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MANAGEMENT OF ROOT ROT DISEASES OF EIGHT WHEAT VARIETIES USING RESISTANCE AND BIOLOGICAL CONTROL AGENTS TECHNIQUES

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Abstract

This study is focused on the screening of eight wheat varieties against *Fusarium graminearum* and *Rhizoctonia solani* root rot fungal diseases and their management through biological control agets (BCA) treatments under greenhouse conditions in Department of Plant Pathology, University of Nebraska, Lincoln (USA). These two soil-borne pathogens contributing to significant yield reduction and enhanced growth and yield of Wheat varieties (WVs) after BCA treatments. Experimental trails were set up by applying agar plug inoculation and BCA culture suspension techniques. Highest rating against *R. solani* was observed in WV Seher-2006 (5.0^{A}) while lowest shown by Galaxy-2013 (1.5^{FG}). On the other hand, maximum rating was observed in Seher-2006 (6.25^{A}) while minimum in Punjab-2011 (3.0^{B-E}) against *F. graminearum.* It was observed that T3 and T4 reduced maximum DS of V_1 as indicated 1.5^{B} and 1.75^{B}. Treatment T_4 also showed maximum fresh weight (FW) (0.11g) and dry weight (DW) (0.28g) of root after BCA treatments against *R. solani*. Maximum FW and DW (0.49g, 0.35g) of V_1 roots were also measured against T_3 treatment while minimum mass (0.05g and 0.04g) was calculated against T_7 (check). However, maximum fresh and dry weight of V_7 and V_8 roots were observed against T_4 treatment. It was noted that all varieties treated with BCA showed more FW and DW of roots as comparison to control (-ve control). However, BCA treatments against all analyzed WVs did not show significant difference. So, all treatments were reduced DS of WVs and increased biomass and yield of wheat plants. Galaxy-2013 variety (V_8) was declared as resistance against *R. solani* for year 2016-17 and three varieties (Faisalabad- 2008, Millat-2011 and Punjab-2011) were announced as moderately resistant to *R. solani* root rot. Comparatively, it was estimated that *F. graminearum* was more vulnerable as comparison to *R. solani*. The biological control activity of *Trichoderma viride* and *Bacillus subtilis* secreted enzymes and secondary metabolites in wheat plants and induced systemic disease resistance against *Fusarium graminearum* and *Rhizoctonia solani*. Therefore, it is concluded that BCA treatments are very effective techniques for the management of fungal pathogens without climatic pollution as in chemical spray form.

Key words: Wheat varieties; *Fusarium graminearum; Rhizoctonia solani;* Biocontrol agents; Disease severity; Pakistan; Azad Kashmir; Bhmiber.

Introduction

Wheat (*Triticum aestivum* L.) is the most important cereal crop after rice in the world's most famous country. It is being the staple food for about one-third of the world population. Human beings eat wheat grains more as compare to other cereal crops. Role of world economy for wheat production is significance in terms of food supply and cultivated land, feeding and commerce. China is expected to first in wheat production ranking in the world and is first in total food grain production (Liu *et al*., 2013).

Pakistan is considered as one of the agricultural country that has different type of climatic conditions and almost 2/3 part of the country indicated arid type of climate. A small area of the country (sub mountainous regions) indicate humid climate. Most of the areas in the central and southern part of Pakistan have maximum arid zone. While northern part of the country is only humid except the extreme mountains, northern areas that are comparatively dry (Chaudhry & Rasul, 2004). In Pakistan, the total cultivable area is 34.54 million hectare and only 23.38 million hectare area is under crop cultivation. It was estimated that agriculture sector contributes about 25% to GDP of Pakistan and almost 44% of the country's peoples are involved to agriculture sector. Wheat cultivation covers largest area (8.6 million hectare) in all cultivated crops. It was final estimates that wheat crop produced 24.0 million tons from the overall cultivated areas of 9.2 million ha (Anon., 2008).

Among different sources responsible for yield loss of wheat crop in Azad Jammu and Kashmir and Pakistan, one of them is fungal diseases that play a vital role in reducing and destroying wheat yield. Like other crops, wheat suffers from a number of seed and soil borne fungal diseases (Ahmed, 1994). Several fungal pathogens that cause wheat root rots by invading and colonizing in the root of wheat plants and enter in tissues of wheat seedlings. In wheat plants, the crown and root tissues that effected by fungal pathogens have been destroyed. Their water receiving and nutrient uptake has been blocked (Mesterhazy *et al*., 2005). The fungi *Rhizoctonia solani* and *Rhizoctonia oryzae* cause a root rotting disease of wheat. Rhizoctonia root rot is currently a major problem in the Pacific Northwest United States, Asia, Australia and parts of Europe (Pumphrey *et al*., 1987; Smiley *et al*., 1990; Weller *et al*., 1986). The pathogen causes localized patchy, stunted areas in the field and is sometimes referred to as bare patch or purple patch (Carling & Kuninaga, 1990; Ogoshi *et al*., 1990).

Rhizoctonia species are common causing factor of root rot and damping off diseases that like moist and cool conditions. These are the major stress factors involves inducing disposition and increasing susceptibility of potential host plants (Grogan, 1981). Sandy soils is very effective culture media for the rapid of this fungus (Gill *et al*., 2000). It was observed that winter wheat suffered mainly by *R. solani* and *R. cerealis* (AG-8) strains in North part of America and some part of Europe (Hamada *et al*., 2011; Guo *et al*., 2012). Similarly, in Australia AG-1 and AG-8 are more effective anastomosis groups while in Turkey other five different anastomosis groups of *R. solani* were effective more against root rot disease (Demirci, 1998; Tunali *et al*., 2008). *R. cerealis* and *R. solani* has observed in South Eastern part of Hungary that destroy spring wheat crop (Oros *et al*., 2013). Similarly, in another study, different biocontrol agents were used against Rhizoctonia root rot and it suppressed the disease severity and consequently enhanced the growth characteristics of tomato crop (Abdeljalil *et al*., 2016).

Fusarium spp. are also the main soil borne pathogens that are more famous to plant diseases and reduced economy in agricultural sector (Bentley *et al*., 2006; Bockus *et al*., 2007). Fusarium diseases are major problems in agricultural fields and ultimately it is a source of yield deterioration in different important crops. Some common diseases of wheat crop caused by Fusarium species are as; dry rot of potato, wilting of plants at seedling level, decline of beans or pea cereals, crown rot of wheat, head blight of wheat and bakanae disease on rice. These all diseases caused by this pathogen are a source of yield losses in agricultural field crops (Saremi *et al*., 2007a). Several Fusarium pathogens cause essentially similar symptoms on different crops such as yellowing, rosette and premature death of infected crop plants. On the other hand, some species of Fusarium produce mycotoxins that are harmful for animals (Summerell *et al*., 2001). Fusarium diseases on Wheat had been controlled through using different management techniques like fertilizers treatment, fungicides treatment and biological treatments. These all techniques played an important role in wheat growth and yield in study area (Akgul & Erkilic, 2016).

