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Okra Seeds Priming with Two *Bacilli spp. and TriHar-6* Isolates Enhanced the Physiological Attributes and Suppressed the *Fusarium oxysporum* f. sp. *vasinfectum*.

Muhammad Rizwan Ashraf[1], Osama Bin Abdul Hafeez[1], Ashir Masroor[1*], Shabbir Ahmad[1], Ibtihaj Zaib[1, 2]

[1]University of Agriculture Faisalabad, Sub-campus Burewala, Punjab, Pakistan.
[2]Department of Plant Pathology, Bahauddin Zakariya University, Multan, Pakistan.

*(Corresponding author's email: masroorakb@gmail.com)

ABSTRACT

This study was aimed to determine the antagonistic effect of biological control agents (BCAs) applied for monitoring antifungal activity against *Fusarium oxysporum* f.sp. *vasinfectum* (*Fov*; causal agent of okra wilt disease). Moreover, growth parameters were also monitored upon BCAs application. Seeds primed with the BCAs (i.e., *Bacillus* sp. BL, *TriHar*-6, and *Bacillus* sp. BS with the 0.5 OD, 1.0 OD, and 0.5 OD concentration respectively) and were sown in pots. Laboratory trials for suppression of *Fov* were conducted in the Plant Pathology Lab of UAF Sub Campus Burewala and Experimental Area of UAF Sub-campus Burewala-Vehari. Although *Bacillus* sp. BL, and *Bacillus* sp. BS was effective in the plant growth enhancement, however, when the seedlings were challenged with the pathogen, they failed to suppress the effect of *Fov* as compared to the control and *TriHar*-6. The disease incidence in the case of *TriHar*-6 remained, 41.29%. Control of plant diseases is very important to minimize the economic losses imparted by the pathogens. Fusarium wilt also causes severe damage to the Okra plant. Chemicals controls are very expensive and toxic to the environment. BCAs application for the control of plant diseases is a very effective, sustainable, and durable means to minimizing huge losses induced every year. *Bacillus* sp. BL, and *Bacillus* sp. BS reported in these studies shows a positive effect on plant growth enhancement. They have been tested here for Okra plants these candidates need to be studied for their effect on other important crop plants. Furthermore, their dissection at a molecular level will uncover their role in plant fitness.

Keywords: *Trichoderma harzianum, Bacillus* sp, Okra, and Fusarium wilt.

Introduction

Okra crop is cultivated in sub-tropical areas and tropical areas of the world (Africa and Asia). Okra has a place with the family *Malvaceae* (Kumar et al., 2010). Okra plants require a temperature of more than 20°C for their germination, vegetative growth, and reproductive growth (El-Kader, Shaaban, & El-Fattah, 2010). Okra fruit contains minerals, proteins, fats, and other nutrients which are necessary for human health. Okra seed contains oil which is used as a nutrient source as well as a flavor source (Martin, 1982). Okra oil is additionally appropriate for biofuel purposes (Anwar, Rashid, Ashraf, & Nadeem, 2010).

During its growth Okra plant is attacked by many fungi, bacteria, nematodes, and viruses. Various diseases of okra include bacterial leaf blight *(Pseudomonas cichorii)*, reddish-brown

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spots (*Pseudomonas syringae* pv. *syringae),* and root-knot nematode (*Meloidogyne* sp.). Various diseases are caused by fungal pathogens in the okra plant such as leaf blight (*Cercospora abelmoschi*), blossom rot (*Choanephora cucurbitarum*), Verticillium wilt (*Verticillium albo-atrum*), damping-off (*Macrophomina phaseolina),* southern blight (*Sclerotium rolfsii*), powdery mildew (*Oidium* sp.), and Fusarium wilts (*Fusarium* sp.; (Momol & Pernezny, 2006).

It has been determined that seed priming with biological control agents is an effective method against wilting diseases (El-Kader et al., 2010). Seed priming plays an important role in seed germination and inhibits plant pathogenic growth in the rhizosphere that ultimately enhances the plant growth of individual seedlings (Harman, Howell, Viterbo, Chet, & Lorito, 2004). Primed seed performs well as compared to the untreated seed by enhancing germination in different crops (Parera & Cantliffe, 1994). Plants are also sensitive to abiotic stress but biopriming decreases the sensitivity against abiotic stress. (Anwar et al., 2010). Seed treated with *Trichoderma* sp. prevent the growth of diseases incited by fungi and improve the quality of seed (Harman et al., 2004). *Bacillus subtilis* can restrict the growth of *Pythium aphanidermatum* which causes the damping-off (Leclère et al., 2005).
Elicitor application is an alternate method to control the disease. Elicitors require in very low amount to come into being a defense for the pathogen in plants. Elicitors are the molecules that trigger the resistance mechanism in plants. The natural advantage of the elicitors is that they can be used without carrying a mechanical injury to the plant. Elicitors may be natural products of plants or can be produced artificially. For example, phenolic compounds and salicylic acid (SA), both regulate plant functions (Raskin, 1992).

Complete soil-borne pathogen control is based on the application of fungicide. Chemical sprays are very dangerous to the environment (Davis, Colyer, Rothrock, & Kochman, 2006). In the proposed research, Okra seeds will be primed with different BCAs and then challenged by *Fusarium osysporum* f. sp. *vasinfectum*.

Materials And Methods Collection of seeds

The seeds of okra were collected from the grain market of Burewala. The healthy-looking seeds were selected after an inspection under a microscope for further processing.

