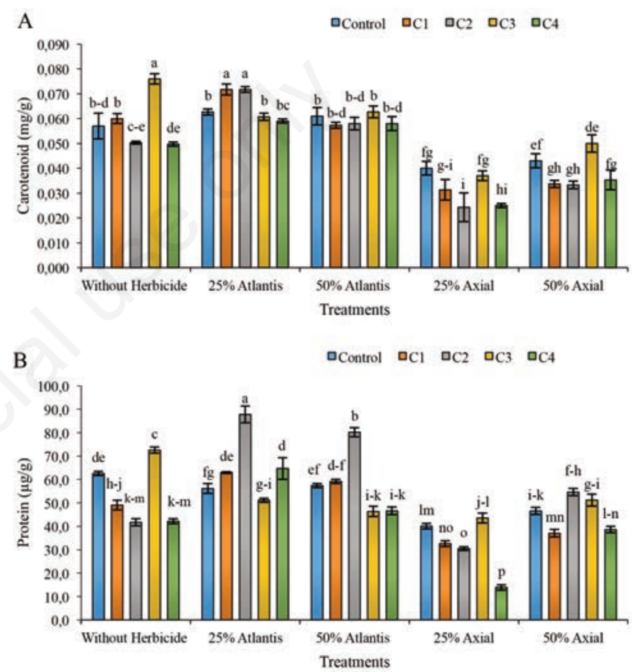

Figure 4. Influence of allelopathic bacterial combinations integrated with chemical herbicides on carotenoid (**A**) and protein contents (**B**) reduction in wild oat. Statistically, Tukey's honestly significant difference test showed no significant difference was found where the means of 3 replications sharing the same letters were at p≤0.05. Control, without bacterial strain; C1, combination of B11 and T75 bacterial strains; C2, combination of T19 and T24 bacterial strains; C3, combination of B11, T24 and T75 bacterial strains; C4, combination of B11, T19, T24 and T75 bacterial strains.

Table 1. Influence of allelopathic bacterial combinations on wheat germination, plant height, root length, and fresh biomass in the pot experiment.

Treatments	Seed germination, %	Plant height, cm	Root length, cm	Plant fresh biomass, g
Control	61.11±9.62^{b}	27.23±0.86^{c}	11.83±0.76^{c}	1.26±0.08^{c}
C1	88.89±9.62^{a}	31.16±1.04^{b}	13.83±0.76^{b}	1.53±0.05^{ab}
C2	72.22±9.62^{ab}	27.16±0.76^{c}	11.66±0.76^{c}	1.23±0.05^{c}
C3	77.78±9.62^{ab}	31.16±1.04^{b}	13.00±0.50^{bc}	1.42±0.06^{b}
C4	88.89±9.62^{a}	33.50±0.50^{a}	16.00±0.50^{a}	1.60±0.06^{a}
HSD (p≤0.05)	25.84	2.325	1.802	0.151

C1, combination of B11 and T75 bacterial strains; C2, combination of T19 and T24 bacterial strains; C3, combination of B11, T24 and T75 bacterial strains; C4, combination of B11, T19, T24 and T75 bacterial strains; HSD, honestly significant difference. [a,b,c]Statistically, Tukey's honestly significant difference test showed no significant difference where the means of 3 replications sharing the same letters were at p≤0.05.

The results showed that there was a significant increase of 23.1% and 16.4% in protein when C3 and C4 combinations were used, respectively. However, a par rise in protein contents by 5% was noted under the C1 combination, and there was a 5.7% decrease in protein under the C2 combination over the uninoculated control.

Field trial

Considering the results obtained from the pot trial, a C4 allelopathic bacterial combination and chemical herbicide Axial were selected, and a field trial was carried out. The following parameters regarding wild oat and wheat were collected to assess the effectiveness of the integrated use of AB with a reduced dose of chemical herbicide. However, when applied with the C4 bacterial combination, the highest decrease in relative wild oat density (70.9%), photosynthetic rate (26.6%), and transpiration rate (25.6%) were observed under 50% Axial. The maximum decline in shoot length (27.1%) and biomass (83.5%) were obtained under a 100% dose of Axial with C4 combination as compared with the weedy control.

Effect on growth and yield attributes of wild oat

The results showed that a significant reduction in growth and yield parameters of wild oat was observed under the integrated application of Axial and C4 allelopathic bacterial combinations (Table 3). The maximum reduction in weed density (82.1%), SPAD value (26.0%), and grain yield (88.2%) were noted under 75% Axial with C4 combination as compared to weedy control.

However, a significant reduction in weed density (34.3%) was observed under the 25% Axial treatment when applied with the C4 bacterial combination as compared with the 25% Axial control (Table 3). Moreover, 33.4% and 32.7% decreases in weed density were observed under 75% and 50% doses of Axial, respectively, when inoculated with C4 as compared with their respective uninoculated controls. The drop in relative wild oat density was observed by 46.1% under 25% Axial treatment when applied with the C4 bacterial combination over 25% Axial control, followed by a 25.0%, 14.3%, and 12.4% decrease in relative wild oat density under 50%, 75%, and 100% Axial doses, respectively, when inoculated with the C4 bacterial combination (Table 3).

