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MICROBIAL POTENTIAL APPRAISAL ASSOCIATED WITH SPENT MUSHROOM COMPOST AGAINST MANAGEMENT OF LENTIL FUSARIUM WILT

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A B S T R A C T

Lentils (*Lens culinaris*) is known as second important cultivated pulse crop in the world. It's a protein-rich pulse. The major area under cultivation is in developing countries and is sown as rain fed crop. This crop can be grown in poor nutrient soils and often faces drought during the period of plant growth. Lentil is known as the second most leguminous important crop grown in Pakistan, which is cultivated on 82000 hectares area which is about 26% on pulse area sown in Pakistan. The annual production of this crop is 30,000 tons. *Fusarium* wilt, is an important diseases of lentil throughout the world. This is also a major factor behind limiting of the successful cultivation of this crop. *Fusarium oxysporum* attacks the roots of plant species that results in the wilting, yellowing or death of the whole plants by colonizing in xylem vessels. It is also noted that, *Fusarium spp*. have a broad host rang and can retain in the soil for a long period of time. Moreover, the source of chemical applications resulted in resistant species and also pollution of the natural ecosystem. Therefore, biological control is being used now a days for controlling of this pathogen, the use of PGPR gave best in this regards. There was total 18 isolates of the pathogen were purified. *F. oxysporum* was isolated, identified and purified on medium. We found 7 isolates to be more virulent as compared to other with varying degrees of virulence. By utilizing serial weakening technique rhizobacterial isolates were disconnected and filtered on Nutrient Agar medium. The complete detached were assessed to known their capability to estrange the shrink pathogen by the method of in vitro. It was screened that of 22 isolates only two (2) detached were discovered proficient and had extensive adversarial impact on the pathogen. These microorganisms selected for further different test. The studies demonstrated that these Plant Growth Promoting Rhizobacteria were assessed their hostile capacity under both research center condition. All the medication was demonstrated the results that these PGPR successfully diminished the rate of illness and bettering further increment in the development of plants. The study demonstrated that among all treatments T7 showed the best results with 0% ailment occurred. T4 and T1 was showed little minimum successful after T7 among themselves respectively. The present study hence demonstrates that the PGPR reduced the attack of *F. oxysporum* also improved the plant growth. The findings of the current research suggested that a there should be mixture of PGPR agents for the development is needed, because it is better to adapted the environmental changes occur globally around the world in the growing season and protect against a broader range of pathogens.

Keywords: Compost; Evaluation; Microbial; Potential

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INTRODUCTION

Worldwide, lentil is grown on a total of 1.8 million hectares, of which 60% is in the South Asian region which include the lentil producing countries of Bangladesh, Burma, India, Nepal, and Pakistan. Within South Asia, Bangladesh, India, and Pakistan are the biggest lentil producers. Pakistan is second in importance with about 9.5% of the growing area. The area sown to lentil in Pakistan varies from 49,000 to

106,000 hectares (FAO, 2017). The present day cultivated lentil originated in western Asia and disseminated to Egypt and southern Europe. Punjab is the main leading territory of lentil development with the high aggregate national region and production. About 75% of the Punjab zone lies in the districts of Chakwal, Rawalpindi, Narowal, Gujrat, and Sialkot. Sindh, Khyber Pakhtunkhwa and Balochistan contribute 14%, 14% and 7% of the region, respectively. Pakistan contributes about 8% of the total lentils produced in the world. The crop responds positively to moderate rainfall, but negatively to heavy rainfall. Lentil can be successfully grown on sandy to clay of medium fertility. Lentils are consumed as food and it nutritional secure millions of people, especially the low earners families in Asia. Among the leguminous crops lentils play a significant role in the crop rotation they enrich the soil with natural fertilizers that results in the increment of fertility in the soil and helps in the productive environment. Lentils are a pulse crop that has been used in farming production for a large deal in the history of human being (Herforth et al., 2020). Besides, the buildups including stems, husks, organic product dividers, dried leaves, and grain (deposits) can be used as a fodder for domestic animals. Lentil deposits contain around half sugar, 4.4% protein, 21.4% fiber, 12.2% fiery remains, 10.2% dampness and 1.8%. Lentils having also have high source of minerals and vitamins such as, zinc and iron and basic amino acids lysine and isoleucine (Sharma et al., 2014).

Several diseases affect lentil causing yield losses. Common fungal diseases of lentil are Fusarium wilt caused by *Fusarium oxysporum* f. sp. *Lentis*, rust caused by *Uromyces fabae*, and ascochyta blight caused by *Ascochyta lentis*. Bacterial disease caused by *Mycobacterium insidiosum* also affect lentil. Lentil is also affected by parasitic flowering plants like *Cuscuta* sp. and *Orobanche* sp. Several viral diseases affect lentil, including pea enation mosaic virus, bean yellow mosaic virus and pea seed borne mosaic virus (PSbMV). Among them, PSbMV is potentially dangerous for lentil (Muehlbauer et al., 1995).

