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Growth response of wheat and associated weeds to plant antagonistic rhizobacteria and fungi

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Abstract

Wheat is the staple cereal crop of Pakistan but its growth is rigorously affected by associated weeds. Present study evaluated the synergistic effect of plant antagonistic rhizobacteria and fungi on growth, yield and suppression of wheat-associated weeds. Wheat associated weeds *Phalaris minor* and *Avena fatua* were grown in pots containing wheat as well. *Pseudomonas aeruginosa* strain PAO1 and *Trichoderma harzianum* T-MN6 were used as amendments to check their effect on two major weeds of wheat. The combined application of PAO1 and T-MN6 reduced the shoot length of *Phalaris minor* up to 30% and *Avena fatua* 40%, root length 22% and 28%, fresh biomass 29% and 31% respectively over their sole application. Similarly, inoculation of PAO1 and T-MN6 alone and in combination considerably enhanced growth, yield and physiological parameters of wheat. It was inferred from this study that the synergistic application of PGPR and fungi is a promising option to suppress major weeds of wheat and to enhance growth and yield of wheat.

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Key words: *Pseudomonas aeruginosa*; *Trichoderma harzianum; Phalaris minor; Avena fatua,* biological weed control; wheat growth.

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Introduction

Wheat (*Triticum aestivum* L.) is the most common staple food in the world with the average production of 672 million tons in 2012 (FAO, 2014). In Pakistan it occupies a substantial place due to the largest area under single crop cultivation. The average yield of wheat in Pakistan does not exceed 30-35% of its potential compared with the higher yields in other wheat growing countries (Khan *et al*., 2002). Plants are continuously exposed to different biotic (pathogens, insects and weeds) and abiotic (temperature, precipitation and limited nutrients) stresses which leads to reduction in crop yields (Mustafa *et al*., 2019). Of all other yield determining factors attack by weeds is a most damaging and costly factor in crop production (Noorka and Shahid, 2013). Among other crop pests weeds cause higher yield reductions (Oerke *et al*., 2006).

In conventional times, weeds were controlled by traditional agronomic and chemical methods. However, there are certain merits and demerits of using such conventionally outdated methods. No doubt herbicides get famed in weed control due to their rapid response upon application, variety of available chemicals and application practices, energy efficiency and lesser costs (Ghorbani *et al*., 2005). Continuous use of chemical herbicides causes contamination of water bodies and pollute natural resources like air, soil and plants thus have damaging effects on non-target species such as wildlife (Geiger *et al*., 2010; Tabaglio *et al*., 2013). Herbicide residues are another serious threat in food and environment related concerns (Crone *et al*., 2009). Similarly, hand weeding is a labour-intensive job, hence cannot be applied on a larger scale. Accordingly, new weed control strategies that are safe, cost effective and environmentally sound are needed. In this context, biological control using bacteria and fungi could be a valuable approach in management of noxious weeds (Mustafa *et al*., 2019).

Different bacterial genera residing in rhizosphere that are widely involved in several biological activities such as sustainability of soil ecosystems and nutrient dynamics are called rhizobacteria (Ahemad and Kibret, 2014). Apart from direct plant growth promotion mechanisms these rhizobacteria differentially colonise in the rhizosphere and produce an array of phytotoxic metabolites that suppress germination and growth of certain plants hence are termed as plant antagonistic bacteria (Sturz and Christie, 2003). A major group of rhizobacteria with potential for biocontrol of weeds is *Pseudomonads*. A number of *Pseudomonas* strains exhibit plant growth promotional activities via production of phytoharmones, solubilisation of phosphate and other nutrients (Vyas *et al*., 2009; Ali *et al*., 2017), production of siderophores and antibiotics such as, phenazines, pyrrolnitrin and pyoluteorin, diacetylphloroglucinol (2,4-DAPG) and biocidal compounds hydrogen cyanide (Raaijmakers *et al*., 2002) and cell wall degrading enzymes (Haas and Defago, 2005).

Trichoderma spp. are most frequently characterised soil borne fungi equipped with several mechanisms to utilise diverse substrates and to survive under plenty of unfavourable chemical compounds in soil-plant-root systems (Harman *et al*., 2004). To- date most of the literature on *Trichoderma spp*. had concentrated their role as biological control agent against nematode and fungal diseases and other plant pathogens including invertebrates and bacteria (Sahebani and Hadavi, 2008; Hanada *et al*., 2009). Whereas, scarce reports are available on biological weed control using *Trichoderma spp.* and the scanty available reports are merely confined to *Trichoderma virens* (Heraux *et al*., 2005). Most obvious underlying mechanisms of *Trichoderma spp.* as biocontrol agents include: mycoparasitism, competition, antibiosis, induced plant defence and production of cell wall lytic enzymes (Howell, 2003). A so-far unexploited category of microorganisms is the use of rhizobacteria and *Trichoderma spp*. for biological weed control.