The highest fall in SPAD value (19.3%) was observed under 50% Axial treatment when applied with the C4 combination as compared with 50% Axial control (Table 3). Whereas 18.0%, 16.2%, and 7.8% decreases in SPAD value were observed under 75%, 25%, and 100% doses of Axial, respectively, when inoculated with the C4 combination compared with their respective uninoculated controls. Hence, a major decrease in shoot length of 9.4% was observed under 75% Axial treatment when applied with the C4 bacterial combination over 75% Axial control, followed by 6.9%, 5.5%, and 2.6% decreases in shoot length under 100%, 50%, and 25% doses of Axial, respectively (Table 3). The reduction in biomass (53.7%) was observed under the 75% Axial treatment when applied with the C4 combination as compared with the 75% Axial control (Table 3). Whereas 48.5%, 24.5%, and 20.9% drops in biomass were observed under 100%, 25%, and 50% doses of

Table 2. Influence of allelopathic bacterial combinations on wheat soil plant analysis development value, chlorophyll a, chlorophyll b, carotenoid and protein contents in the pot experiment.

Treatments	SPAD value	Chlorophyll a, mgg^{-1}	Chlorophyll b, mgg^{-1}	Carotenoid, mgg^{-1}	Protein, μgg^{-1}
Control	28.30±0.79^{bc}	15.55±0.42^{bc}	11.79±0.55^{cd}	0.0121±0.0009^{c}	67.31±1.26^{bc}
C1	27.70±1.57^{c}	20.00±0.72^{a}	10.31±0.49^{d}	0.0178±0.0004^{a}	70.70±3.61^{b}
C2	28.16±2.51^{c}	14.64±0.46^{c}	13.40±0.47^{bc}	0.0146±0.0015^{bc}	63.47±2.39^{c}
C3	32.70±1.39^{ab}	16.32±0.53^{b}	13.94±0.85^{ab}	0.0172±0.0008^{ab}	82.87±1.47^{a}
C4	36.16±1.60^{a}	18.68±0.42^{a}	15.47±0.59^{a}	0.0183±0.0012^{a}	78.34±1.43^{a}
HSD ($p\leq0.05$)	4.480	1.400	1.629	0.00294	5.957

SPAD, soil plant analysis development; C1, combination of B11 and T75 bacterial strains; C2, combination of T19 and T24 bacterial strains; C3, combination of B11, T24 and T75 bacterial strains; C4, combination of B11, T19, T24 and T75 bacterial strains; HSD, honestly significant difference. a,b,cStatistically, Tukey's honestly significant difference test showed no significant difference where the means of 3 replications sharing the same letters were at p≤0.05.

Table 3. Influence of the combination of B11, T19, T24 and T75 bacterial strains integrated with chemical herbicides (Axial) on wild oat under field conditions.

Treatments	Weeds density, m^{-2}	Relative density of wild oat, %	SPAD value	Shoot length, cm	Straw yield, t ha^{-1}	Grain yield, t ha^{-1}
Weed free control	-	-	-	-	-	-
Weedy control	119.3±12.4^{a}	81.8±4.8^{a}	48.5±2.1^{a}	126.7±4.9^{a}	1.89±0.12^{a}	0.68±0.08^{a}
Wheat+weed 100% Axial control	30.0±2.6^{b-d}	29.1±4.2^{cd}	41.2±3.2^{e}	99.3±7.7^{de}	0.61±0.06^{c}	0.18±0.03^{c}
Wheat+weed 100% Axial+C4	22.3±2.3^{d-e}	25.5±2.7^{d}	38.0±3.0^{g}	92.4±7.2^{f}	0.31±0.03^{f}	0.09±0.02^{d}
Wheat+weed 75% Axial control	32.0±3.6^{bc}	32.9±5.7^{c}	43.8±3.4^{d}	104.2±8.1^{cd}	0.74±0.13^{b}	0.20±0.03^{bc}
Wheat+weed 75% Axial+C4	21.3±0.6^{e}	28.2±0.8^{cd}	35.9±2.7^{h}	94.4±7.3^{ef}	0.34±0.03^{e-f}	0.08±0.02^{d}
Wheat+weed 50% Axial control	32.7±3.2^{b}	32.4±4.8^{c}	45.0±3.5^{c}	107.2±8.3^{bc}	0.53±0.08^{cd}	0.19±0.03^{bc}
Wheat+weed 50% Axial+C4	22.0±1.0^{e}	24.3±2.8^{d}	36.3±2.8^{h}	101.3±7.9^{d}	0.42±0.05^{d-f}	0.11±0.02^{d}
Wheat+weed 25% Axial control	37.0±0.0^{b}	50.5±1.6^{b}	47.0±3.7^{b}	112.1±8.7^{b}	0.60±0.07^{c}	0.24±0.02^{b}
Wheat+weed 25% Axial+C4	24.3±2.1^{c-e}	27.2±4.1^{cd}	39.4±3.1^{f}	109.2±8.5^{bc}	0.45±0.01^{de}	0.14±0.01^{cd}
HSD ($p<0.05$)	7.8688	6.8893	0.8283	5.6855	0.1322	0.0601

SPAD, soil plant analysis development; C4, combination of B11, T19, T24 and T75 bacterial strains; HSD, honestly significant difference. a,b,cStatistically, Tukey's honestly significant difference test showed no significant difference where the means of 3 replications sharing the same letters were at p≤0.05.