Nematodes are also impact on lentil. The most important pests that affect lentil and cause economic losses are gram caterpillar (*Heliothis obsoleta*), white ants (*Clotermes* sp.), gram cutworm (*Ochropleura flammatra*), the weevil (*Callosobruchus analis*) and hand bean seed beetle (*Bruchus ervi* ve *Bruchus lentis*) (Duke, 1981).

Fusarium wilt is caused by the fungus *Fusarium* (the full name is *Fusarium oxysporum* f. sp. *cubense*). It is a soil pathogen which infects the root system and goes on to colonise the plant through the vascular system hyphae of the fungus can even reach the leaves. The disease cannot be controlled or cured other than by soil treatments, which unfortunately have such a detrimental effect on the environment that they are prohibited almost everywhere.

Biological methods under development are showing great potential, however. One of the worst effects of *Fusarium* wilt is the production of so called Chlamydospores, or resting spores, which survive in the soil for decades. As soon as a susceptible banana plant is grown nearby, these spores germinate, infect the plant, and kill it. In other words, soil that has been contaminated once becomes unfit for future banana production unless resistant varieties are grown. Biological control was an elective procedure to control *Fusarium* wilt disease (De Cal et al., 1995; Larkin and Fravel, 1996).

A few microscopic organisms that are related with the underlying roots of harvest plants that affect their host and are alluded to the plant development advancing rhizobacteria. Due to the sensational changes occur in Rhizosphere and dynamic idea makes communications that prompt bio-control way of diseases. PGPR is the free living microscopic organisms that may effectively affect plants from colonizing roots, seedling rise, stimulating general plant development, nourishment of minerals, uses of water, and additionally ailment concealment. The PGPR is used as a bio control for the harvest Rhizosphere has been demonstrated extensive guarantee.

The substrate of spent mushroom left after the harvesting of the mushroom is the by product in Mushroom production. It has great diversity of micro-organism, like fungi and bacteria. It is easily decomposed in nature and are economical and easy for application. It has the ability to suppress the pathogens that causes the decline in the yield of the plants. It is used in organic farming and having the potency of soil infiltration in soil, high water holding capacity and aeration. It has high amount of salt and unstable organic material. It remains in the soil for two years after the application. It contains nitrogen 12%, phosphorous 0.2% and potassium 1.3%. After being aged for 18 months, phosphorus and nitrogen do not change, but potassium can decrease.

The spent mushroom compost is known to be the attractive material for improving soil structure in tilled soils. This study aimed to assess the fresh spent mushroom compost associated microbes against *Fusarium* wilt of lentil.

Fusarium oxysporum sp. *melonis* (FOM) is a standout amongst the most serious Phytopathogenic agent, and the reason for huge economic misfortunes to melon crop in Italy and south-east Spain where it finds great climatic

conditions(Gonzalez Torres et al., 1988). Thusly, FOM inhibition is an earnest need of current Mediterranean agriculture but the synthetic substances utilized for its control are either wasteful or unsafe to the environment and, subsequently, hindering to human being (Boulter et al., 2000; Brimner and Boland, 2003). Along these lines, contrasting options to concoction control are currently being widely examined (Abawi and Widmer, 2000; Garcia et al., 2004; Pascual et al., 2002).

Physical and organic properties of SMC change significantly relying upon treating the soil forms, development systems and climate conditions. It do not contain any weed seeds, on account of the high temperatures related with the fertilizing the soil and sanitization forms. SMC additionally contains low levels of pesticides and overwhelming metals, well beneath the points of confinement set by the US EPA. SMC has a mass thickness of in the vicinity of 300 and 600 g/L, and a carbon/nitrogen proportion in the vicinity of 8 and 27. *Streptomyces* species 385 and *Paenibacillus* species 300 were disconnected while screening adversaries of *R. solani* and indicated key opposing movement against *F. oxysporum* and *Cucumerinum* species. These strains deliver chitinases as well as corresponding generation of other anti-fungal substances. Target of the present investigation was to decide the natural control of *Streptomyces* species 385 and *Paenibacillus* species 300 against *Fusarium* wilt diseases of cucumber when utilized as part of mix. The capacity of these disconnects to create chitinases and to lyse cell dividers of *Cucumerinum spp.* and *F. oxysporum* to look at by utilizing somewhat cleansed proteins. It is an estimate that the creation of the complimentary hydrolytic chemicals by these two strains in mix brings about the capacity to stifle sickness as contrasted and each strain utilized exclusively.