Seed Priming

Surface sterilization of seeds was performed to remove dust and surface debris of seeds (Younesikelaki et al., 2016). Okra seeds were surface sterilized in the 0.1% solution of mercuric chloride (Altaf, Khan, Ali, & Bhatti, 2009), solution for 5 minutes, and washed thoroughly before treatment of biocontrol agents (Tomita et al., 1998). Then seeds were dried by spreading them on a clean paper to remove moisture and bring them back to their initial contents. Selected seeds were weighed and were primed with water contained formulations of biocontrol agents with the optical density 0.5, 1.0, 1.5. Thereafter, seeds were primed with the bacterial suspension for 30 minutes by shaking on the orbital shaker. Untreated seeds were considered as control. Then seeds were dried in the incubator for 24 hrs untiled the original weight is occupied.

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Pathogen culture preparation:

The culture of fungus *Fusarium oxysporum* f.sp *vasinfectum* obtained from reliable sources was revived on Potato Dextrose Agar (PDA) culture. Pour 10 ml sterile water into the Petri plate with fungal culture and harvest by scraping the face of media with the help of a sterile loop. Thereafter, pour this water into the beaker containing 140 ml sterile water. Seedlings were challenged with the pathogen (*Fusarium oxysporum* f.sp. *vasinfectum*) 1×10^4 cfu.

Media preparation

For LB (Luria Bertani) media preparation was poured into the flask, and (DW) distilled water was added to make the volume up to 1000ml. To dissolve the ingredients in distilled water, the solution was stirred with the help of a magnetic stirrer until all ingredients dissolved (Dhingra & Sinclair, 1995). Then autoclaved it at 121^{o}C and 15 lbs pressure for 30 minutes.

Inoculum preparation

Bacterial freezes were revived on LB plates. Thereafter, culture was raised from a single colony of bacterium (*Bacillus* sp. BL, and *Bacillus* sp. BS) in an orbital shaker for 24 hrs at a temperature of 27^{o}C. From the revived bacterial culture pick a single colony of bacterial culture and inoculate in the falcon tube. Incubate the inoculated falcon tubes of media at 37^{o}C and 150 rpm in an orbital shaker for 24 hrs. The (OD) optical density of the bacterial suspension was recorded by using a double beam UV-Vis spectrophotometer, PG instrument Ltd (Model T80). Bacterial culture growth readings were recorded at 600nm wavelength using a blank (growth medium only) as a reading reference. Dilution technique was followed as suggested by Alpert and colleagues to set the required concentration of solutions using the mentioned below formula (Alpert, Keiser, & Szymanski, 2012)

$$C_1V_1=C_2V_2$$

Where,

C_1 is the final concentration of dilution.

V_1 is the final volume of the dilution.

C_2 is the initial concentration of the dilution which has been measured by spectrophotometry.

V_2 is the initial volume of the dilution which is added in the cuvette.

Bacterial Growth and their Suspensions

The bacterial handling and cultivation were carried out under the aseptic conditions in the inoculating chamber (Laminar air flow chamber). Bacteria were incubated at 37C^o temperature overnight, culture tubes containing bacteria were placed in an incubator (Model No. IWISP140438) to maintain temperature. Then bacteria suspensions were made in distilled water and the OD of bacteria was checked at 600 nm. Bacteria pellets were obtained after centrifuge at 6000rpm in a centrifuge machine. Then make sure the optical density of bacterial culture is 1.0.

Preparation for invitro trails

Two Beckmann No. 1 filter papers were cut to the size and shape of the plate. Now dip each filter paper in the dilution of pathogen and placed it at the bottom of each petri dish. Okra seeds

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were treated with the bacterial suspension having OD equal to 1.0, were planted in each of the Petri plates in such a way that (the distance between seeds was at least three to five times the seed diameter), except one that was considered as control having seeds without biocontrol agents and also irrigated them with autoclaved distilled water. The plates were covered to ensure that there is no airlock resulting from excess moistures on the cover and transferred out from Laminar Flow Hood. The Petri plates were observed at 24 hours intervals for at least seven days.

Pots preparation

Pots were allotted from the nursery of UAF sub-campus Burewala. Then filled them with the soil and makes the appearance of the ridges in the pots. Seeds were sown in earthen pots containing soil. The pots were irrigated after two days with 100 ml of water. The experiment was conducted in a completely randomized design (CRD) and each treatment was replicated thrice.

Study of the Growth Parameters

The growth parameters of okra plants were recorded. Shoot length of plants of each Petri plate was measured in centimeters (cm) from the base of hypocotyls to the tip of the shoot with the help of a meter rod (Sarathkumar & Dhandhayuthapani, 2016). Similarly, the root length of randomly selected seedlings from each Petri plate was measured in centimeters from the base of hypocotyls to the tip of the longest root with the help of a meter rod (Sarathkumar & Dhandhayuthapani, 2016). For fresh weight, the seedlings were wrapped in filter paper to eradicate any drop of water that is present on the leaves and shoots of plants. Afterward, the digital measuring balance was used for the calculations of fresh weights (Rhoades & Linford, 1959). For recording dry weights, the seedlings were kept in the oven and dried at 80 ^{o}C for the 3 days. Dry weight was recorded by using the digital balance and the average of the dry and fresh weight was calculated (Schuurman & Goedewaagen, 1971). Based on symptomology, the data was collected from each inoculated plant. The pots were kept in a field until clear symptoms were expressed. The evaluation was carried out twentyeight days after inoculation when external symptoms (yellowing, wilt, and leaf fall) appeared in a sub-group of highly susceptible accessions. An ordinal disease severity scale, adapted from (Rao et al., 2014), was used to evaluate the plant responses. The grades of this scale ranged from 0 to 4.