Axial, respectively, when inoculated with the C4 bacterial combination compared with its controls. However, a significant decline in grain yield of 60.0% was observed under 75% Axial treatment when applied with the C4 bacterial combination over 75% Axial control, followed by 50.0%, 42.1%, and 41.7% decreases in grain yield under 100%, 50%, and 25% doses of Axial, respectively, when inoculated with the C4 bacterial combination (Table 3).

Effect on physiological attributes of wild oat

The results illustrated that the AB decreased the physiological attributes of wild oat (Table 4). A significant reduction in photosynthetic rate (21.9%) was observed under 75% Axial treatment when applied with the C4 bacterial combination as compared with 75% Axial control (Table 4). Whereas 20.0%, 12.8%, and 6.2% decreases in photosynthetic rate were observed under 50%, 25%, and 100% doses of Axial, respectively, when inoculated with the C4 bacterial combination as compared with controls. A significant reduction in transpiration rate (17.5%) was observed under 75% Axial treatment when applied with the C4 combination as compared with 75% Axial control, followed by a 17.4%, 8.8%, and 3.4% decrease in transpiration rate under 50%, 100%, and 25% doses of Axial, respectively, when inoculated with the C4 bacterial combination (Table 4). The considerable decline in stomatal conductance by 11.8% was measured under 75% Axial treatment when applied with the C4 bacterial combination as compared with the control (75% Axial control) (Table 4). Whereas a 10.9%, 5.8%, and 3.8% decrease in stomatal conductance was observed under 50%, 100%, and 25% doses of Axial, respectively, when inoculated with the C4 bacterial combination compared with their uninoculated controls.

Effect on growth and yield attributes of wheat

The statistics (Tables 4 and 5) revealed that wild oat infestation significantly reduced wheat SPAD value, number of tillers per plant, transpiration rate, and stomatal conductance by 27.4%, 29.2%, 12.4%, and 25.4%, respectively, under 100% Axial appli-

Table 4. Influence of the combination of B11, T19, T24 and T75 bacterial strains integrated with chemical herbicides (Axial) on photosynthetic attributes of wild oat and infested wheat under field conditions.

Treatments	Wild oat Photosynthetic rate, $\mu mol\ m^{-2}\ s^{-1}$	Transpiration rate, $mmol\ m^{-2}\ s^{-1}$	Stomatal conductance, $mmol\ mm^{-2}\ s^{-1}$	Infested wheat Photosynthetic rate, $\mu mol\ mm^{-2}\ s^{-1}$	Transpiration rate, $mmol\ m^{-2}\ s^{-1}$	Stomatal conductance, $mmol\ m^{-2}\ s^{-1}$
Weed free control	-	-	-	23.1±0.9^{a}	3.72±0.05^{a}	438±1^{a}
Weedy control	20.7±1.6^{a}	3.51±0.27^{a}	313±1^{a}	-	-	-
Wheat+weed 100% Axial control	17.7±1.6^{bc}	3.08±0.11^{b}	274±14^{bc}	22.2±3.1^{a-c}	3.26±0.12^{g}	326±18^{e}
Wheat+weed 100% Axial+C4	16.6±1.2^{d}	2.81±0.19^{c}	258±12^{cd}	23.3±1.1^{a}	3.44±0.05^{de}	358±29^{cd}
Wheat+weed 75% Axial control	19.6±0.8^{a}	3.32±0.14^{ab}	262±18^{cd}	20.7±0.1^{b-d}	3.33±0.07^{fg}	338±16de
Wheat+weed 75% Axial+C4	15.3±0.2^{d}	2.74±0.17^{c}	231±10^{e}	22.7±0.2^{ab}	3.65±0.05^{ab}	388±12^{b}
Wheat+weed 50% Axial control	19.0±0.4^{ab}	3.16±0.08^{b}	275±24^{bc}	19.9±0.2^{d}	3.38±0.03^{ef}	352±4^{c-e}
Wheat+weed 50% Axial+C4	15.2±0.2^{d}	2.61±0.05^{c}	245±13de	21.8±0.8^{a-d}	3.56±0.06^{bc}	379±10^{bc}
Wheat+weed 25% Axial control	19.5±1.8^{ab}	3.24±0.26^{b}	292±17^{ab}	17.0±0.6^{e}	3.36±0.05^{e-g}	331±17de
Wheat+weed 25% Axial+C4	17.0±0.7^{cd}	3.13±0.07^{b}	281±16^{bc}	20.1±0.9^{cd}	3.53±0.07^{cd}	356±21^{cd}
HSD ($p<0.05$)	1.8804	0.2630	26.819	2.2524	0.1064	29.167

C4, combination of B11, T19, T24 and T75 bacterial strains; HSD, honestly significant difference. [a,b,c]Statistically, Tukey's honestly significant difference test showed no significant difference where the means of 3 replications sharing the same letters were at $p\leq0.05$.

Table 5. Influence of the combination of B11, T19, T24 and T75 bacterial strains integrated with chemical herbicides (Axial) on infested wheat under field conditions.