Biological control might be probability for organization of the sickness. The usage of profitable microorganisms considered as respectable strategy for the organization of ailment. Out of different living things used for bio-control, rhizosphere microorganisms may give a cutting-edge hindrance to pathogen and best to use as biocontrol administrator (Lang et al., 2012). A segment of the wild plants are in like manner potential wellspring of detachment of worthwhile microorganisms (Goudjal *et al*., 2014) (Goudjal et al., 2014). Various investigations have shown that the natural control of *Fusarium* wilt can be refined using distinctive productive microorganisms including *Bacillus*, *Pseudomonas* spp., *Streptomyces* spp. (Boukaew et al., 2011).

Impacts analysis of *Bacillus pumilus* and *Rhizobium* species on wilt sickness disease that is resulted by *F. oxysporum* in the lentil development (Akhtar et al., 2010). *F. oxysporum* inoculation reduced nodulation in plants. Immunization with *Bacillus pumilus* and *P. alcaligenes* caused a more noteworthy increment in the development of plants, no. of units, root colonization and nodulation caused by the rhizobacteria, and decreased *Fusarium* wilt. Utilization of *Rhizobium* sp. brought about a more prominent increment in the development of plants, nodulation, no. of cases and diminished wilting greater than *B. pumilus*. Combined use of *P. alcaligenes* and *B. pumilus* with the species of *Rhizobium* brought about the best increment in number of units, development of plant, colonization in roots and nodulation that are resulted by rhizobacteria which decrease wilting in the plants that are immunized to species of the *Fusarium*.

MATERIALS AND METHODS

The current research on the "Use of rhizobacteria isolate from the spent mushroom compost for *Fusarium* wilt management in lentil" was conducted at Department of Plant Pathology, Faculty of Crop and Food Sciences, Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi, Pakistan.

Pathogen isolation

Twenty-five diseased samples of lentil plants collected from different lentil growing areas of district Chakwal, Rawalpindi and NARC. The collected samples were transfer into the laboratory in plastic bags and kept at 4°C in the refrigerator to study the further experiments. The root and stem parts of infected lentil crop were sliced into many pieces to incorporate symptomatic and asymptomatic parts. The sliced pieces treated with surface sanitized with 0.5% sodium hypochlorite, dye arrangement for 2 minutes and then washed by using distilled sterile water. The pieces were dried in disinfected channel paper before putting on PDA medium keeping in mind that the end goal to detach the parasitic pathogen. The petri dishes that are immunized were then fixed and labeled properly and left for incubated at (28±2°C) room temperature, for 7-10 days. The colonies of the fungal growth were purified by using sub culturing and by coming about pure colonies were kept up on slants.

Identification of pathogen

The microscopic and cultural characteristic that appears on PDA was utilized for distinguishing proof for parasitic fungal pathogens up to the level of species. The pathogenicity tests were used to identify the speciales and races of the pathogen. Colonies displaying the ordered

highlights of *F. oxysporum* were distinguished. Cultural and microscopic identification were based on the colour and the mycelia growth pattern of the pathogen on PDA as well as characteristics such as shape, size, structure and diversity of the macroconidia, microconidia and colony growth traits were examined under the compound microscope.

Pathogenicity

To check the pathogenicity of *F. oxysporum* culturing and fungal growth of conidial suspensions were prepared on PDA at temperature (28±2°C) for 10 days. After which 50 ml of sterile distilled water was dispensed into the pure cultures on Petri plates and the surface of the cultures was scrapped slightly with a sterile scalpel to dislodge the conidia from the mycelium then filtered through a double layer of sterilized cheese cloth to remove mycelia fragments, into sterile beakers, then mixed thoroughly and re-suspended in sterile distilled water. The micro conidia were counted with a hemocytometer and the concentration 10^6 per ml was adjusted. For each count, 0.01 ml of the conidial suspension will be one the hemocytometer covered with its cover slip.

Isolation of plant growth promoting rhizobacteria from spent mushroom compost

For the isolation of rhizobacteria sample of SMC with two degree of decaying fresh and six month old take from National Agriculture Research Council (NARC). Peptone, meat concentration and glucose were added to 500 ml of distilled water and separated. Agar was added gradually to a substitute carafe having 500 ml water. Both were mixed to make l liter. Media was then autoclaved at 15 lbs psi and 121^{o}C for 20 minutes. In the wake of autoclaving media was permitted to chill off to 50^{o}C. When it is refined this temperature media (Peptone 5 g, Agar 15 g, Beef Extract 3 g and Glucose 2.5 g) was poured in Petri plates and let bond.