Where,

Stage of Disease	Symptom on Plants
0	No symptoms
1	No symptoms of wilt or yellowing, but with darkened vascular bundles
2	Intensely darkened vascular bundles and with wilt or yellowed leaves
3	Plants with severe wilt, yellowing, and premature leaf drop
4	Dead plants

From the grades, a disease index (DI) was calculated as described by (McKinney, 1923).

$$DI\ (\%) = 100.\Sigma[(f.v)/\ (n.x)]$$

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Where,

	DI = disease index
f = number of plants with the same grade	n = total number of plants evaluated
v = observed grade	x = maximum grade on the scale

These DI data were grouped into classes according to the reaction to the pathogen observed in each treatment:

Classes	Disease Incidence
0%	Like an immune-like response (SI)
0.01-25%	High level of resistance (HR)
26-50%	Intermediate resistance (IR)
51-75%	Intermediate susceptibility (IS)
76-100%	High level of susceptibility (HS)

(Rao et al., 2014)

Data collection

Seedling growth, phenology, were recorded. One plant from each pot was randomly selected for recordings the data. Plant height (cm), root length (cm), shoot length (cm), plant fresh weight (g plant-1) was observed, the whole plant dry weight (g plant-1). After twenty-one days of sowing the plants were uprooted and data on plant height weight and root length were recorded according to the described in the in vitro or Petri plates experiment. Data were collected at 28 dpi.

Experimental Design and Data Analysis

The experiment was laid out in a completely randomized design (CRD) replicated three times. Data were analyzed using the analysis of variance (ANOVA) technique. Comparative analysis of treatment means was carried out using Tukeys HSD test at a 5% level of significance.

Results and Discussion

The study was conducted at UAF Sub Campus Burewala-Vehari. Experiments were conducted in two rounds; the first experiment was conducted in *in-vitro* conditions and the second round was conducted in the *invivo* conditions. The First-round comprise the dose optimization experiment in which seeds were primed with the different doses of BCAs to find the best dose affecting the physiological characters of the okra seedlings

1. Dose Optimization Experiment / In vitro experiment

1.1. The *invitro* effect of *Bacillus* sp. BL on Okra seedlings

The data presented in Table 1 revealed the effect of *Bacillus* sp. BL application on the root length of Okra seedlings, the three concentrations of BL (0.5 OD, 1.0 OD, 1.5 OD, and control) used here harvested different root lengths of Okra seedlings. It shows varied root lengths of Okra seedling (6.36, 6.16, 6.01, and 5.67). These results resembled those presented by (Bai, Zhou, & Smith, 2003), showing that some *Bacillus* strains enhance the plant growth in Soybean (Bai et al., 2003). Moreover, the data also show that among the three bacterial loads applied the optical density of 0.5 (the minimum load) has shown the best effect. The result also shows

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that upon increasing the bacterial load (OD 1-1.5) there is no enhancement in the root length of Okra seedling.

Similarly, the data on shoot length of Okra seedlings, among the three concentrations used showed different shoot lengths of Okra seedlings. i.e., 6.4, 6.24, 6.09 and 5 cm. Among them, *Bacillus* sp. BL suspension at 0.5 OD performed best. In our study, *Bacillus* sp. BL primed seeds at OD 0.5 bacterial suspension comparatively enhanced the shoot length of Okra seedling as compared to the control and other doses (1.0 OD and 1.5 OD). It has been previously reported that seed treated with *Bacillus subtillus* enhances the height of seedling (Murunde & Wainwright, 2018; Okereke, Rehua, & Godwin-Egein). The effect of *Bacillus* sp. BL application on the fresh weight of Okra seedling the three concentrations of *Bacillus* sp. BL used here showed the varied fresh weight of Okra seedling (0.14g, 0.13 g, 0.11 g, and 0.08 g) when okra seeds were treated. Among them *Bacillus* sp. BL suspension at 0.5 OD performed best. As the plant height is increasing the fresh weight is also increasing. It has been reported that as the shoot length is increasing the fresh weight is also increasing (Ayub, Khan, Hussain, Ahmad, & Khan, 2018).

1.2. The *invitro* effect of *TriHar*-6 on Okra seedlings

The effect of *TriHar*-6 application on root length, shoot length and fresh weight of Okra seedling was monitored after the application of three different concentrations of *TriHar*-6. The root lengths of control, 0.5 OD, 1.0 OD, and 1.5 OD remained 5.67 cm, 5.64 cm, 5.64, and 5.67 cm respectively. The shoot lengths of control, 0.5 OD, 1.0 OD, and 1.5 OD remained 5.0 cm, 5.1 cm, 5.1 cm, and 5.2 cm respectively. However, the fresh weights of Okra seedlings remained 0.1g, 0.1g, 0.1g, and 0.1g for all the applied treatments. This suggests that no significant difference for root length, shoot length and fresh weight of Okra seedling was observed for the control or among the treatments. It has been reported that *Trichoderma harzianum* increases the root length of Okra seedlings (Dubey, Suresh, & Singh, 2007).

Table 1: Response of different dozes of BCAs on Okra seedlings.