Treatments	SPAD value	Shoot length, cm	No. of tillers per plant t ha^{-1}	Biological yield, t ha^{-1} t ha^{-1}	Straw yield, t ha^{-1}	Grain yield, t ha-$^{-1}$	1000 grain weight, g
Weed free control	49.7±0.4^{a}	122.3±1.5^{a}	11.3±1.5^{a}	10.23±0.29^{a}	6.17±0.24^{a}	4.07±0.04^{a}	40.20±0.95^{ab}
Weedy control	-	-	-	-	-	-	-
Wheat+weed 100% Axial control	36.1±2.2^{e}	111.3±4.9^{bc}	8.0±1.0^{d}	7.61±0.14^{d}	4.99±0.10^{de}	2.61±0.07^{e}	36.50±1.61^{c}
Wheat+weed 100% Axial+C4	40.2±0.9^{c-e}	116.3±0.6^{ab}	10.3±0.6^{a-c}	8.75±0.24^{c}	5.37±0.28^{bc}	3.39±0.07^{c}	41.07±2.76^{a}
Wheat+weed 75% Axial control	37.6±0.7^{de}	109.0±3.0^{bc}	8.7±1.2^{cd}	7.15±0.23^{e}	4.49±0.18^{f}	2.65±0.08^{e}	36.40±1.47^{c}
Wheat+weed 75% Axial+C4	47.7±0.7^{ab}	120.0±3.6^{a}	11.0±1.0^{ab}	9.49±0.23^{b}	5.59±0.18^{b}	3.89±0.07^{b}	41.27±0.47^{a}
Wheat+weed 50% Axial control	41.5±4.7^{cd}	106.7±5.7^{c}	9.0±1.0^{cd}	7.73±0.12^{d}	4.84±0.09^{e}	2.90±0.06^{d}	37.00±2.36^{bc}
Wheat+weed 50% Axial+C4	47.1±6.0^{ab}	114.7±2.5^{a-c}	12.0±1.7^{a}	9.14±0.14^{b}	5.29±0.09^{b-d}	3.84±0.07^{b}	39.17±2.07^{a-c}
Wheat+weed 25% Axial control	44.1±0.7^{bc}	111.0±7.5^{bc}	9.3±0.6^{b-d}	7.24±0.24^{e}	4.69±0.23^{ef}	2.54±0.05^{e}	35.97±4.12^{c}
Wheat+weed 25% Axial+C4	48.7±2.9^{ab}	115.0±6.1^{ab}	10.3±0.6^{a-c}	8.68±0.28^{c}	5.23±0.24^{cd}	3.44±0.07^{c}	37.93±1.89^{a-c}
HSD ($p<0.05$)	4.6248	8.2070	1.9882	0.3688	0.3327	0.0601	3.5019

SPAD, soil plant analysis development; C4, combination of B11, T19, T24 and T75 bacterial strains; HSD, honestly significant difference. [a,b,c]Statistically, Tukey's honestly significant difference test showed no significant difference where the means of 3 replications sharing the same letters were at $p\leq0.05$.

cation. The major declines in shoot length, biological yield, and straw yield by 10.9%, 30.1%, and 27.4% were observed in infested wheat under a 75% dose of Axial as compared with weed-free control treatment. The highest declines in grain yield, 1000 grain weight, and photosynthetic rate of infested wheat were observed by 37.6%, 10.4%, and 26.4%, respectively, under a 25% dose of chemical herbicide over weed-free control treatment.

The highest rise in SPAD value up to 26.9% was observed under 75% Axial with C4 combination than their uninoculated treatment (75% Axial control), followed by 13.5%, 11.4%, and 10.4% increase under 50%, 100%, and 25% Axial dose as compared to their respective uninoculated control (Table 5). The application of the C4 bacterial combination enhanced shoot length (10.1%) under 75% Axial as compared with their uninoculated control, followed by 7.5%, 4.5%, and 3.6% increases under 50%, 100%, and 25% Axial, respectively, as compared to their respective control treatments (Table 5).

The inoculation of the C4 bacterial combination improved tillering (33.3%) under 50% Axial as compared with their uninoculated control treatment, followed by 28.8%, 26.4%, and 10.8% increases under 100%, 75%, and 25% Axial, respectively, as compared to their respective control treatments. The rise in biological and straw yield was observed by 32.7% and 24.4% under 75% Axial application as compared with their respective uninoculated controls. However, the highest increase in grain yield, up to 46.8%, was observed under 75% Axial treatment when applied with the C4 bacterial combination over 75% Axial control. Whereas, a 35.4% rise in grain yield was measured under a 25% dose of Axial when inoculated with the C4 bacterial combination. The maximum increase in 1000 grain weight by 13.5% was observed under 75% application of Axial when inoculated with the C4 combination as compared to the uninoculated control, followed by a 12.6% increase at 100% Axial application (Table 5).

Effect on physiological attributes of wheat

The outcomes illustrated that the AB boosted the physiological attributes of wheat (Table 4). The highest rise in photosynthetic process was up to 18.2% under a 25% Axial treatment when applied with the C4 bacterial combination, as compared with 25% Axial control. The maximum elevation in transpiration rate and stomatal conductance of 9.6% and 14.7% were noted under 75% Axial when applied with the C4 bacterial combination over their own uninoculated control treatment. The results showed that there was a non-significant difference in the rise of growth and yield attributes of wheat under 50 and 75% Axial treatment under the C4 combination.

Correlation between growth and yield attribute of wheat and wild oat

Pearson's analysis illustrated that the shoot length, SPAD value, straw yield, grain yield, and photosynthetic rate of wheat were positively correlated with each other and negatively correlated with wild oat growth parameters (Table 6). The wheat shoot length is positively correlated with the SPAD value (R=0.9670), straw yield (R=0.9852), grain yield (R=0.9235), and photosynthetic rate (R=0.9761) of wheat while negatively correlated with relative wild oat density (R=-0.5884) and wild oat biomass (R=-0.9525).