Many agronomists are trying to produce resistant varieties of wheat against fungal diseases through screening and biological control agents (Corrazza *et al*., 1987; Ahmed and Bakar, 1991; Harlapur *et al*., 1993, Ahmed *et al*., 2009). In biological control agents (BCA), induced systemic resistance (ISR) by plant growth promoting rhizobacteria (PGPR) has been observed in few crops. PGPR have been applied in different experimental trails under greenhouse and field for ISR against fungal diseases in different crops (wheat, corn, tomato, cucumber, tobacco and radish etc.) as described by Chen *et al*., (1995). Marleny *et al*., (2008) found that PGPR are beneficial bacteria that enhance growth of plants by protecting it from certain pathogenic diseases. Some scientists are implementing or establishing beneficial microbial communities such as PGPR to promote health of soil ecosystem that suppress the fungal pathogens and other dangerous pests (Kloepper and Ryu, 2006; Lucy *et al*., 2004). Gasoni *et al*., (1998) studied that some bacteria belongs to Pseudomonas and Bacillus used as BCA. Yehia *et al*., (1988) observed the antagonistic effect of *Trichoderma viride* against *Fusarium solani*. Seed dress with a fungus, *Trichoderma viride* promoted growth characteristics such weight and length of shoots, root length and weight roots and nodules number. Hamza *et al*., 2016 and Nelsson (1992) had reported that *Trichoderma viride* is a specific BCA against Rhizoctonia solani root rot pathogens which can eradicate it effectively. It was evaluated the positive effects of *Bacillus subtilis* and *Trichoderma harizianum* alone or in combined with different chemicals (Captan-400 and Vitavax-200) as BCA treatments against the dry bean root rot pathogens (Jensen *et al*., 2002).

Various methods are available for the control of soil-borne diseases (root rot diseases) of wheat varieties. However, development of resistant varieties and management of root rot diseases of wheat crop through biological control agents (BCA) are methods that are more reliable. Hence, the present research work focused on the screening of root rot diseases caused by *Fusarium graminearum* and *Rhizoctonia solani* of selected wheat varieties under greenhouse conditions. To find out any resistant/tolerant variety against these root rot diseases of wheat for future cultivation in Bhimber, Azad Jammu and Kashmir. To apply different BCA techniques for management of root rot diseases under greenhouse conditions and evaluate their impacts on growth parameters of wheat crop.

Materials and Methods

Collection of seed germplasm: Seeds of eight wheat varieties, namely Fareed-2006, Seher-2006, Lasani-2008, Faisalabad-2008, Millat-2011, Aas 2011, Punjab-2011 and Galaxy-2013 were collected for screening of soil-borne diseases from Agricultural Department of Azad Jammu and Kashmir, Pakistan. Then, these seeds germplasm were surface sterilized, packed in sterile bag and brought to Dr. Tony Adesemoye Laboratory, Department of Plant Pathology, University of Nebraska, Lincoln (USA) for further experimental trails under greenhouse conditions.

Collection of fungal isolates and their sub-culturing: One strain of *Rhizoctonia solani* and one strain of *Fusarium graminearum* were obtained from the preserved stock of Dr. Tony Adesemoye Laboratory, Department of Plant Pathology, University of Nebraska, Lincoln, USA. These strains were sub-cultured on already autoclaved PDA-t medium in aseptic conditions under laminar flow hood. Then these strains incubated at room temperature (28°C±2) for seven days in growth chamber for further inoculation on wheat seeds sowing in pots under greenhouse conditions (Backman *et al*., 1975; Botha *et al*., 2005).

Biocontrol agents collection: All the biocontrol agents (*Burkholderia ambifara, Bacillus subtilis, Trichoderma viride,* CruiserMaxx and Actinovate) were also obtained from the preserved stock of Dr. Tony Adesemoye Laboratory, Department of Plant Pathology, University of Nebraska, Lincoln, USA. These *Burkholderia, Bacillus* and *Trichoderma viride,* were sub-cultured under aseptic conditions for further use in experimental trials.

Screening by agar plug inoculation method: In the current technique, culture medium (sterile soil) was put in each pot under greenhouse conditions. Then dig up a hole in the center of each pot. After that put a single seed of each variety in each hole. At the same time two holes were dug up in each pot and inoculated two plugs of one pathogen in each pot. Similar procedure was repeated for each pot. The experiment arranged as 8x3 factorial with 4 replicates using pots. In this way, 96 pots were used. Thirty two pots were inoculated with *Fusarium graminearum* 2mm agar plug, 32 pots inoculated with *Rhizoctonia solani* 2mm agar and 32 pots did not inoculate (control). After that all these pots were irrigated according to given time frame (15 minutes twice a day). Harvesting have done after 3 weeks interval after inoculation. Then, severity of root rot diseases was rated against each pathogen for analysis of susceptibility or resistance of eight wheat varieties. After observations, it was concluded that which variety is better for future sowing (Dhingra and Sinclair, 1995; Michereff *et al*., 2008 and Goudjal *et al*., 2014).

Preparation of biocontrol agents suspension: We prepared suspensions of two bacteria (*Burkholderia ambifara* and *Bacillus subtilis*), one fungal species (*Trichoderma viride*), combined suspension of three organisms, suspension of fungicide (Cruiser Maxx) and commercially available product (Actinovate) for treatments to wheat varieties. i. *Burkholderia ambifara* cells suspension was prepared from already grown culture in nutrient broth (TSB media) before 48 hours. The bacterial cells washed by centrifugation at 3500 x *g* for 15min, twice re-suspending each bacterial pellet in 10ml sterile distilled water. The concentration of washed cells adjusted to 10^6 CFU mL^{-1}. Then the bacterial cells transferred in 40ml distilled water for bacterial suspension. ii. Similar procedure repeated for *Bacillus subtilis* suspension as described above for *Burkholderia ambifara* (Monteiro *et al*., 2014) iii. For *Trichoderma viride* suspension, it was sub-cultured on sterilized PDA media for 5-7 days. After that 10ml, distilled water injected by sterile pipette into those petri dishes that containing already *Trichoderma viride* culture. Scraped the fungal mycelium by sterile needle and passed through sterilized cheesecloth in autoclaved conical flask. Then 40ml more ddH_2O added to the suspension and shacked gently for making good fungal culture suspension of 50ml (Hicks *et al*., 2001). iv. Suspension of these three organisms was prepared collectively by using equal amount of each BCA. 10ml Concentrated suspension of each was transferred in 120ml ddH2O. v. CruiserMaxx suspension prepared by using recommended procedure (10 oz. /100lb) by Knodel & Boetel, 2016). By adjusting this as added 25ml of the product to 100ml distilled water and shacked gently for uniform suspension. vi. Commercial product "Actonovate" suspension was also prepared by recommended-dilute 5.0 oz. product/100 gallons of water. By adjusting this, transferred 1g of the product in 2L sterilized water (Knodel & Boetel, 2016).

Biocontrol agents activity

Seed dressing and BCA treatments: Seeds of each wheat variety were mixed thoroughly in each prepared BCA suspension for 30mint under laminar flow. After that, treated seeds were allowed to dry for 2 days before planting. Then two seeds of each variety were planted in each pot. Similar procedure repeated for each variety and total four replicates were sown. At the same time, 2ml suspension of each BCA pipped on each seed again for soil drenching. Pathogen inoculated by using agar plug inoculation method as described above. After that all these pots were irrigated 15 minutes twice a day. Harvesting have done after 4 weeks interval after treatments and measured results (Smith *et al*., 2003; Paulitz *et al*., 2002).

Significant importance (statistical analysis): The experimental trails set up in the current project as completely randomized block design (CRBD). Each values is a mean of four replicates. By the help of SAS, software designed analysis of variance (ANOVA). Means of all results were compared by least significant difference (LSD) values for their significance.