Strains	Bacterial Load (OD)	Root Length (cm)	Shoot Length (cm)	Fresh Weight (g)
Bacillus sp. BL	0	5.67 d	5.00 d	0.08 c
	0.5	6.36 a	6.40 a	0.14 a
	1	6.16 b	6.24 b	0.13 a
	1.5	6.01 c	6.09 c	0.11 b
		*	*	*
TriHar-6	0	5.67	5.0	0.11
	0.5	5.64	5.1	0.11
	1	5.64	5.1	0.11
	1.5	5.67	5.2	0.11
		ns	*ns*	*ns*

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	0	5.67 d	5.00 d	0.11 b
Bacillus	0.5	7.12 a	6.69 a	0.16 a
sp. BS	1	6.92 b	6.49 b	0.15 a
	1.5	6.77 c	6.34 c	0.10 a
		*	*	*

* Significant at 5% level of significant *ns* Non-Significant at 5% level of significance

He also concluded that the plant *Trichoderma harzianum* produces enzymes that promote the growth of the plant. In our study, *TriHar*-6 has not performed well in enhancing the growth of the Okra plant. The root length was near or equal to the control which means that our *Trichoderma harzianum* isolate *TriHar*-6 may not be considered as a plant growth regulator. On the other hand, (Dawar, Sattar, & Zaki, 2008) reported that a seed priming with *Trichoderma harzianum* influences the shoot length of Okra seedling when compared to the control. Our study revealed that *TriHar*-6 does not enhance or inhibit the shoot length of Okra seedlings.

1.3. The *invitro* effect of *Bacillus* sp. BS on Okra seedlings

Effect of *Bacillus* sp. BS The data revealed the effect of *Bacillus* sp. BS application on the root length of Okra seedlings, the three concentrations (0.5 OD, 1.0 OD, 1.5 OD, and control) used here harvested different root lengths of Okra seedlings. It shows varied root lengths of Okra seedling i.e., 7.12 cm, 6.92 cm, 6.77 cm, and 5.67 cm. The result shows that *Bacillus spp*. exerts the plant growth improving effect on the mung bean plant under the in-vitro experiment. Similarly, the data on shoot length of Okra seedlings, among the three concentrations used showed different shoot lengths of Okra seedlings. i.e., 6.69 cm, 6.49 cm, 6.34 cm and 5.0 cm. Among them, *Bacillus* sp. BS suspension at 0.5 OD performed best. In our study *Bacillus* sp. BS primed seeds at OD 0.5 bacterial suspension comparatively enhanced the shoot length of Okra seedling as compared to the control and other doses (i.e., 1.0 OD and 1.5 OD). The effect of *Bacillus* sp. BS application on the fresh weight of Okra seedling the three concentrations of *Bacillus* sp. BS used here showed the varied fresh weight of Okra seedling (0.16 g, 0.15 g, 0.10 g, and 0.11g) when okra seeds were treated. Among them *Bacillus* sp. BS suspension at 0.5 OD performed best. As the plant height is increasing the fresh weight is also increasing. It is concluded that as the shoot length is increasing the fresh weight is also increasing (Ayub et al., 2018). It has been established that the seeds treated with the plant growth-promoting rhizobacteria enhance the fresh weight when compared to the control (Domenech et al., 2006). Our study revealed that the fresh weight of Okra seedling was more than 0.16g as compared to the control at 0.11g. Tank and Saraf, experiments confirmed that *Bacillus subtillus* screats growth hormones that enhance the physical parameters of the tomato plant (Tank & Saraf, 2010). He also revealed that the use of *Bacillus subtillus* as a growth promoter also boosts the dry matter of the tomato plant.

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2. Pots experiment / *Invivo* Experiment

The second round was conducted under *invivo* conditions in which seeds were primed with optimized doses of BCAs i.e., 0.5 OD bacterial load. After the germination of inoculated seeds, one lot of seedlings was challenged with the Okra wilt pathogen *Fov* and the other lot was kept as such. Seven days after the inoculation of the pathogen the data was collected (from without *Fov* plants and *Fov* inoculated plants) with the seven days intervals and the data was collected till 28 dpi. Recorded parameters include total plant height, root length, shoot length, fresh weight, dry weight, and disease incidence.

2.1. **Okra seedlings shoot length after *Fov* application in a time-course experiment**

Okra seedlings were treated with different BCAs (under *in vivo* conditions) to see the effect of these treatments on plant growth. Out of the treatments three BCAs (i.e., *Bacillus* sp. BL, *TriHar*-6, *Bacillus* sp. BS) and a control (water treated), *Bacillus* sp. BL application resulted in maximum shoot length (3.8 cm) after seven dpi. However, the shoot length in the case of *Bacillus* sp. BS and *TriHar*-6 remained 2.6 cm and 2.9 cm respectively. The shoot length for the control was recorded at 2.5 cm. Similar results are shown by the same treatments at 14, 21, and 28 dpi where bacterial isolates *Bacillus* sp. BL contributed best to the physical attributes of the plant as concluded previously in Petri plate experiments. However, the plant did not respond to the application of *TriHar*-6 and *Bacillus* sp. BS in terms of plant shoot length (Pahari, Pradhan, Maity, & Mishra, 2017). Nevertheless, it has been reported that an isolated *Bacillus pumilus* SE-34 supports the growth of pearl millet (Raj et al., 2003).