Discussion

Wheat is a staple diet all over the world, but the potential yield is limited due to pest attacks (especially weeds). Wild oat is considered one of the most noxious weeds, which reduces the yield of main crops by 70% and results in huge economic losses (Beckie *et al.*, 2012). Different weed control strategies, such as manual, mechanical, chemical, and biological, are being used, but each has its own limitations. Hence, the current study evaluates the potential of the AB under axenic conditions in integration with chemical herbicides (Axial and Atlantis) at a lower dose for growth inhibition of wild oat to cut down on the excessive use of chemical herbicides.

The best-performing bacterial combination of B11, T19, T24, and T75 strains (C4) and herbicide (Axial) at different levels was further tested under field conditions. The significant reduction in germination percentage, plant height, fresh biomass, SPAD value, and chlorophyll contents of wild oat was observed under axenic conditions by the integration of biological (C4 combination) and chemical (Axial) control strategies. While a significant reduction in wild oat density, biomass, grain yield, and photosynthetic traits was observed in field conditions under a reduced dose of Axial when applied with the C4 bacterial combination as compared with uninoculated control treatments, a similar reduction in seed germination, root length, and shoot length of wild oat was also recorded by Abbas *et al.* (2020) and Dar *et al.* (2020). Mustafa *et al.* (2019) reported that *Pseudomonas* sp. has the potential to suppress weed growth and biomass, which supports our findings. Similar results were found by Abbas *et al.* (2017a) for the drop in weed density

Table 6. Correlation coefficients among different parameters of wheat and wild oat under the combined application of the combination of B11, T19, T24 and T75 bacterial strains and chemical herbicides (Axial) under field conditions.

	Wheat shoot length, cm	Wheat SPAD	Wheat straw yield, t ha^{-1}	Wheat grain yield, t ha-$^{-1}$	Wheat photosynthetic rate, µmol m^{-2} s^{-1}	Relative wild oat density, %	Wild oat SPAD	Wild oat biomass, t ha^{-1}
Wheat SPAD	0.9670							
Wheat straw yield	0.9852	0.9677						
Wheat grain yield	0.9235	0.9522	0.9619					
Wheat photosynthetic rate	0.9761	0.9139	0.9757	0.9255				
Relative wild oat density	-0.8584	-0.8459	-0.9187	-0.9216	-0.9036			
Wild oat SPAD	-0.3732	-0.4320	-0.5058	-0.5669	-0.4106	0.7322		
Wild oat biomass	-0.9525	-0.9536	-0.9881	-0.9675	-0.9441	0.9351	0.5957	
Wild oat photosynthetic rate	-0.3697	-0.4379	-0.5044	-0.5669	-0.3995	0.7201	0.9977	0.5970

SPAD, soil plant analysis development.

and growth of broad-leaved dock when 4 bacterial strains (T42, W9, $7O_0$ and L9) were applied. In accordance with our results, Omer and Balah (2011) also reported a reduction in shoot and root lengths, seedling biomass, and germination of weeds through the application of AB and fungi. Suppression in germination percentage, root length, and growth of weeds due to allelopathic rhizobacteria was reported by many scientists (Li and Kremer, 2006; Ali *et al.*, 2017). The fall in germination percentage of wild oat might be due to competitive root colonization, and their ability to release secondary metabolites like phytotoxins, antibiotics, and flavonoids in the rhizosphere soil is very likely to play a vital role in weed control (Kremer *et al.*, 1990; Mitchell, 1991; Ali *et al.*, 2017).

All these works depicted that cyanide production (impair cytochrome c oxidase in the root cells) by *Pseudomonas* sp. is the responsible mechanism for the weed suppression; moreover, they also suggested other mechanisms like the production of lytic enzymes and excessive indole acetic acid (IAA) production as major weed suppressing metabolites in *Pseudomonas* spp. (Dar *et al.*, 2023). AB *Pseudomonas fluorescens* have the ability to stop the germination process after the emergence of plumule and coleorhiza due to the secretion of various compounds known as the germination arrest factor (Banowetz *et al.*, 2008). The reduction in growth was caused by the interruption of the respiration process due to the production of cyanide by AB affecting the electron transport chain (Siedow and Umbach, 2000). Cyanide is also involved in the disruption of electron transport during aerobic respiration, which imbalances the synthesis of adenosine triphosphate (ATP) and proton motive force (Umbach *et al.*, 2006). The photosynthetic process was also suppressed due to cyanide production through inhibition of ATP synthase, elevated biosynthesis of auxin, and its transport (Rasi-Caldogno *et al.*, 1978). Cyanide can bind with plastocyanin and reduce photosynthetic processes such as carbon dioxide and NO_3 metabolism (Grossmann, 1996). AB affect the growth of weeds through the release of indole 3-acetic acid and antibiotics such as phenazine and 2,4-diacetylphloroglucinol (Keel *et al.*, 1992; Sarwar and Kremer, 1995; Kremer and Souissi, 2001). Production of phytohormones (like IAA) in higher concentrations acts as an herbicide because of their minute quantity requirements for the proper functioning (root development) of the plants (Nehl *et al.*, 1997).