Results

A preliminary screening of eight wheat varieties against two root rot causing pathogens *Fusarium graminearum* and *Rhizoctonia solani* are conducted under greenhouse conditions (controlled conditions). After that, different biological control agents (BCA) were used as a treatment for the control of these two pathogens under similar conditions. Wheat seeds dressing with different BCA treatments were given before two days sowing and then similar treatments were repeated at the time of sowing as soil drenching. All experimental trails were harvested and measured disease severity, root fresh and dry weight after 4 weeks. Repetition of experimental trails were shown similar results. Therefore, did not expressed repeated results in the article.

Preliminary screening of wheat varieties: Screening of eight-wheat varieties (WVs) germplasm against *Fusarium graminearum* and *Rhizoctonia solani* was presented in Table 1. It was observed that one WV (Galaxy-2013) indicated resistance against *R. solani* and three (Faisalabad-2008, Millat-2011 and Punjab-2011) were moderately resistant. Other five varieties were shown susceptibility against *R. solani*. Highest rating against *R. solani* was observed in WV Seher-2006 (5.0^{A}) while lowest was shown by Galaxy-2013 (1.5^{FG}). On the other hand, *F. graminearum* was more vulnerable to WVs. None of the observed varieties showed resistant against *F. graminearum.* Only Punjab-2011 was rated as moderately resistant to *F. graminearum. It was* estimated that three WVs were moderately susceptible, three susceptible and one highly susceptible against *F. graminearum.* Maximum rating was observed in Seher-2006 (6.25^{A}) while minimum in Punjab-2011 (3.0^{B-E}) as indicated in Table 1. Pathogenicity test against both of these inoculated pathogens showed significance difference as mentioned in Fig. 2. The field data of trials conducted in US green house are presented in Figs. 5-7.

Table 1. Screening of eight wheat varieties inoculated with two-root rot causing fungal pathogens under greenhouse conditions (Harvested after 3 weeks).

Wheat Varieties	Fungal pathogens inoculation and disease rating					
	R. solani	**Control**	***Status**	***F. graminearum***	**Control**	***Status**
Fareed-2006	4.25^{AB}	1.5^{FG}	S	3.25^{BCD}	2.0^{CDE}	MS
Seher-2006	5.0^{A}	2.25^{DEFG}	S	6.25^{A}	2.25^{CDE}	HS
Lasani-2008	4.0^{ABC}	2.25^{DEFG}	MS	3.75^{BC}	3.0^{BCDE}	MS
Faisalabad-2008	2.75^{CDEF}	1.5^{FG}	MR	4.75^{AB}	2.0^{CDE}	S
Millat-2011	3.0^{BCDE}	1.75^{EFG}	MR	4.25^{B}	1.5^{DE}	S
Aas-2011	3.5^{BCD}	2.0^{EFG}	MS	4.5^{AB}	2.0^{CDE}	S
Punjab-2011	2.5^{DEF}	1.5^{FG}	MR	3.0^{BCDE}	1.75^{DE}	MR
Galaxy-2013	1.5^{FG}	1.0^{G}	R	3.75^{BC}	1.25^{E}	MS
LSD (5%)	**1.33**			**1.79**		

Key: Different letters show significance difference; ***Status**: 1-2= Resistant (R), 2-3= Moderately Resistant (MR), 3-4= Moderately Susceptible (MS), 4-5= Susceptible (S), 5-7= Highly Susceptible (HS); **Rating Scale (1-7*)**: 1= No visible symptoms, 2= Less than 10% infection, 3= 11-30% infection, 4= 31-50% infection, 5= 51-70% infection, 6= 71-90% infection, 7= More than 90% infection

Table 2. Effect of different biological control agents (BCA) on severity of *Rhizoctonia solani* against wheat varieties under greenhouse conditions (Harvested after 4 weeks).

Treatments	*Rating (1-7) of wheat varieties inoculated with *Rhizoctonia solani*							
	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8
T1	2.0^{BC}	1.5^{C}	1.75^{B}	2.0^{B}	2.25^{AB}	2.0^{B}	1.75^{B}	1.75^{AB}
T2	2.25^{BC}	2.75^{B}	1.75^{B}	1.75^{B}	2.0^{AB}	2.25^{B}	1.25^{B}	1.5^{AB}
T3	1.75^{BC}	1.75^{BC}	1.5^{B}	1.25^{B}	1.75^{AB}	1.75^{B}	1.5^{B}	1.25^{B}
T4	2.75^{B}	1.5^{C}	1.5^{B}	2.0^{B}	1.75^{AB}	2.0^{B}	1.75^{B}	1.5^{AB}
T5	1.25^{C}	1.75^{BC}	1.75^{B}	1.5^{B}	1.5^{B}	1.5^{B}	1.75^{B}	1.5^{AB}
T6	2.5^{B}	2.25^{BC}	1.75^{B}	2.0^{B}	2.25^{AB}	2.25^{B}	2.5^{B}	2.25^{AB}
T7	4.0^{A}	4.25^{A}	4.75^{A}	4.5^{A}	3.0^{A}	3.75^{A}	4.75^{A}	2.5^{A}
T8	2.75^{B}	2.25^{BC}	1.5^{B}	1.5^{B}	2.0^{AB}	1.5^{B}	1.5^{B}	1.75^{AB}
LSD (5%)	**1.14**	**1.21**	**0.97**	**0.96**	**1.33**	**1.36**	**1.29**	**1.07**

Key: Different letters show significance difference; **Treatments:** T1= *Trichoderma viride*, T2= *Bacillus subtilis*, T3= *Burkholderia ambifara*, T4= Combination of three treatments (T1, T2 and T3), T5= CruiserMaxx (Fungicide), T6= Actinovate (Commercially available biocontrol product), T7= With pathogen but without BCA (Negative Control), T8= Without pathogen and BCA (Positive Control); ***Rating Scale (1-7)**: 1= No visible symptoms, 2= Less than 10% infection, 3= 11-30% infection, 4= 31-50% infection, 5= 51-70% infection, 6= 71-90% infection, 7= More than 90% infection

Correlations of biocontrol agents with *Rhizoctonia solani*: The disease severity (DS) of *R. solani* was measured after BCA treatments by using 1-7 rating scale (Fig. 1). Highest severity was shown by negative control (T7) in all WVs. It was estimated that all BCA treatments minimized DS, significantly. Biocontrol treatments against Fareed-2006 (V_1) indicated significant difference as compared to control. It means that DS of V_1 was minimized after different BCA treatments significantly. Much more minimum DS 1.25 was observed after T5 (CruiserMaxx) treatment. Similarly, lowest DS 1.5 was observed in Seher-2006 (V_2) after T1 (*Trichoderma viride*) and T4 (BCA combined treatment) treatments. Significantly, a little bit difference was shown all BCA treatments against V_2. Lasani-2008 (V_3) severity was reduced significantly but all treatments were indicated similar disease reduction against V_3. Similar results were observed against Faisalabad-2008 (V_4), Millat-2011(V_5), Aas-2011 (V_6), Punjab-2011 (V_7) and Galaxy-2013 (V_8) as indicated in Table 2. It means that they did not show significant difference among treatments and all treatments have equal probability of *Rhizoctonia* root rot disease reduction (Fig. 3).