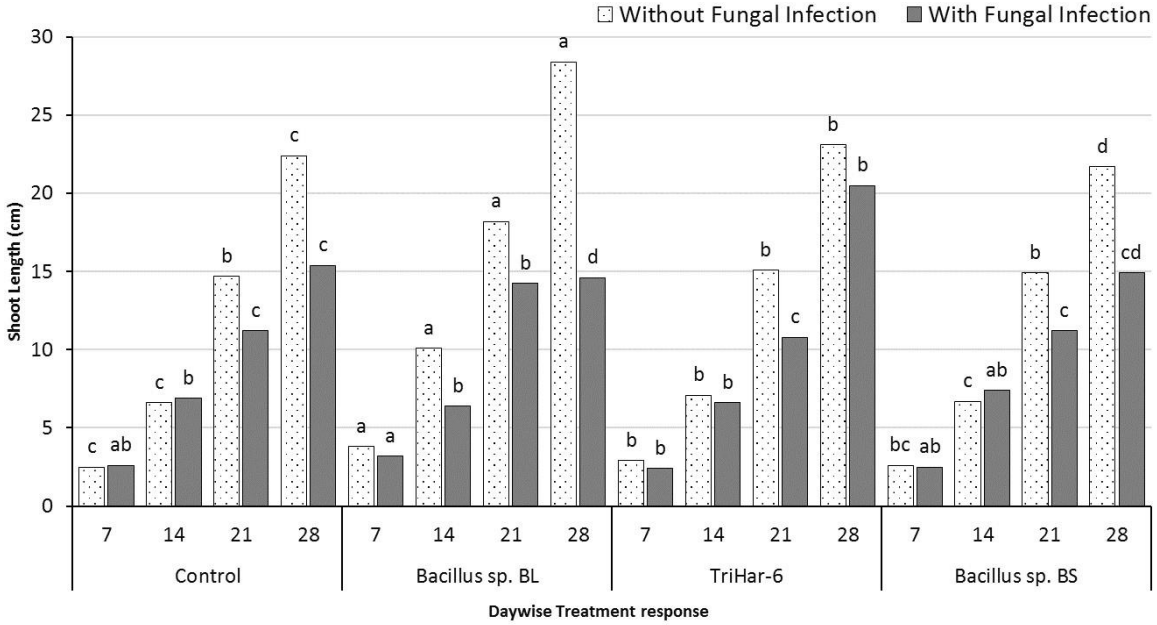

Figure 1: Effect of *Fov* and BCA application on shoot length of Okra**. (**Statistical analysis of strains and lettering was carried out for 7, 14, 21, and 28 days separately)

However, the data recorded after the application of *Fov* on plants already primed with the BCAs didn't show clear results after 7dpi of the *Fov*. Data showed that none of the BCAs applications could sustain the height of the Okra plants after the same plants were challenged with the *Fov* (Fig. 1). The results become even clearer on 21 and 28 dpi of *Fov*. The shoot length of pre-

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primed potted plants after the *Fov* challenge on 21 dpi remained 14.2 cm, 10.8 cm, 11.2 cm, and 11.2 cm for *Bacillus* sp. BL, *TriHar*-6, *Bacillus* sp. BS, and control respectively. However, in the absence of the pathogen shoot length remained 18.2 cm, 15.1, 14.9 cm, and 14.7 respectively.

Similarly, at 28 dpi shoot length of Okra plants primed with *Bacillus* sp. BL, but without *Fov* stress reached 27 cm. the *TriHar*-6 and *Bacillus* sp. BS showed 21.7 cm and 23.1 cm respectively. The shoot length of the control remained 22.4 cm. However, the shoot length of Okra after the *Fov* challenge in *Bacillus* sp. BL, *TriHar*-6, *Bacillus* sp. BS, and controltreated plants remained 14.6 cm, 20.5 cm, 14.9 cm, and 15.4 cm, respectively. Therefore, in this study, the height of the plant treated with *TriHar*-6 was the least affected by the *Fov*. However maximum shoot length was maintained with *Bacillus* sp. BL treatment with *Fov* challenge.

2.2. Okra seedlings root length after *Fov* application

Bacillus spp. has been proved to increase the shoot length of the mung bean plant due to the plant growth-promoting effect on the plant (Bai et al., 2003). On 28 dpi plants were harvested to record the effect of BCAs on root length (under *in vivo* conditions). The data show that *Bacillus* sp. BL, *TriHar*-6, *Bacillus* sp. BS and control-treated plants show 18.9 cm, 15.4 cm, 14.4 cm, and 14.9 cm root length respectively. However, the root length in *Fov* treated plants as in the above order remained 9.7 cm, 13.6 cm, 9.9 cm, and 10.3 cm respectively (Fig.2).