On the other hand, the application of allelopathic bacterial combinations significantly enhances the germination and growth of wheat crops. The maximum increase in germination percentage, growth, and photosynthetic parameters was found under the C4 allelopathic bacterial combination as compared with their uninoculated treatment, as described by Dobbelaere *et al.* (2002) and Dar *et al.* (2020). Bacterial inoculation improved the growth and yield of major crops through their growth-promoting traits (Kozdrój *et al.*, 2004; Gravel *et al.*, 2007). A similar axenic trial was conducted by Abbas *et al.* (2017a), and the results showed that the AB were not inhibitory for wheat crops and significantly improved the germination percentage, fresh biomass, and growth of wheat. The field studies showed that the increase in shoot length, SPAD value, straw, and grain yield was measured when inoculated with the C4 allelopathic bacterial combination, and our findings are in line with the results of Abbas *et al.* (2017b). The production of phytohormones such as auxin in the rhizosphere of wheat plays an important role in enhancing root growth, which ultimately increases the uptake of water and nutrients and improves plant growth and yield (Príncipe *et al.*, 2007). AB also possess P-solubilization ability phosphorous by the release of various acidic compounds such as salicylate and benzene acetate, which ultimately enhance its availability to plant roots (Yao *et al.*, 2004; Qin *et al.*, 2011; Chen *et al.*, 2014). An increase in growth due to the solubility of nutrients, the production of phytohormones, and a decreased heavy metal toxicity by rhizobacteria was reported by (Burd *et al.*, 2000). Allelopathic rhizobacteria can act as plant growth promoters, which increase the growth and physiological parameters by various mechanisms such as siderophore production, antibiotic production, growth regulators or phytohormones, and solubilization of phosphorus (Zahir and Arshad, 2003; Wu *et al.*, 2005; Han and Lee, 2006; Zaidi and Khan, 2006).

Conclusions

The results revealed that the application of cyanide-producing AB reduced plant length, physiological attributes such as SPAD value, chlorophyll a and b, carotenoid and protein contents, and yield parameters of wild oat, and improved these traits in wheat crops, especially when used at different doses of chemical herbicides, especially Axial. However, it has been concluded that the C4 bacterial combination proved to be the best bacterial consortia, significantly reducing the growth and development of wild oat and increasing wheat growth as compared to all other bacterial combinations, especially when applied with reduced doses of axial herbicide (25% and 50% of the recommended dose). Therefore, the integrated application of AB as a bioherbicide with a 50% reduced dose of Axial will be the better choice to cope with wild oat infestation and ultimately enhance the yield of the wheat crop under sustainable agricultural practices. Moreover, due to the great potential of AB towards weed suppression, we have planned to test these bacterial strains for different cropping systems to find their suppressive ability for other weeds without damaging crop plants, which may lead to the formation of multi-target bioherbicides.

References

Ab Rahman SFS, Singh E, Pieterse CM, Schenk PM, 2018. Emerging microbial biocontrol strategies for plant pathogens. Plant Sci. 267:102-11.

Abbas T, Naveed M, Siddique S, Aziz MZ, Khan KS, Zhang J, Mustafa A, Sardar MF, 2020. Biological weeds control in rice (Oryza sativa) using beneficial plant growth promoting rhizobacteria. Int. J. Agric. Biol. 23:522-8.

Abbas T, Zahir ZA, Naveed M, 2017a. Bioherbicidal activity of allelopathic bacteria against weeds associated with wheat and their effects on growth of wheat under axenic conditions. Biocontrol 62:719-30.

Abbas T, Zahir ZA, Naveed M, Aslam Z, 2017b. Biological control of broad-leaved dock infestation in wheat using plant antagonistic bacteria under field conditions. Environ. Sci. Pollut. Res. Int. 24:14934-44.

Abd-Alla MH, Morsy FM, El-Enany AWE, Ohyama T, 2012. Isolation and characterization of a heavy-metal-resistant isolate of Rhizobium leguminosarum bv. viciae potentially applicable for biosorption of Cd2+ and Co2+. Int. Biodeter. Biodegr. 67:48-55.

Ali MA, Naveed M, Mustafa A, Abbas A, 2017. The good, the bad, and the ugly of rhizosphere microbiome. In: Kumar V, Kumar M, Sharma S, Prasad R (eds.) Probiotics and plant health. Springer, New York, USA, pp. 253-90.