Correlations of biocontrol agents with *Fusarium graminearum*: The disease severity (DS) of *F. graminearum* was also observed after BCA treatments by using same rating scale as indicated in Fig. 1. Maximum DS was shown by T7 (without treatment/-ve control) against all WVs. It was observed that T3 and T4 reduced maximum DS of V_1 as indicated 1.5^{B} and 1.75^{B}. Similarly, T3 and T4 BCA treatments were minimized DS more in other seven WVs ranged from 1.5^{B}-1.75^{BC} as comparison to other applied treatments. Although, a small difference was shown against all BCA treatments. However, biocontrol treatments against all analyzed WVs did not show significant difference among BCA treatments. So, all treatments were depicted that similar disease severity reduction results against all WVs and therefore all treatments have equal chance of *Fusarium* root rot disease control. It was also estimated that all BCA treatments minimized DS significantly when compared to control (T7). It means that all treatments were effective against *F. graminearum* pathogen (Table 3). Statistical analysis among wheat varieties were measured through SAS software by using GLM procedure (Fig. 4).

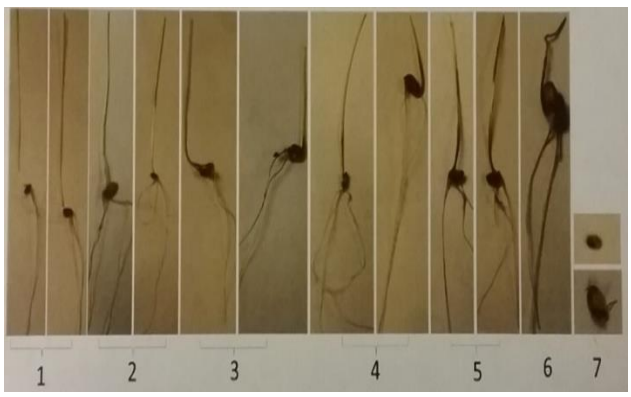

Fig. 1. Experimental Field Survey Showing Occurrence of Disease Severity on Whaet Varieties; Rating scale (1-7) used in the current study: 1= No visible symptoms, 2= Less than 10% infection, 3= 11-30% infection, 4= 31-50% infection, 5= 51-70% infection, 6= 71-90% infection, 7= More than 90% infection.

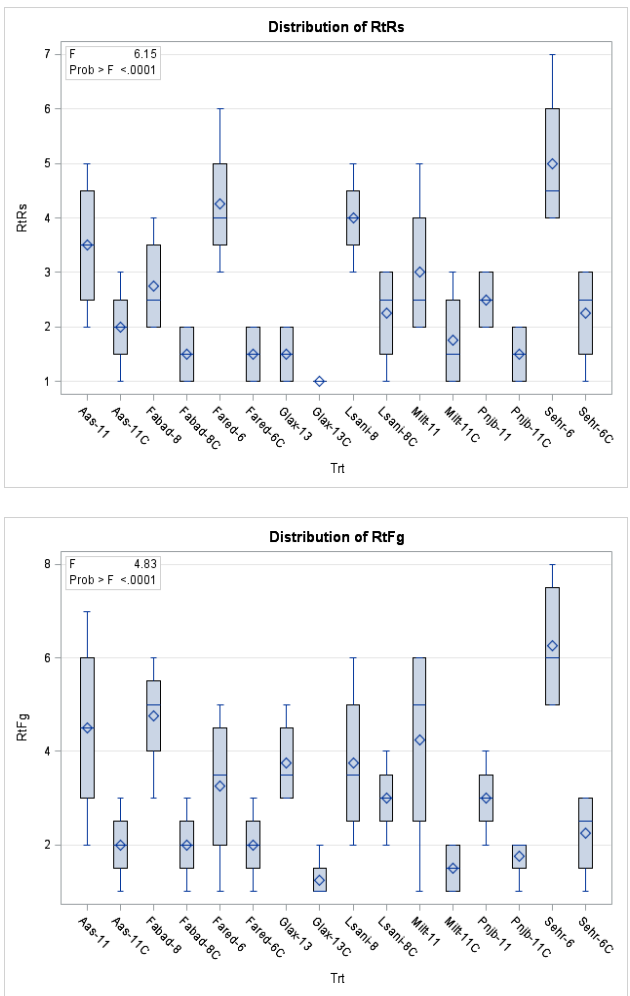

Fig. 2. Statistical analysis of rating of Diseases Severtiy casued by *Rhizoctonia solani* (RtRs) and *Fusarium graminearum* (RtFs) on eight wheat varieties.

Impact of BCA on root fresh and dry weight (*R. solani*): Seeds of different wheat varieties were treated with different BCA, harvested after 4 weeks and measured fresh and dry weight of root. As indicated in Table 4, root fresh weight (FW) and dry weight (DW) of V_1 variety was increased after BCA treatments as compared to control (pathogen treatment). Treatment T4 showed maximum FW (0.11g) and DW (0.28g). Variety V2 indicated different results as FW and DW did not show significant difference between treated and non-treated samples. Similar results were expressed among all other observed varieties. However, it was noted that all varieties treated plants with BCA showed more FW and DW of roots as comparison to control (-ve control). If we compared BCA treatments, maximum increased FW and DW of roots against T4 treatment as mentioned in Table 4.

Impact of BCA on root fresh and dry weight (*F. graminearum*): Fresh weight (FW) and dry weight (DW) of roots were affected by attack of pathogen *F. graminearum* as indicated in Table 5. Maximum FW and DW (0.49g, 0.35g) of V_1 roots were measured against T3 treatment while minimum mass (0.05g and 0.04g) was calculated against T7 (Table 5). Variety V_2 indicated highest FW and DW (0.19g and 0.17g) against T2 while minimum weight (FW=0.06g and DW=0.05g) observed against T6, T7 and T8. Lasani-2008 (V_3), maximum FW and DW weight (0.23g and 0.12g) was observed against T3 and minimum (0.07g and 0.06g) indicated against T7. Variety V_4 showed maximum FW and DW (0.13g and 0.12g) against T2. Similarly, V_5 and V_6 indicated highest FW and DW against T3. However, maximum fresh and dry weight of V_7 and V_8 roots was observed against T4 BCA treatment. It was noted that treatments T3 and T4 increased better FW and DW among all BCA treatments. Overall, it was noted that all treatments were effective against *F. graminearum* pathogen and increased biomass of root and shoot (Table 5). The lab and green trails are being shown here depicting impact of rot disease and its control by BCA (Figs. 5-7).

Discussion

Wheat crop screening against pathogens is a key procedure to categorized the cultivars as resistance or susceptible. For that purpose, pathologists should regularly check the susceptibility of wheat varieties every year and then recommend resistant varieties for next year sowing. This research work focused on management of two pathogens, *Fusarium graminearum* and *Rhizoctonia solani* against eight wheat varieties grown in district Bhimber Azad Jammu and Kashmir, Pakistan by using different biocontrol agents (BCA) treatments under greenhouse conditions in University of Nebraska Lincoln, USA.

Screening of wheat varieties: In recent study, screening of eight-wheat varieties (WVs) was completed against *Rhizoctonia solani* and *Fusarium graminearum* root rot causing pathogens in February 2017. Galaxy-2013 (V_8) was declared as resistance against *R. solani* for year 2016-17 and three varieties (Faisalabad- 2008, Millat-2011 and Punjab-2011) were announced as moderately resistant to *R. solani* root rot. Rhizoctonia root rot can causes serious yield losses of wheat crop. It was reduced yield upto 23% in pathogen inoculated experimental trails. It should be controlled by tillage or applying non-selective herbicide at least two weeks before planting. These techniques can reduce possibility of infection by Rhizoctonia root rot if it

is present in the soil (Neate, 1989, Botha *et al*., 2005). Overall susceptibility of one respective variety influenced only the frequency of various symptoms of disease caused by *Rhizoctonia* and on taxonomic traits or origin of various strains of *R. solani*. Hypersensitive reaction (HR) suggesting the defense mechanism against *Rhizoctonia* attack rapidly activates (Oros *et al*., 2013). Further studies in future should be conduct with special reference to wheat varieties that response to *Rhizoctonia* infection from soil as the manipulation of HR into different breeding programs. This may be an open door towards to the management of *Rhizoctonia* root rot disease (Lucas *et al*., 1993; Iakimova *et al*., 2005).