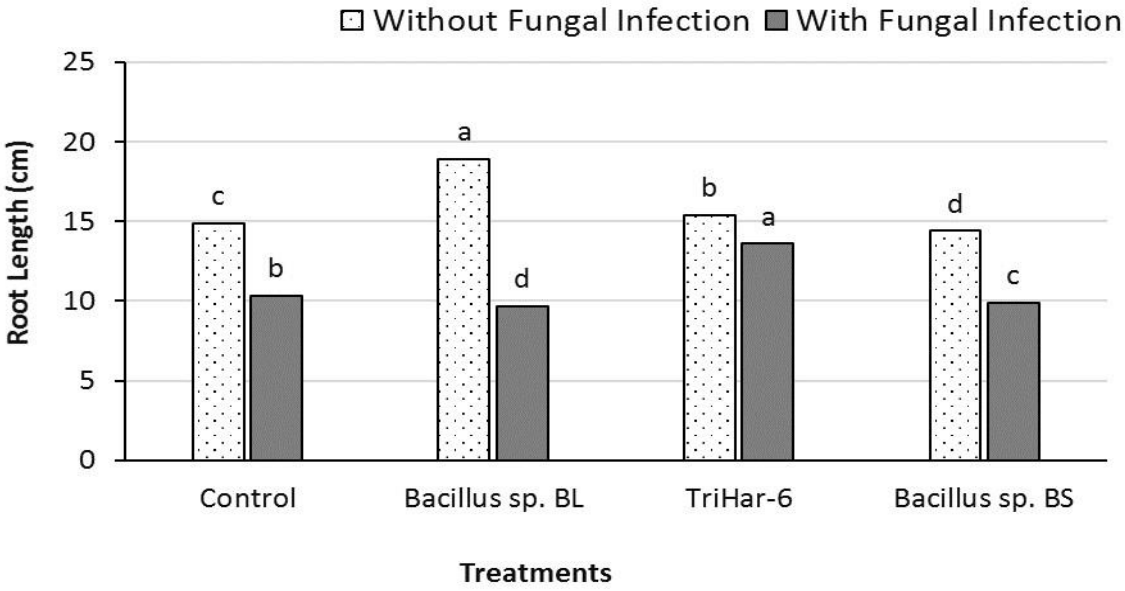

Figure 2: Effect of *Fov* and BCA application on root length of Okra**.** (Statistical analysis for without and with fungal infection treatments were carried out separately).

The result presented here shows that *Bacillus* sp. BL application results in maximum root length, but the case of *TriHar*-6 and *Bacillus* sp. BS no enhancement was recorded. However, the *TriHar*-6 was revealed as the best biocontrol against Fusarium wilt in Okra.

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2.3. **Total Plant Length at 28 days of okra seeds treated with BCAs**

Upon harvest total plant length was recorded carefully. The maximum total plant length 47.4 cm was recorded for *Bacillus* sp. BL treated Okra plants. However, the *TriHar*-6 and *Bacillus* sp. BS showed 38.5 cm and 36.1 cm total plant length respectively. The total plant length of 37.4 cm was recorded for the control. The total plant length of the plant treated with *Bacillus* sp. BL, *TriHar*-6, *Bacillus* sp. BS and control when challenged with *Fov* remained 24.3 cm, 34.1 cm, 24.9 cm, and 25.7 cm respectively (Fig.3). This shows that among the bacterial isolates used *Bacillus* sp. BL contributed best to the physical attributes of the plant however, the plant did not respond to the application of *TriHar*-6, *Bacillus* sp. BS. However, the *TriHar*-6 application resulted in *Fov* inoculated plants tolerating that load of the pathogen to some extent.

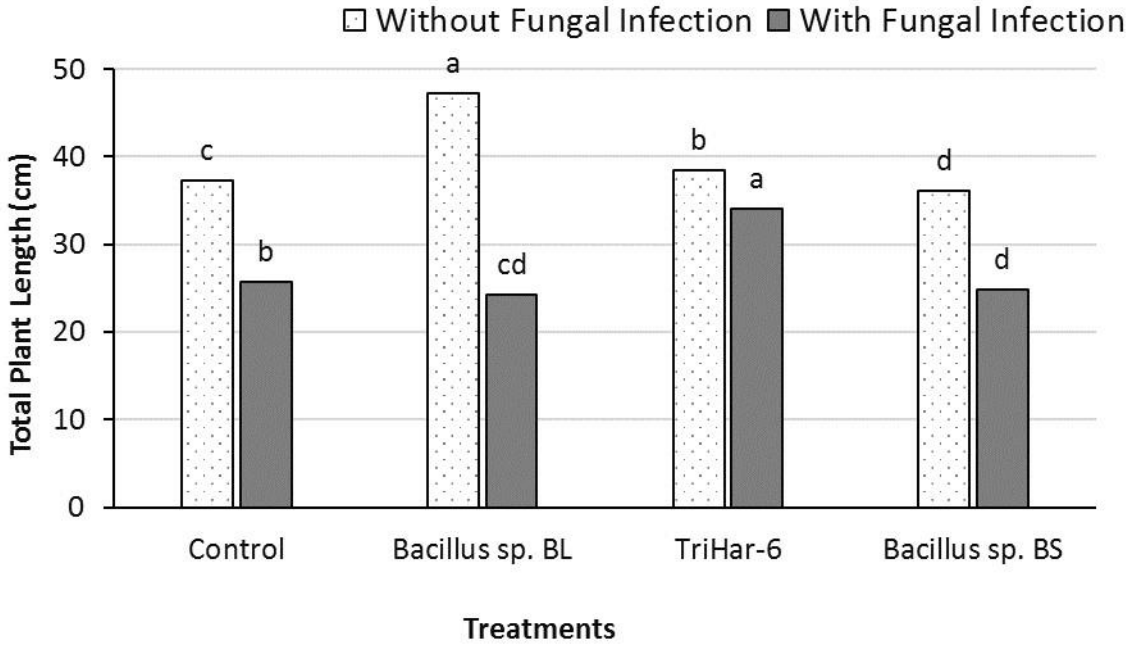

Figure 3: Effect of *Fov* and BCA application on the total length of Okra**. (**Statistical analysis for without and with fungal infection treatments was carried out separately).

Previously it has been reported that *Fusarium oxysporum* f.sp. *lycopercie* causing wilting and poor growth in tomatoes due to mycotoxins. *Trichoderma* spp secretes the hydrolytisc enzymes to inhibit the disease (Keswani, Bisen, Chitara, Sarma, & Singh, 2017). Furthermore, *Bacillus* spp. had been shown to enhance plant growth in many plants (Pahari et al., 2017).