Ashiq M, Aslam Z, 2014. Weeds and weedicides. Department of

Agronomy, Ayub Agricultural Research Institute, Pakistan
Azam A, Shafique M, 2017. Agriculture in Pakistan and its Impact on Economy. Int. J. Adv. Sci. Technol. 103:47-60.
Bailey K, Boyetchko S, Derby J, Hall W, Sawchyn K, Nelson T, Johnson D, Spencer N, 2000. Evaluation of fungal and bacterial agents for biological control of Canada thistle (pp 203-8). Proc. 10th ISBCW. Bozeman, MT, USA.
Bailey K, Boyetchko S, Längle T, 2010. Social and economic drivers shaping the future of biological control: a Canadian perspective on the factors affecting the development and use of microbial biopesticides. Biol. Control 52:221-9.
Banowetz GM, Azevedo MD, Armstrong DJ, Halgren AB, Mills DI, 2008. Germination-arrest factor (GAF): biological properties of a novel, naturally-occurring herbicide produced by selected isolates of rhizosphere bacteria. Biol. Control 46:380-90.
Beckie HJ, Francis A, Hall LM, 2012. The biology of Canadian weeds. 27. Avena fatua L.(updated). Can. J. Plant Sci. 92:1329-57.
Bender C, Rangaswamy V, Loper J, 1999. Polyketide production by plant-associated pseudomonads. Annu. Rev. Phytopathol. 37:175-96.
Blair A, Ritz B, Wesseling C, Freeman LB, 2015 Pesticides and human health. Occup. Environ. Med. 72:81-2.
Burd GI, Dixon DG, Glick BR, 2000. Plant growth-promoting bacteria that decrease heavy metal toxicity in plants. Can. J. Microbiol. 46:237-45.
Charudattan R, 2005. Ecological, practical, and political inputs into selection of weed targets: what makes a good biological control target? Biol. Control 35:183-96.
Chauvel B, Guillemin JP, Gasquez J, Gauvrit C, 2012. History of chemical weeding from 1944 to 2011 in France: changes and evolution of herbicide molecules. Crop Prot. 42:320-6.
Chen Y, Fan J-B, Du L, Xu H, Zhang QH, He YQ, 2014. The application of phosphate solubilizing endophyte Pantoea dispersa triggers the microbial community in red acidic soil. Appl. Soil Ecol. 84:235-44.
Culliney TW, 2005. Benefits of classical biological control for managing invasive plants. Crit. Rev. Plant Sci. 24:131-50.
Dar A, Were E, Hilger T, Zahir ZA, Ahmad M, Hussain A, Rasche F, 2023. Bacterial secondary metabolites: possible mechanism for weed suppression in wheat. Can. J. Microbiol. 69:103-16.
Dar A, Zahir ZA, Asghar HN, Ahmad R, 2020. Preliminary screening of rhizobacteria for biocontrol of little seed canary grass (Phalaris minor Retz.) and wild oat (Avena fatua L.) in wheat. Can. J. Microbiol. 66:368-76.
De Prado R, Osuna MD, Fischer AJ, 2004. Resistance to ACCase inhibitor herbicides in a green foxtail (Setaria viridis) biotype in Europe. Weed Sci. 52:506-12.
Dobbelaere S, Croonenborghs A, Thys A, Ptacek D, Okon Y, Vanderleyden J, 2002. Effect of inoculation with wild type Azospirillum brasilense and A. irakense strains on development and nitrogen uptake of spring wheat and grain maize. Biol. Fert. Soils 36:284-97.
Farooq M, Jabran K, Cheema ZA, Wahid A, Siddique KH, 2011. The role of allelopathy in agricultural pest management. Pest Manag. Sci. 67:493-506.
Farooq M, Nawaz A, Ahmad E, Nadeem F, Hussain M, Siddique KH, 2017. Using sorghum to suppress weeds in dry seeded aerobic and puddled transplanted rice. Field Crop. Res. 214:211-8.
Foley JA, Ramankutty N, Brauman KA, Cassidy ES, Gerber JS, Johnston M, Mueller ND, O'Connell C, Ray DK, West PC, Balzer C, Bennett EM, Carpenter SR, Hill J, Monfreda C, Polasky S, Rockström J, Sheehan J, Siebert S, Tilman D, Zaks DRM, 2011. Solutions for a cultivated planet. Nature 478:337-42.
Gravel V, Antoun H, Tweddell RJ, 2007. Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with Pseudomonas putida or Trichoderma atroviride: possible role of indole acetic acid (IAA). Soil Biol. Biochem. 39:1968-77.
Grossmann K, 1996. A role for cyanide, derived from ethylene biosynthesis, in the development of stress symptoms. Physiol. Plantarum 97:772-5.
Han HS, Lee K, 2006. Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. Plant Soil Environ. 52:130-7.
Iqbal M, Khan MF, Suhail M, Zaman Q, 2017. Determinants of various factors for wheat production. J. Agric. Res. 55:379-85.
Jabran K, Mahmood K, Melander B, Bajwa AA, Kudsk P, 2017. Weed dynamics and management in wheat. Adv. Agron. 145:97-166.
Kao-Kniffin J, Carver SM, DiTommaso A, 2013. Advancing weed management strategies using metagenomic techniques. Weed Sci. 61:171-84.
Keel C, Schnider U, Maurhofer M, Voisard C, Laville J, Burger U, Wirthner PJ, Haas D, Défago G, 1992. Suppression of root diseases by Pseudomonas fluorescens CHA0: importance of the bacterial secondary metabolite 2, 4-diacetylphloroglucinol. Mol. Plant Microbe In. 5:4-13.
Korres NE, Norsworthy JK, Mauromoustakos A, 2019. Effects of Palmer amaranth (Amaranthus palmeri) establishment time and distance from the crop row on biological and phenological characteristics of the weed: implications on soybean yield. Weed Sci. 67:126-35.
Kozdrój J, Trevors J, Van Elsas J, 2004. Influence of introduced potential biocontrol agents on maize seedling growth and bacterial community structure in the rhizosphere. Soil Biol. Biochem. 36:1775-84.
Kremer RJ, 2006. The role of allelopathic bacteria in weed management. In: Mukerji KG (ed.) Allelochemicals: biological control of plant pathogens and diseases. Springer, New York, USA, pp 143-55.
Kremer RJ, 2013. Interactions between the plants and microorganisms. Allelopathy J. 31:51-70.
Kremer RJ, Begonia MFT, Stanley L, Lanham ET, 1990. Characterization of rhizobacteria associated with weed seedlings. Appl. Environ. Microbiol. 56:1649-55.
Kremer RJ, Caesar AJ, Souissi T, 2006. Soilborne microorganisms of Euphorbia are potential biological control agents of the invasive weed leafy spurge. Appl. Soil Ecol. 32:27-37.
Kremer RJ, Souissi T, 2001. Cyanide production by rhizobacteria and potential for suppression of weed seedling growth. Curr. Microbiol. 43:182-6.
Lacey LA, Shapiro-Ilan DI, 2008. Microbial control of insect pests in temperate orchard systems: potential for incorporation into IPM. Annu. Rev. Entomol. 53:121-44.
Li J, Kremer RJ, 2006. Growth response of weed and crop seedlings to deleterious rhizobacteria. Biol. Control 39:58-65.
Mahajan G, Chauhan BS, 2021. Interference of wild oat (Avena fatua) and sterile oat (Avena sterilis ssp. ludoviciana) in wheat. Weed Sci. 69:485-91.
Mitchell T, 1991. Colonising Egypt: with a new preface. 1st ed. University of California Press, Berkeley, CA, USA.
Montgomery DC, 2017. Design and analysis of experiments. John