In our previous studies, the plant antagonistic rhizobacterial strains were used to suppress wheat associated weeds (Abbas *et al.,* 2017b). In present study, we hypothesised that the synergistic use of plant antagonistic rhizobacteria and fungi may have differential effects on wheat and its associated weeds. Therefore, rhizobacterial strain PAO1 characterised as (*Pseudomonas aeruginosa*) inhibitory to weeds but not to wheat and a fungus T-MN6 (*Trichoderm harzianum*) were used in consortium to check their bio herbicidal potential against major weeds of wheat and their effects on wheat. These agents were applied in pots containing wheat and weeds (*Avena fatua*) and (*Phalaris minor*).

Materials and methods

Collection of plant antagonistic rhizobacterial and Trichoderma strain

393 strains of presumed rhizobacterial plant antagonists were isolated from wheat fields heavily infested with associated weeds across District Faisalabad, Pakistan. These strains were isolated from rhizosphere of both the wheat as well as its associated weeds. The strains were tested in vitro for production of phytotoxic substances and in vivo to check their effects inhibitory on weeds and growth promoting effects in wheat in sterilised agar plates in our previous studies (Abbas *et al.,* 2017a, 2017b). Whereas, the previously isolated plant growth promoting fungus *Trichoderma harzianum* T-MN6 was friendly donated by Plant Pathology Department, University of Agriculture, Faisalabad, Pakistan that was further purified in laboratory. In present study, based on the efficiency to inhibit germination (data not shown) and growth of weeds the rhizobacterial *Pseudomonas* aeruginosa PAO1 strain was selected to further test its inhibitory effect on two major weeds of wheat (*Avena fatua* and *Phalaris minor*) in synergism with previously isolated and purified plant growth promoting fungus (*Trichoderma harzianum* T-MN6) under weedy and weed free pot conditions.

Culture preparation and mycelial suspension

For *Pseudomonas aeruginosa* PAO1 the culture containing strains was prepared in (King's B broth) and stored in Erlenmeyer flasks (King *et al*., 1954). Mature Growth of the strain was then transferred to flasks using sterilised bacteriological loop, following incubation in a shaking incubator (Firstek Scientific, Tokyo, Japan) at 100 rev. per minute for 48 h at 28±1°C. The optical density (O.D) of the prepared culture was then measured using a spectrophotometer (Nicolet Evolution 300 LC, Cambridge, UK) at (wavelength 600 nm), and adjusted to 0.5 to attain an identical population of bacteria (10^8-10^9 cfu mL^{-1}). Whereas, for *Trichoderma harzianum* T-MN6 culture was prepared on sterile potato dextrose broth in Erlenmeyer flasks and incubated for 8 days at 27°C and 160 rpm min^{-1} (Javaid and Adrees, 2009). The culture of *Trichoderma harzianum* T-MN6 was then filtered to obtain culture filtrates and residue (mycelia). The mycelia were then washed three times by sterile water followed by dilution with sterile distilled water to maintain uniform concentration of (10^6-10^7 mycelia mL^{-1}).

Inoculation

Inoculum of the *Pseudomonas aeruginosa* strain PAO1 and *Trichoderma harzianum* T-MN6 were mixed with three times autoclaved and sterilised peat at the ratio of 1.25:1 followed by incubation at 28±1°C for overnight. Seed coating of *A. fatua*, *P. minor* and wheat were performed using a mixture containing inoculated peat and sterilised sugar solution. Whereas, only wheat seeds were dipped in broth to maintain a weed free control. Inoculated fifteen viable seeds of *A. fatua* and *P. minor* were seeded together below soil surface under pot condition. After that, 25 mL fresh culture of selected bacterial strain and 25 mL mycelial suspension of *Trichoderma harzianum* T-MN6 were applied on soil surface of relevant pots sown with wheat and weeds followed by a thin surface layer of sand. For weed free control, 25 mL of King's B and potato dextrose broth were sprayed onto soil surface of pots followed by a thin apparent layer of sand (Vargas and O'Hara, 2006). All the pots were placed in rain protected wire house and maintained at field capacity.