It was observed that none of the varieties created complete resistant against *F. graminearum.* Although, one wheat variety (Punjab-2011) was rated as moderately resistant against *F. graminearum.* Comparatively, it was estimated that *F. graminearum* was more vulnerable to analyzed WVs as comparison to *R. solani*. According to statistical analysis, all wheat varieties were shown significance difference. Many plant pathologists described and supported these results (Smith *et al*., 2003; Paulitz *et al*., 2002; Vajna & Oros, 2005; Gill *et al*., 2002; Al-Abdalall *et al*., 2010). Thus the importance of selection of resistant wheat cultivars increased yield significantly (Bruton 1998; Bentley *et al*., 2006; Oros *et al*., 2013).

Correlations of biocontrol agents with *Rhizoctonia solani*: It was estimated that all BCA treatments minimized disease severity (DS) of *R. solani*. The treatment of fungicide 'CruiserMaxx' control much more DS. Similar results were obtained by Kader *et al*., (2012). Significantly, a small difference was observed against all BCA treatments. It means that all treatments reduced disease severity and effective against *R. solani*. Similar results were observed many other researchers (Smith *et al*., 2003; El-Fiki *et al*., 2008; Alamri *et al*., 2012; Matloob & Juber, 2013; Zaman *et al*., 2015). Some other scientists were agreed that seed dressing treatment with BCA before sowing only sufficient control of pathogens threatening the cultivated plants in early stage of development (Harman, 1991; Soleimani *et al*., 2005; Oros *et al*., 2013).

Correlations of biocontrol agents with *Fusarium graminearum*: Different biocontrol agents (BCA) were used for control of disease severity (DS) of *F. graminearum*. It was observed that T3 and T4 treatments were reduced maximum DS. However, BCA treatments against all analyzed WVs did not show significant difference. So, all treatments were reduced DS of WVs and therefore all treatments have equal power to control of DS of *Fusarium* root rot. It was also estimated that all BCA treatments minimized DS significantly when compared to control. These results were reflected that all treatments were effective against *F. graminearum* pathogen. These findings correlated with results of Yu *et al*., (2006) and Han *et al*., (2012). They justified that transgenic expression of gene 'lactoferrin' in wheat cultivars imparts visible resistance against root rot diseases cause by *F. graminearum* both in vitro and in vivo. Biocontrol agents promoted expression of gene 'lactoferrin'. This is a new approach to control a potentially dangerous wheat disease caused by *F. graminearum*. Paulitz *et al*., (2002) was also analyzed and screening of cereal pathogens having soil-borne nature. They described correlation of DS with management strategies. Some other scientists have been provided similar solid reasons that supports current study (Amini *et al*., 2010; Foroutan, 2013; Noumavo *et al*., 2015).

Impact of BCA on root fresh and dry weight (*R. solani*): Root fresh weight (FW) and dry weight (DW) significantly reduced after *R. solani* inoculation as indicated in Table 4. It was observed that all varieties treated with BCA showed more FW and DW of roots as comparison to control (treated with pathogen). If we compared between BCA treatments, maximum increased FW and DW of roots against T4 (mixture of *Trichoderma viride*, *Bacillus subtilis* and *Burkholderia ambifara*) treatment. It means that all BCA treatments were reduced or eradicated the infection of *R. solani* (causal agent of root rot). Many plant pathologists were agreed and supported these findings (Lewis *et al*., 1996; Cook *et al*., 2002; Moubarak *et al*., 2011; Alamri *et al*., 2012; Mavrodi *et al*., 2012; Noumavo *et al*., 2015).

Table 3. Evaluation of different biological control agents' activity against *Fusarium graminearum* of wheat varieties under greenhouse conditions (Harvested after 4 weeks).

Treatments	*Rating (1-7) of wheat varieties inoculated with *F. graminearum*							
	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8
T1	2.0^{B}	3.0^{B}	2.5^{B}	2.25^{AB}	2.0^{B}	2.25^{B}	2.0^{AB}	1.25^{B}
T2	2.0^{B}	2.0^{BC}	2.25^{B}	1.75^{AB}	2.5^{B}	2.25^{B}	1.75^{B}	1.75^{B}
T3	1.5^{B}	1.75^{BC}	1.75^{B}	1.5^{B}	1.75^{B}	1.5^{B}	1.5^{B}	1.5^{B}
T4	1.75^{B}	1.75^{BC}	2.0^{B}	1.5^{B}	1.75^{B}	1.75^{B}	1.5^{B}	1.5^{B}
T5	2.0^{B}	2.0^{BC}	2.5^{B}	2.25^{AB}	2.0^{B}	2.25^{B}	2.0^{AB}	1.75^{B}
T6	3.0^{AB}	2.0^{BC}	2.75^{B}	1.75^{AB}	3.0^{B}	2.5^{B}	2.0^{AB}	2.25^{B}
T7	3.75^{A}	5.5^{A}	4.5^{A}	3.0^{A}	5.25^{A}	4.75^{A}	3.25^{A}	3.5^{A}
T8	1.75^{B}	1.5^{C}	1.75^{B}	2.0^{AB}	1.5^{B}	1.75^{B}	1.5^{B}	1.25^{B}
LSD 5%	**1.40**	**1.32**	**1.74**	**1.36**	**1.64**	**1.75**	**1.47**	**1.22**

Key: Different letters show significance difference; **Treatments:** T1= *Trichoderma viride*, T2= *Bacillus subtilis*, T3= *Burkholderia ambifara*, T4= Combination of three treatments (T1, T2 and T3), T5= CruiserMaxx (Fungicide), T6= Actinovate (Commercially available biocontrol product), T7= With pathogen but without BCA (Negative Control), T8= Without pathogen and BCA (Positive Control); ***Rating Scale (1-7)**: 1= No visible symptoms, 2= Less than 10% infection, 3= 11-30% infection, 4= 31-50% infection, 5= 51-70% infection, 6= 71-90% infection, 7= More than 90% infection