wiley & sons, Hoboken, NJ, USA.
Mustafa A, Naveed M, Saeed Q, Ashraf MN, Hussain A, Abbas T, Kamran M, Minggang X, 2019. Application potentials of plant growth promoting rhizobacteria and fungi as an alternative to conventional weed control methods. In: Hasanuzzaman M, Carvalho Minhoto Teixeira Filho M, Fujita M, Rodrigues Nogueira TA (eds.) Sustainable crop production. IntechOpen, London, UK.
Nehl D, Allen S, Brown J, 1997. Deleterious rhizosphere bacteria: an integrating perspective. Appl. Soil Ecol. 5:1-20.
Omer AM, Balah MA, 2011. Using of rhizo-microbes as bioherbicides of weeds. Glob. J. Biotech. Biochem. 6:102-11.
Peng G, Byer KN, 2005. Interactions of Pyricularia setariae with herbicides for control of green foxtail (Setaria viridis). Weed Technol. 19:589-98.
Pimentel D, 2005. Environmental and economic costs of the application of pesticides primarily in the United States. Environ. Dev. Sustain. 7:229-52.
Príncipe A, Alvarez F, Castro MG, Zachi L, Fischer SE, Mori GB, Jofré E, 2007. Biocontrol and PGPR features in native strains isolated from saline soils of Argentina. Curr. Microbiol. 55:314-22.
Qin L, Jiang H, Tian J, Zhao J, Liao H, 2011. Rhizobia enhance acquisition of phosphorus from different sources by soybean plants. Plant Soil 349:25-36.
Rasi-Caldogno F, Cerana R, Pugliarello M, 1978. Effects of anaerobiosis on auxin-and fusicoccin-induced growth and ion transport. Experientia 34:841-2.
Razzaq A, Cheema ZA, Jabran K, Hussain M, Farooq M, Zafar M, 2012. Reduced herbicide doses used together with allelopathic sorghum and sunflower water extracts for weed control in wheat. J. Plant Prot. Res. 52:281-5.
Sahil, Mahajan G, Loura D, Raymont K, Chauhan BS, 2020. Influence of soil moisture levels on the growth and reproductive behaviour of Avena fatua and Avena ludoviciana. PLoS One 15:e0234648.
Sargent JA, 1986. Herbicide-induced microbial invasion of plant roots. Weed Sci. 34:50-3.
Sarwar M, Kremer RJ, 1995. Enhanced suppression of plant growth through production of L-tryptophan-derived compounds by deleterious rhizobacteria. Plant Soil 172:261-9.
Scavo A, Mauromicale G, 2020. Integrated weed management in herbaceous field crops. Agronomy 10:466.
Siedow JN, Umbach AL, 2000. The mitochondrial cyanide-resistant oxidase: structural conservation amid regulatory diversity. Biochim. Biophys. Acta 1459:432-9.
Steel RGD, Torrie JH, Dicky DA, 1997. Principles and procedures of statistics: a biometrical approach. 3rd ed. McGraw Hill, New York, USA, pp 352-8.
Sturz A, Christie B, 2003. Beneficial microbial allelopathies in the root zone: the management of soil quality and plant disease with rhizobacteria. Soil Tillage Res. 72:107-23.
Umbach AL, Ng VS, Siedow JN, 2006. Regulation of plant alternative oxidase activity: a tale of two cysteines. Biochim. Biophys. Acta 1757:135-42.
Wu S, Cao Z, Li Z, Cheung K, Wong MH, 2005. Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma 125:155-66.
Zahir ZA, Ahmad M, Hilger TH, Dar A, Malik SR, Abbas G, Rasche F, 2018. Field evaluation of multistrain biofertilizer for improving the productivity of different mungbean genotypes. Soil Environ. 37:182-8.
Zahir ZA, Arshad M, Frankenberger W, 2003. Plant growth promoting rhizobacteria: applications and perspectives in agriculture. Adv. Agron. 81:97-168.
Zaidi A, Khan MS, 2006. Co-inoculation effects of phosphate solubilizing microorganisms and Glomus fasciculatum on green gram-Bradyrhizobium symbiosis. Turk. J. Agric. For. 30:223-30.
Zeller SL, Brandl H, Schmid B, 2007. Host-plant selectivity of rhizobacteria in a crop/weed model system. PLoS One 2:e846.