Experimental set up

Present study was conducted in pots placed in wire house of Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad (31.438976° N and 73.069029° E) to assess the ability of chosen plant antagonistic bacterial strain (*Pseudomonas. aeruginosa* PAO1) and plant growth promoting fungus (*Trichoderma harzianum* T-MN6*)* for suppression of wheat-associated weeds and subsequent enhancement of growth and yield of wheat. Pots with uniform diameter 30 cm were filled with air-dried and sieved soil at 8 kg per pot. There were four treatments (Control, PAO1, T-MN6 and PAO1 + T-MN6) replicated in triplicates. The pots were arranged in three sets, *viz*.: i) weed free conditions containing wheat only with a separate control; ii) *Phalaris minor* infested pots co-seeded with wheat having a *P. minor* containing control; iii) *Avena fatua* infested pots co-seeded with wheat having an *A. fatua* containing control. The merged soil sample was examined for different physico-chemical characteristics. The texture of soil was sandy clay loam (Typic Haplocambid), pH 7.4, extract electrical conductivity (ECe) 1.5 dS m^{-1}, saturation percentage 30.2%, organic matter 0.88%, total N 0.037%, available P 7.81 mg kg^{-1}, and extractable K 158 mg kg^{-1}. Seeds of wheat (Galaxy 2013) were collected from Department of Agronomy, University of Agriculture, Faisalabad and seeds of weeds were friendly donated by Ayub Agriculture Research Institute, Faisalabad. Clean seeds were dipped in water; floating seeds were discarded and the seeds settled in the bottom were taken for the trial. Eight seeds of wheat were sown in each pot. Fifteen seeds of *A. fatua* and *P. minor* were sown in each pot except weed free conditions. Pots were placed in the wire house under ambient light and temperature by using completely randomised design. Chemical fertilisers were applied as N, P and K at the rate of 120-90-60 kg ha^{-1} as urea, diammonium phosphate and sulphate of potash, respectively. Whole PK fertilisers were applied at the time of sowing while N was applied in two splits. Data regarding growth and yield parameters were collected following standard procedure.

Growth and yield parameters of wheat and weeds

Data regarding growth and yield parameters including plant height, 1000-grains weight, root length, number of total tillers, productive tillers and spike length of wheat were recorded. Similarly, data regarding shoot length, root length and fresh biomass of weeds were recorded. Shoot length and root length were measured at the time of harvesting and uprooting the plant, respectively. Grain yield per pot was measured after harvesting the plant.

Plant chemical analysis

At physiological maturity grain samples of wheat were collected from pots for determination of nitrogen, extractable phosphorus and potassium. All the samples were ground and digested (Wolf, 1982). Total nitrogen was measured by using Kjeldahl ammonium distillation apparatus. Phosphorus was measured by adding 10 mL Barton reagent in 5 mL sample through spectrophotometer (T80 UV/VIS Spectrometer PG Instruments Ltd). Actual concentration of phosphorus was measured following standard curve. Potassium was simply measured by flame photometer (Jenway PFP-7, England) and its concentration was derived by using calibration curve.

Physiology of plants

Chlorophyll contents of wheat were measured using SPAD-502 m (Konica-Minolta, Japan) at 60 DAS. Values given by instrument are represented as SPAD values, and index directly related to chlorophyll contents in leaves (Coste *et al*., 2010).

Relative water content (RWC) of leaves was determined by using the following formula as described by Mayak *et al*. (2004).

$$\text{Relative water content (RWC)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fully turgid weight} - \text{Dry weight}} \times 100$$

The fully turgid weight of leaf was taken after putting it in 100% humidity in the dark at 4° C for 48 h.

Statistical analysis

Data regarding growth and NPK contents of infected wheat plants were analysed statistically following standard procedures. Means were compared to figure out significant differences among all treatments by using least significant difference test (LSD) test (Steel *et al*., 1997). All the statistical analyses were performed by using Statistix 8.1 using linear models.

Results

Growth response of test weeds

Data on growth parameters (Table 1) of both weeds indicated significant reduction in shoot length, root length and fresh biomass of the tested weeds upon sole and synergistic application of strain PAO1 and T-MN6 In *Phalaris minor* containing control (no amendment), the sole application of PAO1 and T-MN6 reduced the shoot length up to 20 and 11% respectively, whereas their effect was more obvious when these strains were applied in combination yielding 30% reduction in shoot length as compared to control. Similarly, in *A. fatua* containing pots the separate application of PAO1 and T-MN6 reduced shoot length up to 20 and 16% as compared to respective controls, whilst these results are comparable with significant reduction of shoot length upon combined application resulting in 40% reduced shoot length of *A. fatua.* These results revealed shoot length of tested weeds was considerably reduced with the combined application of PAO1 and T-MN6 as compared to their sole application.

Alone inoculation of PAO1 and T-MN6 reduced root length of *Phalaris minor* up to 14 and 12% respectively, while the maximum reduction (22%) resulted with co-inoculation of these isolates in *Phalaris minor* containing pots as compared to respective controls. Likewise, in *A. fatua* containing pots the separate application of PAO1 and T-MN6 resulted in 20 and 18% reduction as compared to controls but the effect of interactive application was more pronounced resulting in 28% root length reduction as compared to their sole application.

Data regarding fresh biomass revealed similar trend. The separate application resulted in considerable reduction 14 and 11% of fresh biomass of *P. minor* as compared to controls and the consortium application yielded 29% reduction in fresh biomass as compared to sole applications. However, the effect of their single application was statistically non-significant to each other. Similarly, in *A. fatua* infested pots the separate application of PAO1 and T-MN6 resulted in 20 and 15% reduction as compared to containing control. The synergistic application resulted in maximum 31% fresh biomass reduction as compared to sole applications.