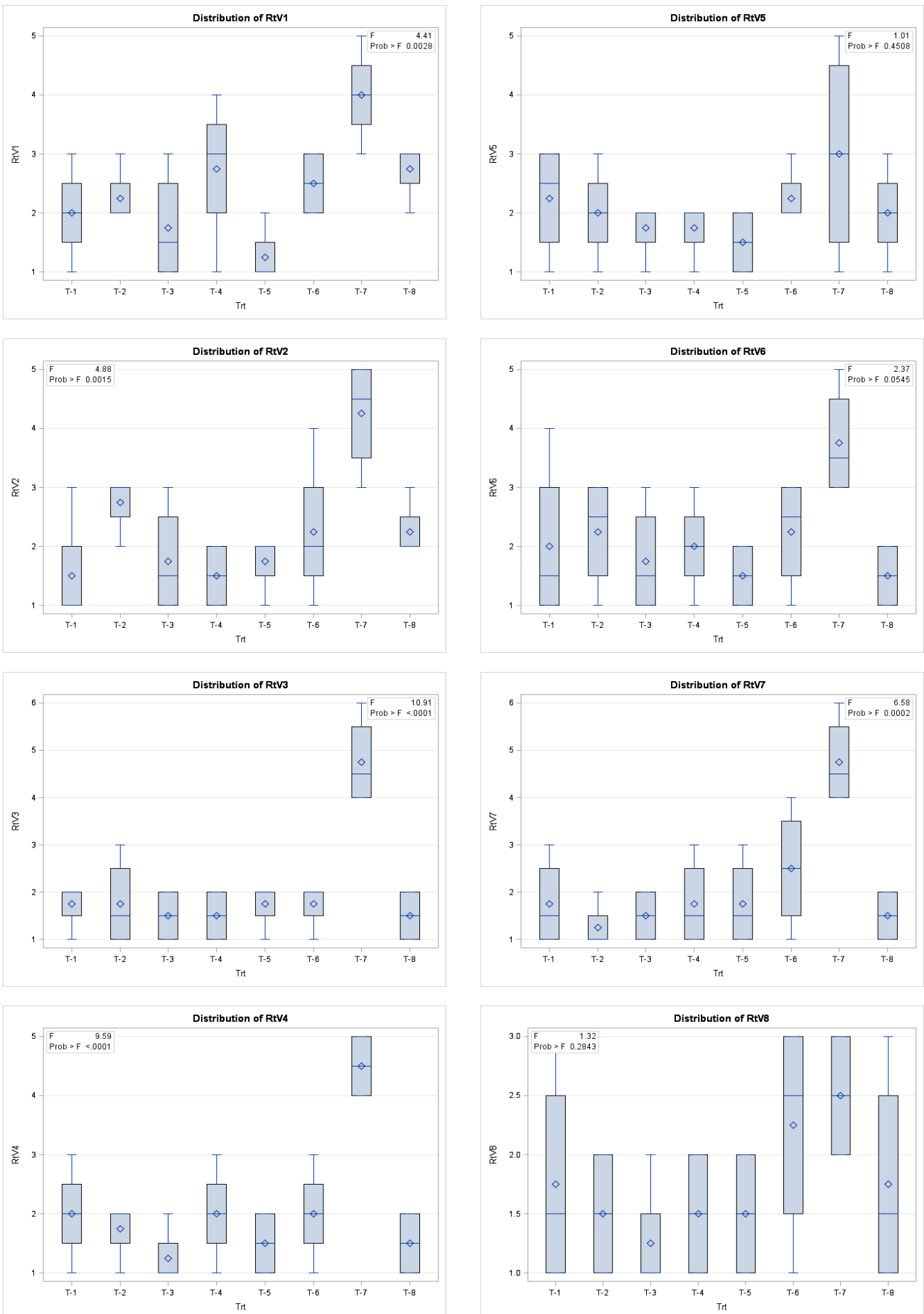

Fig. 3. Statistical representation of different BCA treatments (T1-T8) against severity of *Rhizoctonia* root rot disease of wheat varieties.

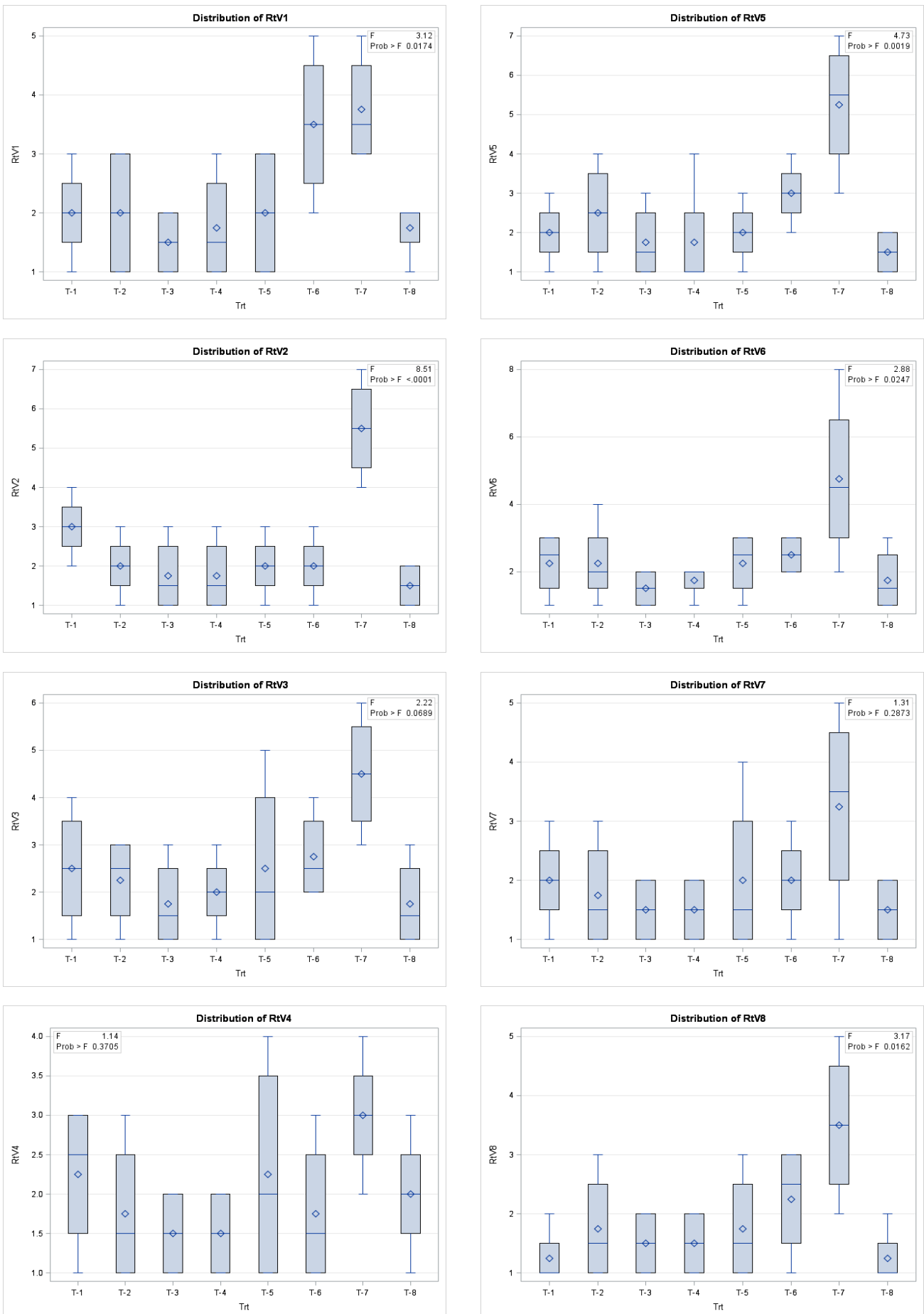

Fig. 4. Statistical representation of different BCA treatments (T1-T8) against severity of *Fusarium* root rot.

Table 4. Measurement of wheat varieties weight after treatments of BCA against *Rhizoctonia* root rot under greenhouse conditions (Harvested after 4 weeks).