2.4. **Fresh Weight after 28 days of okra seeds treated with different BCAs.**

On 28 dpi plants were harvested to record the effect of BCAs on fresh weight (under *in vivo* conditions). The data show that *Bacillus* sp. BL, *TriHar*-6, *Bacillus* sp. BS and control treated plants show 17.5 g, 14.2 g, 13.36 g, and 13.8 g fresh weight respectively. However, the fresh weight in *Fov* treated plants as in the above order remained 8.7 g, 12.3 g, 8.9 g, and 9.2 g respectively (Fig. 4).

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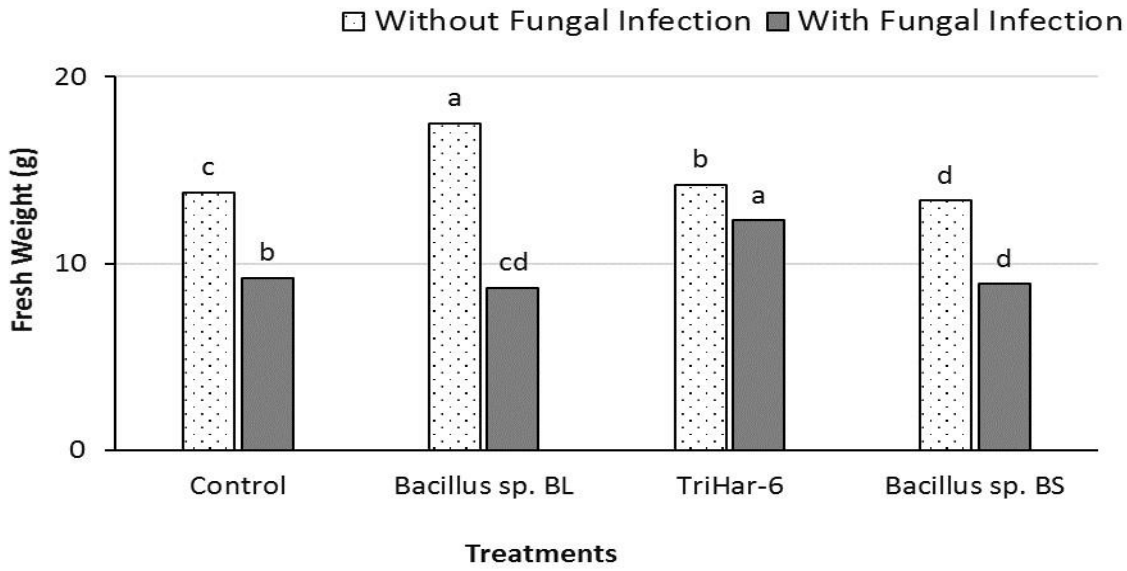

Figure 4: Effect of *Fov* and BCA application on fresh weight of Okra. **(**Statistical analysis for without and with fungal infection treatments was carried out separately).

The result presented here shows that *Bacillus* sp. BL application results in maximum root length, but the case of *TriHar*-6 and *Bacillus* sp. BS no enhancement was recorded. However, the *TriHar*-6 was revealed as the best biocontrol against Fusarium wilt in Okra. Total biomass of rice plant gained maximum biomass when seeds were treated with bacillus spp. (Bai et al., 2003).

3. **Disease incidence of Okra against *Fov* using different BCAs**

A comparative study of different BCAs against the *Fov* inoculation challenge was conducted under *invivo* conditions. Results showed no significant differences in *Fov* disease incidence in Okra seedlings after the seven dpi, as all treated plants remained symptomless. Control potted plants were not challenged with any pathogen, that showed no incidence till seven, 14, 21 days, and on the 28th day, DI percentage was recorded 8.15% only. The DI % in the case of only *Fov* treated plants remained 29.16 % after 14 dpi, however, after 21 dpi and onwards, the DI % reached 100 % (Fig. 5). The control treatment and only *Fov* treated plants were used as positive and negative control references. The data showed that *Fov* disease incidence dropped in plants that were pretreated with the BCAs. The data show that in the case of *Bacillus* sp. BS treated plants the DI remained 91.65 % after 28 dpi of *Fov*. Furthermore, *Bacillus* sp. BL treated plants showed almost the same DI (i.e., 83.13%) when these plants were challenged with *Fov*. Interestingly, the *TriHar*-6 application lowers the effect of the pathogen by dropping the disease incidence to 41.29% (Fig. 5). This supports the fact that *TriHar*-6 isolate is the best BCA in controlling the *Fov*.