Table 1. Effect of plant antagonistic rhizobacteria and fungi on suppression of shoot length, root length and fresh biomass of test weeds.

Treatment	Shoot length (cm)		Root length (cm)		Fresh biomass (g pot^{-1})	
	A. fatua	*P. minor*	*A. fatua*	*P. minor*	*A. fatua*	*P. minor*
Control	39.7^{a}	29.07^{c}	13.7^{a}	12.33^{b}	34.80^{a}	26.47^{c}
PAO1	31.7^{b}	23.20^{e}	11.0^{c}	10.63^{c}	27.87^{b}	22.87^{e}
T-MN6	33.6^{b}	25.87^{d}	11.2^{c}	10.87^{c}	29.43^{b}	23.47^{d}
PAO1+T-MN6	23.9^{de}	20.30^{f}	9.9^{d}	9.60^{d}	23.93^{d}	18.70^{f}

Values sharing same letter(s) are statistically non-significant with each other at (P<0.05). PAO1, *Pseudomonas aeruginosa* and T-MN6, *Trichoderma harzianum* T-MN6.

Growth and yield response of wheat

Data indicated that separate application of PAO1 and T-MN6 increased shoot length of wheat grown without weeds up to 16 and 13% respectively as compared to control but the effect was more significant with the combined application of PAO1 and T-MN6 resulting in 21% increased shoot length as compared to sole applications. Similarly, in *P. minor* infested wheat the individual application increased shoot length up to 14 and 11% as compared to *P. minor* containing control. The maximum increase (17%) in this case was obtained when PAO1 and T-MN6 were applied together. Likewise, in *A. fatua* infested pots the sole application of PAO1 and *Trichoderma sp.* resulted in 13 and 11% increased shoot length of wheat as compared to *A. fatua* containing control. The integrative application of PAO1 and T-MN6 gave maximum (15%) increase in shoot length as compared to alone applications in *A. fatua* infested wheat (Table 2).

It was observed that the single application of PAO1 and T-MN6 significantly improved the root length of wheat grown without weeds about 21 and 44% respectively over weed free control (Table 2). But the combined application gave maximum results in terms of 75% increase in root length of wheat as compared to sole application. In *Phalaris minor* containing pots 29 and 54% increase was observed in root length of wheat with the single application of PAO1 and T-MN6 over *Phalaris minor* containing control. The maximum increase 74% was observed with the consortium application as compared to the sole application of PAO1 and T-MN6 Similarly, in *Avena fatua* containing pots the individual application of PAO1 and T-MN6 caused an increase of 29 and 54% respectively as compared to *Avena fatua* containing control. However, the synergistic effect of PAO1 and T-MN6 resulted in maximum 68% increase in root length as compared to the sole application.

Data illustrated that separate application of PAO1 and T-MN6 increased spike length of wheat grown without weeds up to 20 and 16% respectively as compared to control but the effect was more significant with the combined application of PAO1 and T-MN6 resulting in 51% increased spike length as compared to sole applications. Similarly, in *P. minor* infested wheat the individual application increased spike length up to 17 and 14% as compared to *P. minor* containing control (Table 2). The maximum increase (40%) in this case was obtained when PAO1 and T-MN6 were applied together. Likewise, in *A. fatua* infested pots the sole application of PAO1 and T-MN6 resulted in 15 and 13% increased spike length of wheat as compared to *A. fatua* containing control. The integrative application of PAO1 and T-MN6 gave maximum (35%) increase in spike length as compared to alone applications in *A. fatua* infested wheat.

The obtained data specified increasing trend in total tillers of wheat grown with and without weeds (Table 2). Single inoculation of PAO1 and T-MN6 increased number of total tillers up to 19 and 14% under weed free wheat conditions as compared to control. But the co-inoculation maximally (33%) increased the number of total tillers per pot as compared to their sole inoculation. In case of *P. minor* containing pots the individual application of PAO1 and T-MN6 resulted in 17 and 13% increase in number of total tillers as compared to *P. minor* containing control. Whereas, the combined application gave maximum results in terms of 27% increased number of total tillers as compared to sole applications. In *A. fatua* infested pots alone application of PAO1 and T-MN6 increased the number of total tillers up to 15 and 11% respectively as compared to *A. fatua* containing control. The combined application in this case resulted in 23% increase in number of total tillers as compared to their alone applications.