Treatments	Measurement of root fresh and root dry weight (gm) of wheat varieties															
	V_1		V_2		V_3		V_4		V_5		V_6		V_7		V_8	
	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW
T1	0.07^{AB}	0.04^{B}	0.05^{A}	0.04^{A}	0.08^{A}	0.07^{A}	0.07^{A}	0.06^{AB}	0.11^{A}	0.09^{A}	0.08^{AB}	0.07^{A}	0.05^{BC}	0.04^{B}	0.10^{AB}	0.08^{AB}
T2	0.09^{AB}	0.04^{B}	0.09^{A}	0.08^{A}	0.07^{A}	0.06^{A}	0.07^{A}	0.06^{AB}	0.10^{AB}	0.30^{A}	0.09^{AB}	0.07^{A}	0.09^{A}	0.08^{AB}	0.10^{AB}	0.09^{AB}
T3	0.07^{AB}	0.06^{AB}	0.09^{A}	0.06^{A}	0.09^{A}	0.08^{A}	0.08^{A}	0.06^{AB}	0.10^{AB}	0.29^{A}	0.13^{AB}	0.08^{A}	0.08^{AB}	0.12^{AB}	0.09^{AB}	0.08^{AB}
T4	0.11^{A}	0.28^{A}	0.08^{A}	0.04^{A}	0.09^{A}	0.08^{A}	0.08^{A}	0.06^{AB}	0.10^{AB}	0.51^{A}	0.11^{AB}	0.09^{A}	0.09^{AB}	0.28^{A}	0.12^{A}	0.10^{A}
T5	0.05^{BC}	0.04^{B}	0.06^{A}	0.03^{A}	0.08^{A}	0.07^{A}	0.08^{A}	0.03^{B}	0.09^{AB}	0.48^{A}	0.14^{A}	0.11^{A}	0.05^{BC}	0.04^{B}	0.07^{BC}	0.06^{B}
T6	0.08^{AB}	0.04^{B}	0.07^{A}	0.05^{A}	0.09^{A}	0.07^{A}	0.07^{A}	0.05^{B}	0.06^{B}	0.05^{A}	0.07^{B}	0.06^{A}	0.03^{C}	0.02^{B}	0.06^{C}	0.05^{B}
T7	0.06^{B}	0.08^{AB}	0.05^{A}	0.03^{A}	0.07^{A}	0.05^{A}	0.09^{A}	0.27^{A}	0.07^{AB}	0.05^{A}	0.08^{AB}	0.06^{A}	0.06^{BC}	0.05^{B}	0.07^{BC}	0.06^{B}
T8	0.07^{AB}	0.06^{AB}	0.06^{A}	0.04^{A}	0.09^{A}	0.07^{A}	0.09^{A}	0.07^{AB}	0.07^{AB}	0.05^{A}	0.09^{AB}	0.07^{A}	0.05^{BC}	0.04^{B}	0.09^{AB}	0.08^{AB}
LSD 0.05%	**0.057**	**0.235**	**0.048**	**0.052**	**0.043**	**0.040**	**0.034**	**0.209**	**0.047**	**0.489**	**0.061**	**0.055**	**0.039**	**0.226**	**0.038**	**0.032**

Key: Different letters show significance difference; **Treatments:** T1= *Trichoderma viride*, T2= *Bacillus subtilis*, T3= *Burkholderia ambifara*, T4= Combination of three treatments (T1, T2 and T3), T5= CruiserMaxx (Fungicide), T6= Actinovate (Commercially available biocontrol product), T7= With pathogen but without BCA (Negative Control), T8= Without pathogen and BCA (Positive Control); **Wheat varieties**: V_1= Fareed-2006, V_2= Seher-2006, V_3= Lasani-2008, V_4= Faisalabad- 2008, V_5= Millat-2011, V_6= Aas 2011, V_7= Punjab-2011, V_8= Galaxy-2013, FW= Fresh weight, DW= Dry weight

Table 5. Measurement of wheat varieties growth characteristics after treatments of different BCA against *Fusarium* root rot under greenhouse experimental trails.

Treatments	Measurement of root fresh and root dry weight (gm) of wheat varieties															
	V_1		V_2		V_3		V_4		V_5		V_6		V_7		V_8	
	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW
T1	0.09^{B}	0.08^{B}	0.09^{BC}	0.07^{B}	0.08^{AB}	0.07^{AB}	0.08^{B}	0.04^{A}	0.08^{B}	0.07^{AB}	0.09^{AB}	0.08^{AB}	0.09^{A}	0.07^{AB}	0.12^{AB}	0.10^{AB}
T2	0.10^{B}	0.09^{B}	0.19^{A}	0.17^{AB}	0.11^{AB}	0.09^{AB}	0.13^{A}	0.12^{A}	0.08^{B}	0.06^{B}	0.13^{AB}	0.11^{A}	0.09^{A}	0.07^{AB}	0.15^{A}	0.12^{A}
T3	0.49^{A}	0.35^{A}	0.14^{AB}	0.11^{AB}	0.23^{A}	0.12^{A}	0.10^{AB}	0.09^{A}	0.10^{A}	0.09^{A}	0.14^{A}	0.12^{A}	0.08^{A}	0.07^{B}	0.13^{A}	0.11^{AB}
T4	0.09^{B}	0.11^{B}	0.09^{BC}	0.08^{B}	0.11^{AB}	0.09^{AB}	0.11^{A}	0.09^{A}	0.09^{AB}	0.07^{AB}	0.10^{AB}	0.09^{AB}	0.11^{A}	0.09^{AB}	0.16^{A}	0.13^{A}
T5	0.08^{B}	0.06^{B}	0.11^{BC}	0.09^{B}	0.11^{AB}	0.09^{AB}	0.09^{AB}	0.05^{A}	0.09^{AB}	0.06^{B}	0.09^{AB}	0.07^{AB}	0.07^{A}	0.06^{B}	0.12^{AB}	0.10^{AB}
T6	0.06^{B}	0.05^{B}	0.06^{C}	0.05^{B}	0.08^{AB}	0.07^{AB}	0.10^{AB}	0.07^{A}	0.09^{AB}	0.08^{AB}	0.09^{AB}	0.08^{AB}	0.08^{A}	0.07^{AB}	0.09^{B}	0.08^{C}
T7	0.05^{B}	0.04^{B}	0.06^{C}	0.05^{B}	0.07^{B}	0.06^{B}	0.04^{C}	0.03^{A}	0.04^{C}	0.03^{B}	0.07^{B}	0.05^{B}	0.07^{A}	0.05^{B}	0.09^{B}	0.08^{C}
T8	0.06^{B}	0.11^{B}	0.06^{C}	0.05^{B}	0.09^{AB}	0.07^{AB}	0.09^{AB}	0.08^{A}	0.07^{B}	0.06^{B}	0.08^{AB}	0.07^{AB}	0.09^{A}	0.08^{AB}	0.09^{B}	0.08^{C}
LSD 0.05%	**0.209**	**0.35**	**0.062**	**0.22**	**0.046**	**0.164**	**0.029**	**0.407**	**0.021**	**0.020**	**0.063**	**0.056**	**0.026**	**0.174**	**0.035**	**0.031**

Key: Different letters show significance difference; **Treatments:** T1= *Trichoderma viride*, T2= *Bacillus subtilis*, T3= *Burkholderia ambifara*, T4= Combination of three treatments (T1, T2 and T3), T5= CruiserMaxx (Fungicide), T6= Actinovate (Commercially available biocontrol product), T7= With pathogen but without BCA (Negative Control), T8= Without pathogen and BCA (Positive Control); **Wheat varieties**: V_1= Fareed-2006, V_2= Seher-2006, V_3= Lasani-2008, V_4= Faisalabad- 2008, V_5= Millat-2011, V_6= Aas 2011, V_7= Punjab-2011, V_8= Galaxy-2013, FW= Fresh weight, DW= Dry weight