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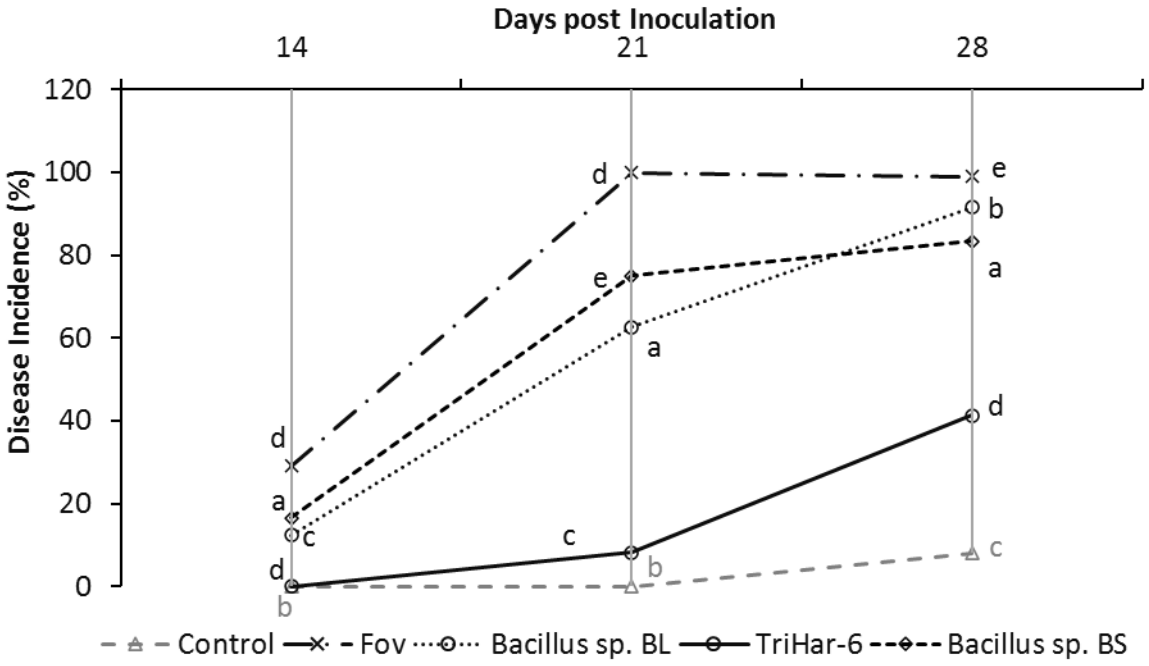

Figure 5: Disease incidence of *Fov* in Okra plant bio-primed with BCAs**. (**Y-axis expresses the disease incidence percentage. The X-axis represents the days post-inoculation of *Fov*)**.**

Previous Studies showed that *Trichoderma harzianum* inoculation has effectively controlled the soil-borne pathogen (Fusarium wilt of melon) (Ashrafizadeh, Etebarian, & Zamanizadeh, 2005). Additionally, it has been reported that *Trichoderma harzianum* produces volatile components and non-volatile antibiotics to ensure the antagonistic activity against the soilborne pathogen (Harman et al., 2004). Maximum inhibition of the *Fusarium oxysporum* f.sp. *lycopersci* (*Fol*) was observed when the seeds of okra were primed with the TH as compared to the control. In our study *TriHar*-6 has been found effective against the soil-borne pathogen of Okra (*Fov*). Plant height was recorded at the seven days of seedling showing their positive effects on the health of the plant but when the plant was challenged with the pathogen, BCAs did not show their effect on plant physiological characters. *TriHar*-6 only controlled the pathogen *Fov*.

Hens, Biological Control Agents are easy to handle, increase the activity of the organism in the field, cost-effective and convenient for field applications. For this purpose, this study was conducted bio-agent dependent technology, screening of microbes for desirable traits, selection of potential strains and inoculum development are important steps. To evaluate the biological seed treatment, bio priming along with its effect to control *Fov* are studied here.

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their effect on plant physiological characters. *Trichoderma harzianum* is only controlling the pathogen *Fov*.

The main objective of this research was to test the performance of *Trichoderma harzianum, Bacillus Large,* and *Bacillus Spore* and also to check these BCAs against suppressing *Fusarium oxysporum* f.sp. *vasinfectum* under field conditions (in pots).

Conclusion

The present research work was conducted to evaluate the effect of different biological control agents (*Bacillus* Large, *Bacillus* Spore, and *Trichoderma harzianum*) on the productivity and rhizosphere of okra (*Abelmoschus eschulentus)*. Laboratory trials for suppression of Fusarium wilt of Okra (*Fusarium oxysporum* f.sp. *vasinfectum*) were conducted in the Plant Pathology Lab of UAF Sub Campus Burewala and Experimental Area of UAF Sub Campus Burewala. Two sets of experiments were conducted; the first *invitro* trial was carried out in the Plant Pathology Lab of UAF Sub Campus Burewala and the second one-pot trial was conducted in the research area of UAF Sub Campus Burewala, during 2018. This study was therefore aimed at determining the antagonistic effect of *Bacillus* spp. and *Trichoderma harzianum* applied at different concentrations on wilt pathogens of potted okra in the pots to show antifungal activity against seed-borne pathogens.

From this investigation, it is concluded that *TriHar*-6 application could reduce the *Fov* population in the soil, which can protect the Okra crop from the *Fusarium* wilt disease. Disease incidence and disease severity were significantly lower with the application of *TriHar*-6 at the concentration of 1.0 OD in the earthen pots of the 12 inches. It implies that fungal control in the pots experiment may have practical application in the biological control programs and potentially replace the use of chemicals. however, further confirmation and other necessary tests need to work on. Nevertheless, the study conducted revealed that both *Bacillus* spp. tested show plant growth-promoting characteristics. However, the *Bacillus* spps. treated plants when challenged with the *Fov* pathogen failed to perform as potential BCAs. *TriHar*-6 on the contrary did not show any growth-promoting characteristics in *invitro* conditions. *TriHar*-6 disease suppressing bioagent of the *Fusarium oxysporum* f.sp.
vasinfectum.

Authors Contribution

The research work was conducted by Ibtihaj Zaib under the supervision of Ashir Masroor, Ashir Masroor wrote the paper, Osama bin Abdul Hafeez helped in statistics and graphs, Muhammad Rizwan Ashraf helped in manuscript preparation, Shabbir Ahmad helped in English and data analysis.

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Conflict of Interest

The authors declare no conflict of interest with anyone.

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