It was observed that the single application of PAO1 and T-MN6 significantly improved the number of productive tillers of wheat per pot grown without weeds about 33 and 21% respectively over weed free control (Table 2). But the combined application gave maximum results in terms of 63% increase in number of productive tillers of wheat as compared to sole applications. In *Phalaris minor* containing pots 29 and 19% increase was observed in number of productive tillers of wheat with the single application of PAO1 and T-MN6 over *Phalaris minor* containing control. The maximum increase 48% was observed with the consortium application as compared to the sole application of PAO1 and T-MN6 Similarly, in *Avena fatua* containing pots the individual application of PAO1 and T-MN6 caused an increase of 22 and 47% respectively as compared to *Avena fatua* containing control. However, the synergistic effect of PAO1 and resulted in maximum 39% increase in productive tillers as compared to the sole application.

Obtained data depicted that separate application of PAO1 and T-MN6 increased 1000 grains weight of wheat grown without weeds up to 21 and 16% respectively as compared to control but the effect was more significant with the combined application of PAO1 and T-MN6 resulting in 33% increased spike length as compared to sole applications. Similarly, in *P. minor* infested wheat the individual application increased 1000 grains weight up to 16 and 14% as compared to *P. minor* containing control. The maximum

Table 2. Effect of plant antagonistic rhizobacteria and fungi on growth and yield parameters of wheat.

Treatment	Weed free	*P. minor*	*A. fatua*	Weed free	*P. minor*	*A. fatua*	Weed free	*P. minor*	*A. fatua*
	Wheat plant shoot length (cm)			Wheat plant root length (cm)			Wheat spike length (cm)		
Control	68.80^{cd}	66.83^{fg}	61.17^{i}	16.67^{f}	14.50^{g}	13.27^{g}	9.60^{d}	8.40^{fg}	7.67^{g}
PAO1	79.67^{b}	76.33^{de}	69.13^{gh}	20.17^{de}	18.70^{e}	17.07^{f}	11.50^{bc}	9.87^{de}	8.80^{ef}
T-MN6	77.67^{c}	74.00^{ef}	67.67^{h}	24.07^{b}	22.33^{c}	20.40^{d}	11.17^{c}	9.57^{d-f}	8.67^{g}
PAO1+ T-MN6	83.33^{a}	78.40^{cd}	70.50^{fg}	29.23^{a}	25.30^{b}	22.33^{c}	14.50^{a}	11.73^{b}	10.37^{c}
Treatment	**No. of total tillers (pot^{-1})**			**No. of productive tillers (pot^{-1}).**			**1000 grains weight (g pot^{-1})**		
Control	12.00^{cd}	10.00^{ef}	8.67^{f}	8.00^{de}	7.00^{ef}	6.00^{f}	40.00^{e}	36.50^{fg}	34.33^{h}
PAO1	14.33^{b}	11.67^{de}	10.00^{de}	10.67^{cd}	9.00^{de}	7.33^{ef}	48.50^{b}	42.17^{e}	39.00^{fg}
T-MN6	13.67^{bc}	11.33^{de}	9.67^{ef}	9.67^{cd}	8.33^{ef}	7.00^{ef}	46.30^{c}	41.67^{f}	38.00^{gh}
PAO1+T-MN6	16.00^{a}	12.67^{b}	10.67^{bc}	13.00^{a}	10.33^{b}	8.33^{bc}	53.30^{a}	45.83^{c}	42.17^{d}

Values sharing same letter(s) are statistically non-significant with each other at ($P<0.05$). PAO1, *Pseudomonas aeruginosa* and T-MN6, *Trichoderma harzianum* T-MN6.

increase (26%) in this case was obtained when PAO1 and T-MN6 were applied together. Likewise, in *A. fatua* infested pots the sole application of PAO1 and T-MN6 resulted in 14 and 11% increased 1000 grains weight of wheat as compared to *A. fatua* containing control. The integrative application of PAO1 and T-MN6 gave maximum (23%) increase in 1000 grains weight as compared to alone applications in *A. fatua* infested wheat (Table 2).

Chemical parameters of wheat

Plants inoculated with PAO1 and T-MN6 alone and in combination showed improved nitrogen, phosphorous and potassium contents in wheat grains as compared to respective controls in weedy and weed free conditions as well (Table 3).

Data regarding nitrogen contents in grains of wheat grown without weeds indicated that alone application of PAO1 and T-MN6 increased grain nitrogen content up to 18 and 15% respectively as compare to control but the effect was more significant with the combined application of PAO1 and T-MN6 resulting in 27% increased grain nitrogen content as compared to sole applications. Similarly, in *P. minor* infested wheat the individual application increased grain nitrogen content up to 14 and 11% as compared to *P. minor* containing control. The maximum increase (20%) in this case was obtained when PAO1 and T-MN6 were applied together. Likewise, in *A. fatua* infested pots the sole application of PAO1 and T-MN6 resulted in 12 and 10% increased grain nitrogen content of wheat as compared to *A. fatua* containing control. The integrative application of PAO1 and T-MN6 gave maximum (19%) increase in grain nitrogen content as compared to alone applications in *A. fatua* infested wheat.