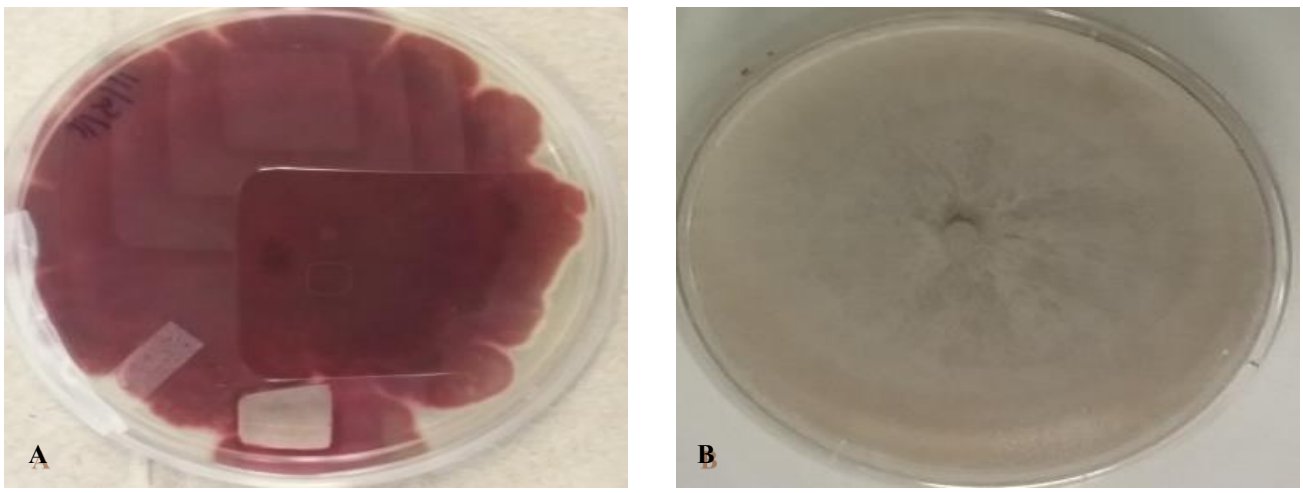

Fig. 5. Culture of two pathogens used in the experimental trails under greenhouse conditions; A. *Fusarium graminearum* B. *Rhizoctonia solani*.

Fig. 6. Biocontrol agents used in experimental trails; 1. *Trichoderma viride,* 2. *Burkholderia ambifara* and 3. *Bacillus subtilis*.

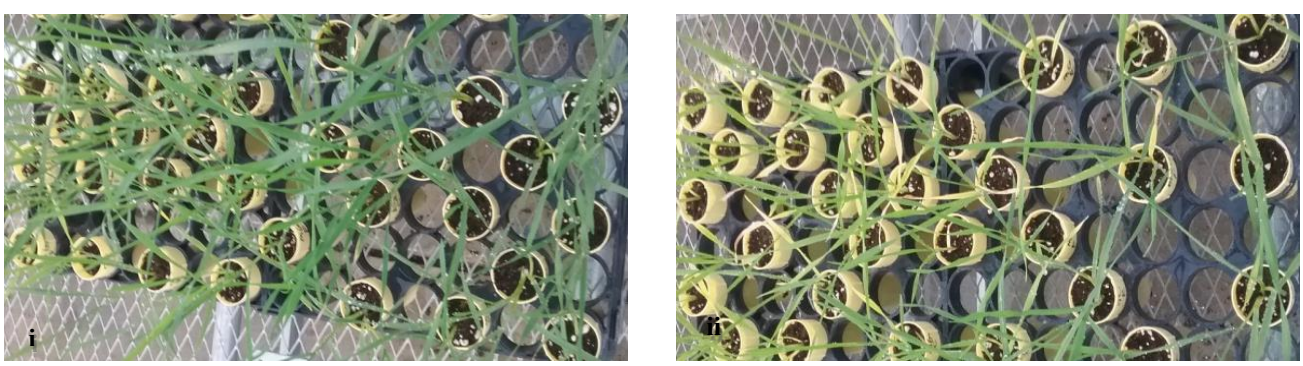

Fig. 7. Experimental trail under greenhouse conditions; i. Wheat seeds growth in conetainers treated with biocontrol agents and ii. Negative control (Pathogen treatment).

Impact of BCA on root fresh and dry weight (*F. graminearum*): It was estimated that fresh weight (FW) and dry weight (DW) of roots were reduced by attack of *F. graminearum* fungal pathogen as indicated in Table 5. After BCA treatments, reduced DS of the pathogen and increased root FW and DW. It was noted that treatments T3 (*Burkholderia ambifara*) and T4 (combination of *Trichoderma viride*, *Bacillus subtilis*, *Burkholderia ambifara*) increased more FW and DW among all BCA treatments. Overall, it was observed that all BCA treatments were effective against *F. graminearum* pathogen and increased biomass of wheat plants root. Cook, (2001) and Bradley *et al*., (2002), obtained similar results. Some other scientists were also supported and justified these findings (Kloepper, 1993; Moubarak *et al*., 2011; Amini *et al*., 2010).

Some scientists described that biocontrol agents have antifungal metabolites and enzymes that induced resistance against root rot diseases. The action of *Trichoderma viride* and *Bacillus* sp. released enzymes and secondary metabolites on pathogens and created binding with the pathogenic organisms. They produced sugar linkage and begins to secrete extracellular protease and lipase (Soleimani *et al*., 2005 and Zaghloul *et al*., 2007;

Ali *et al*., 2009; Abdel-Monaim, 2010). Therefore, it is concluded that BCA treatments are very effective management strategies for the control of fungal pathogens in respective to current and future studies.

Conclusion

This study focused on the screening of eight wheat varieties against *Fusarium graminearum* and *Rhizoctonia solani* root rot fungal diseases. The effect of these pathogens and BCA treatments on growth and yield promotion of WVs were investigated under greenhouse conditions. Galaxy-2013 variety (V_8) was declared as resistance against *R. solani* for year 2016-17 and three varieties (Faisalabad- 2008, Millat-2011 and Punjab-2011) were announced as moderately resistant to *R. solani* root rot. Comparatively, it was estimated that *F. graminearum* was more vulnerable as comparison to *R. solani*. If we compared between BCA treatments, T3 (*Burkholderia ambifara*) an T4 (mixture of *Trichoderma viride*, *Bacillus subtilis* and *Burkholderia ambifara*) treatments increased more FW and DW of roots. However, BCA treatments against all analyzed WVs did not show significant difference. So, all treatments were reduced DS of WVs and increased biomass and yield of wheat plants. Therefore all treatments have equal chance to control DS of *Fusarium* and *Rhizoctonia* root rot diseases and can also enhance growth and yields of wheat crop. The biological control activity of *Trichoderma viride* and *Bacillus subtilis* secreted enzymes and secondary metabolites in wheat plants and induced systemic disease resistance, probably by the activation of the induced systemic resistance pathway in plants against *Fusarium graminearum* and *Rhizoctonia solani*. It is estimated that the management strategies under 'integrated pest management' both agro-chemicals and biocontrol agents influenced on disease suppression and could be considered as disease control agents. Therefore, it is concluded that BCA treatments are very effective management strategies for the control of fungal pathogens in respective to present and future studies. Moreover, pathologists should regularly check the susceptibility of wheat varieties every year and then recommended resistant varieties for next year sowing.

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