It was observed that the single application of PAO1 and T-MN6 considerably enriched the phosphorous concentration in grains of wheat grown without weeds about 13 and 29% respectively over weed free control. But the combined application gave maximum results in terms of 47% increase in phosphorous concentration in grains of wheat as compared to sole application. In *Phalaris minor* containing pots there was 12 and 21% increase observed in phosphorous contents in grains of wheat with the single application of PAO1 and T-MN6 over *Phalaris minor* containing control. The maximum increase 30% was observed with the consortium application as compared to the sole application of PAO1 and T-MN6. Similarly, in *Avena fatua* containing pots the individual application of PAO1 and T-MN6 caused an increase of 17 and 25% respectively as compared to *Avena fatua* containing control. However, the synergistic effect of PAO1 and T-MN6 resulted in maximum 30% increase in phosphorous concentration in grains as compared to the sole application.

In weed free conditions alone inoculation of PAO1 and T-MN6 resulted in 16 and 12% increased potassium concentration in grains as compared to control. Whereas, combined application in this case gave maximum (26%) increase in grain potassium concentration as compared to sole applications. However, in *P. minor* containing pots the sole application of PAO1 and T-MN6 resulted in 14 and 12% increase in grain potassium concentration as compared to control infested with *P. minor*, with the combined application resulting in 18% increase as compared to sole applications. In *A. fatua* infested pots sole inoculation resulted in 12 and 10% increase in grain potassium contents as compared to control containing *A. fatua*. The combined application in this case resulted in 17% increased potassium contents in wheat grains as compared to sole applications.

Physiological parameters of wheat

Physiological parameters of wheat were improved significantly upon inoculation with PAO1 and T-MN6 alone and in synergism as compared to control in weed infested and weed free conditions. Maximum (24%) improvement in chlorophyll (SPAD value) contents of wheat was observed with consortium application of PAO1 and T-MN6 as compared to sole inoculation. Whereas, sole application of PAO1 and T-MN6 resulted in 16 and 13% increased chlorophyll contents of wheat as compared to control under weed free conditions. However, in *P. minor* infested conditions sole application of PAO1 and T-MN6 resulted in 15 and 12% improvement in chlorophyll contents as compared to *P. minor* infested control. The combined application of PAO1 and T-MN6 gave maximum (19%) chlorophyll contents as compared to sole inoculation. Similarly, in *A, fatua* containing pots 13 and 10% increase in chlorophyll contents was observed with sole application of PAO1 and T-MN6 as compared to *A. fatua* containing control. The combined application resulted in 16% improvement in chlorophyll contents as compared to sole applications (Figure 1).

Data regarding relative water contents of wheat grown without weeds indicated that alone application of PAO1 and T-MN6 increased relative water contents up to 18 and 14% respectively as compared to control but the effect was more significant with the combined application of PAO1 and T-MN6 resulting in 24% increased relative water contents as compared to sole applications. Similarly, in *P. minor* infested wheat the individual application increased grain relative water contents up to 14 and 12% as compared to *P. minor* containing control. The maximum increase (20%) in this case was obtained when PAO1 and T-MN6 were applied together. Likewise, in *A. fatua* infested pots the sole application of PAO1 and T-MN6 resulted in 12 and 11% increased relative water contents of wheat as compared to *A. fatua* containing control. The integrative application of PAO1 and T-MN6 gave maximum (17%) increase in relative water contents as compared to alone applications in *A. fatua* infested wheat (Figure 2).

Discussion

Present study highlighted the bioherbicidal potential of previously isolated and pre-characterised *Pseudomonas aeruginosa*

Table 3. Effect of plant antagonistic rhizobacteria and fungi on NPK contents in grains of infested and weed free wheat.

Treatment	Weed free	*P. minor*	*A. fatua*	Weed free	*P. minor*	*A. fatua*	Weed free	*P. minor*	*A. fatua*
	Grain N (%)			Grain P (%)			Grain K (%)		
Control	2.21^{cd}	1.98^{fg}	1.91^{g}	0.24^{fg}	0.23^{g}	0.21^{h}	1.52^{e-g}	1.47^{gh}	1.42^{h}
PAO1	2.60^{b}	2.27^{de}	2.13^{e-g}	0.27^{cd}	0.26^{d-f}	0.25^{e-g}	1.76^{b}	1.67^{e}	1.59^{ef}
T-MN6	2.55^{c}	2.20^{ef}	2.10^{fg}	0.31^{b}	0.28^{c}	0.26^{c-e}	1.70^{d}	1.65^{fg}	1.57^{gh}
PAO1+ T-MN6	2.80^{a}	2.38^{cd}	2.27^{d}	0.35^{a}	0.30^{b}	0.27^{c-d}	1.92^{a}	1.74^{cd}	1.66^{bc}

Values sharing same letter (s) are statistically non-significant with each other at ($P<0.05$). PAO1, *Pseudomonas aeruginosa* and T-MN6, *Trichoderma harzianum* T-MN6.

PAO1 in conjunction with *Trichoderma harzianum* T-MN6 against two wheat associated weeds (*Phalaris minor* and *Avena fatua*) and wheat growth promotion under weed free and weed infested pot conditions. The *Pseudomonas aeruginosa* PAO1 strain used in current study had already been characterised to produce phytotoxic substances, phosphate solubilisation, weeds suppression with non-inhibitory effects on wheat in our previous study (Abbas *et al.*, 2017a). The key objective of this study was to evaluate the synergistic effect of *Pseudomonas aeruginosa* PAO1strain and *Trichoderma harzianum* T-MN6 on suppression of wheat associated weeds and growth and yield enhancement of wheat. Inoculation with PAO1 and T-MN6 significantly reduced growth parameters (shoot length, root length and fresh biomass) of both weeds alone and in combination. Maximum suppression in growth parameters of both weeds was observed with combined use of PAO1 and T-MN6 followed by PAO1 and T-MN6 respectively. However, the effect of PAO1 and T-MN6 remained non-significant to each other. The inhibition of growth parameters of both weeds might be due to the ability of PAO1 and T-MN6 to competitively colonise and produce phytotoxic metabolites in the rhizosphere. In this study maximum inhibition was observed in *Phalaris minor* than *Avena fatua*. This might be due the differential colonisation of PAO1 in roots enabling more targeted weed control (Kennedy *et al*., 2001) and higher suppression of *Phalaris minor* (Table 1) is related to better colonisation of the applied strains in the rhizosphere of *P. minor* (Abbas *et al.*, 2017a). Whereas, the low inhibition of *Avena fatua* in our study is related to exposure to increased competition of introduced strains with the indigenous microbes and fluctuations in environmental conditions affecting the survival and efficacy of these strains (Kremer and Kennedy, 1996; Horwath *et al*., 1998). Similar to our findings are the results reported by Harris and Stahlman (1996) they reported less reduction of wheat associated jointed goatgraas, Japanese brome and downy brome weeds in non-sterile than sterile soil. In nut shell our results depicted significant reduction in growth parameters of both weeds upon inoculation with PAO1 and T-MN6. These results are supported by other studies: as Shukorjuraimi *et al.* (2005) reported reduced growth and dry weight of barnyard grass ecotypes PK-04, L-01 and B-04 upon application of *Exserohilum longirostratum.* Similarly, Weissmann (2003) showed strong bioherbicidal activity of *Serratia plymuthica*, strain A153 against multiple broad-leaved weeds upon foliar spray. Li and Kremer (2006) reported reduction in *Ipomea species* and *Convolvulus arvensis* weeds in wheat and soybean upon inoculation with *Pseudomonas fluorescens.*

In our study the biological control potential of T-MN6 is found to be less than PAO1. This might be due the reason that bacteria took advantage over *Trichoderma* by showing accelerated growth rates and aggressive colonisation with simple growth requirements (Lee *et al*., 2003). Our results are in agreement with (Javaid and Sajjad, 2011) they reported a significant inhibition of shoot and root length of *Avena fatua* upon application of culture filtrates of four *Trichoderma spp.* namely *T. harzianum*, *T. pseudokoningii*, *T. reesei* and *T. viride*. Siddiqui *et al*. (2010), explored fungal involvement in *Chenopodium album* L. suppression under wheat field conditions using *Alternaria alternata.* Application of *Alternaria alternata* significantly (90%) reduced the biomass of *Chenopodium album* L. Akbar and Javaid (2012) reported that application of fungal filtrate of four *Drechslera* sp. reduced germination and growth attribute of two wheat associated weeds *Chenopodium album* L. and *Avena fatua* L.

In this study, significant reduction has been observed in growth, physiology and chemical parameters of wheat grown with weeds as compared to weed free conditions. However, the applied strains ameliorated the negative effects of weeds on wheat crop up to a significant extent. The applied strains were found non-inhibitory to wheat. This may be on part due to the least sensitivity of wheat to the phytotoxic metabolites of applied strains (Owen and Zdor, 2001) rendering increased reduction of weeds growth resultantly, on other part is due to the production of plant growth promoting substances by these strains. Improvement in weeds infected wheat growth due to synthesis of plant growth promoting substances in rhizosphere by rhizobacteria might have evoked the competitive ability of crop against weeds (Zahir *et al.,* 2004; Mejri *et al*., 2010). Similar to bacterial strain T-MN6 might have triggered the systemic or localised resistance responses in crop through production of elicitor-like substances (Harman *et al*., 2004; Ali *et al*., 2017). The production of other plant growth promoting substances siderophores, VOCs and synthesis of IAA by *Trichoderma spp*. are also well-known possible reasons of plant growth promotion under biotic and abiotic stresses (Vinale *et al*., 2008).

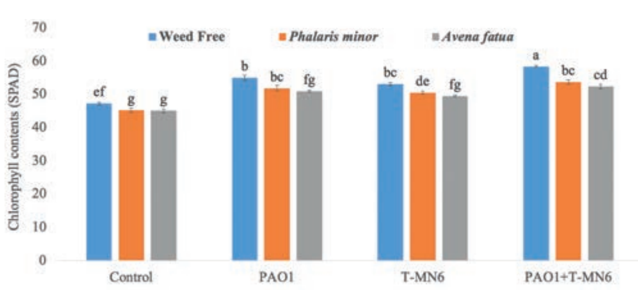

Figure 1. Effect of plant antagonistic rhizobacteria and fungi on chlorophyll contents of infested and weed free wheat. PAO1, *Pseudomonas aeruginosa* and T-MN6, *Trichoderma harzianum* T-MN6. Values sharing same letter(s) are statistically non-significant with each other at (P<0.05).

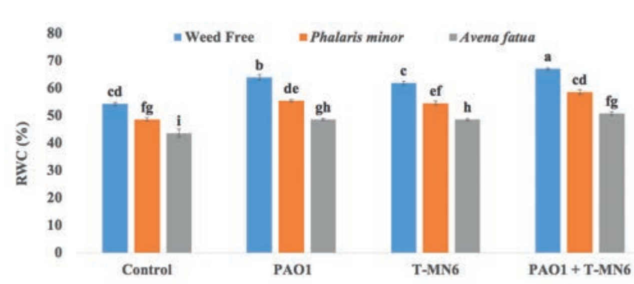

Figure 2. Effect of plant antagonistic rhizobacteria and fungi on relative water contents of infested and weed free wheat. PAO1, *Pseudomonas aeruginosa* and T-MN6 = *Trichoderma harzianum* T-MN6. Values sharing same letter(s) are statistically non-significant with each other at (P<0.05).

In our study, T-MN6 significantly improved root growth of wheat in weed free and weed infested conditions probably in higher amounts than bacterial strain. This may be due to the production of IAA and its derivatives that has promoted root length of wheat. These results are in accordance with Contreras-Cornejo *et al*. (2009) they reported, increased lateral roots of Arabidopsis by *Trichoderma virens* Gv29.8 and *T. atroviride* IMI206040 through the production of IAA and its equivalent compounds. Similarly, data revealed an increased phosphorous content in grains of wheat in pots inoculated with T-MN6 than PAO1 in weed free and weedy conditions as well. However, the trend was as follows: PAO1 + T-MN6 > T-MN6 > PAO1. This may be due to increased production of IAA by both microorganisms that in turn might have modified root architecture, resulting in increased root mass and increased surface area available for microbial colonisation and larger root system enhancing nutrient uptake by plants (Spaepen *et al*., 2007; Berg, 2009; Contreras-Cornejo *et al.,* 2009; Puia *et al*., 2017). Increased phosphorous contents of wheat grains are also obvious from the fact that certain plant growth promoting rhizobacteria and fungi have the ability to solubilise inorganic phosphate from the soil hence results in increased availability of phosphorous to plants leading to increased growth and yield (Soriano *et al.,* 2009; Candido *et al*., 2013; Ali *et al*., 2017). The applied strains in our study also improved chlorophyll and relative water contents of infested as well weed free wheat. The underlying mechanism is the improved availability of iron to main crop by sequestration of iron through production of siderophores by rhizobacteria (Yang *et al*., 2009) and by *Trichoderma* (Segarra *et al*., 2010), as iron is the key component of chlorophyll molecule. So enhanced availability of iron may have increased the chlorophyll contents of wheat grains. Due to the host specific nature of the applied strains, these may have scavenged iron from the environment rendering it unavailable for the competing plants and even microbes.

Overall, the applied strains improved growth, yield and physiology of wheat while significantly inhibiting wheat associated *weeds* (*A. fatua* and *P. minor*). Since the maximum improvement in growth and yield parameters were reflected by the combined use of PAO1 and T-MN6 so, our hypothesis that synergistic use of plant antagonistic rhizobacteria and fungi may improve growth and yield of wheat by inhibiting growth of associated weeds is advocated from the obtained data.

Conclusions

This study concluded that *Pseudomonas aeruginosa* PAO1 in combination with *Trichoderma harzianum* T-MN6 have a great potential to suppress wheat associated weeds along with enhancement in growth, physiology and yield of wheat. The above-mentioned strains through multiple mechanisms can improve the competitive ability of main crops against weeds. Specifically, this study has demonstrated the synergistic use of bacterial and fungal strains in biological weeds suppression and improvement in wheat growth. Therefore, further in-depth studies are required to develop these potent biological agents into suitable formulations in order to achieve environmental and agricultural sustainability, food security and resource conservation.

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