Isolation of rhizobacteria

Make 9 test tubes of distilled water with the volume of 9 ml each. Make the dilution of SMC by adding 1gm SMC in 10 ml distilled water in a beaker and mix it on vortex mixer. Making first dilution of 10^{-1} by adding 1 ml water in 1st test tube taking from the beaker and mix it on vortex mixer. After the first dilution take 1 ml of water from 1st test tube and mix it in 2nd test tube for the 2nd dilution of 10^{-2}. By repeating this procedure solution was diluted up to 10^{-8} dilution. Number dilution at 3, 5, 7, and 8 at 10^{-3}, 10^{-5}, 10^{-7} and 10^{-8} respectively on spread to Petri plates having NA media. To avoid contamination taped Petri plates with paraffin. Incubate the plate in incubator at the temperature of 28 to 30 ^{o}C. All the above activities were done in sterile conditions under sterilized laminar flow which is near to the spirit lamp.

Purification of PGPR

The composite culture of microorganisms that come about because of the spreading inoculation of dilution each bacterial state will be refined on independent nutrient agar NA plates utilizing streaking strategy. Take loop on the flame to make it red hot and then cool it. Single colony of bacteria was picked with the loop by slightly touching it. Inoculating loop immediately was streaked very gently on the plate by back and forth motion. After first take loop on the flame to make it red hot and cool it. Second streak started from the end of the first streak. Similarly repeating the same procedure again and again for the further streak till last one.

***In vitro* evaluation of rhizobacteria for their antagonistic activity**

In vitro antagonistic effects of plat growth promoting rhizobacteria against *F. oxysporum* will be carried. PDA media was used in order to favor the growth of *F. oxysporum* and nutrient agar was used for the potential growth promoting rhizobacteria. Dual culture measure was completed as starter screening test to choose the rhizobacterial secludes with high opposing impacts against *F. oxysporum*. The secludes of pathogen was confined on the media after recognizable proof. Dual culture method is used to check the opposing action of rhizobacterial segregates against *Fusarium oxysporum*. Bacterial disengages were streaked at one side of the Petri dish having PDA. 9 mm of the mycelial plate from 7 days old culture of PDA of *F. oxysporum* were put at contrary side of petri plate opposite of bacterial streak individually and incubated for 5-7days at 27±2 ^{o}C. Petri dishes vaccinated with fungal plates alone filled in as a control. 3 numbers of replications were kept up for each segregate. Perceptions on width of restraint zone and mycelial development of the tested pathogen were recorded and per cent hindrance of pathogen development was used to calculate by utilizing the formula. Per cent inhibition (I) = C-T/C ×100 (C is mycelial growth of pathogen in control; T is mycelial growth of pathogen in dual culture plate).

Bio-chemical characterization of PGPR

Different biochemical test were performed to characterize the antagonistic rhizobacteria.

Gram staining

Pick a sphere over streaming with bacterium was spread on glass slide and then settle by warming on a low fire. Spread watery significant stone violet strategy (0.5%) over the spread for 20-30 second and after that washed using tape water for 1 minute. Affirming that washing flooded with

iodine for one moment, flushed in tape water and decolorized with 95% ethanol until dull over stream. In the wake of washing the outline was counter recolored with safranin for around 10 second, washed with water, dried and watched minutely.

Potassium hydroxide test

Place three percent drop of Potassium Hydroxide course of action was put on the glass slide by the assistance of dropper. Utilizing a tooth or loop pick evacuate an unmistakable measure of new microorganisms from a settlement on an agar plate. Spread on a glass slide and mixed it in a drop of potassium hydroxide for 10 seconds. Industriously mixing for couple of minutes, thick solution was watched and made a gooey and "strings out" advancement.

Siderophore production of bacterial isolates

Siderophores are extracellular, little low weight particles that have high partiality for press (Fe3+). The siderophores are by and large produce by both facultative anaerobic and oxygen consuming microorganisms and monocotyledonous plants under low-press pressure conditions. Chrome Azurol S (CAS) agar medium was used for the rhizobacterial isloates as depicted by Schwyn and Neiland (1987) to recognize the production of siderophore. Chromo Azurol S with ME agar medium was vaccinated in the plate for 1 day old microscopic organisms and left for incubation for 72 hr at 30^{o}C. The medium from blue shade to orange or nearness of light orange to yellow radiance about the colony demonstrates the siderophore production.

Phosphorus solubilizing test

Limit of the isolates to solubilized phosphate was evaluated subjectively using PDYG containing normally supported calcium phosphate ($Ca_3(PO_4)_2$) of sterile 50 mL, disodium hydrogen phosphate that is 10% (wt./vol) and Calcium Chloride of 100 ml sterile 10% (wt./vol) were added in the sterile PDYA per liter to create an encourage of calcium hydrogen phosphate. Each bacterial society was spot immunized in the purpose of meeting of a PDYA-Cap plate and incubated at 32±2 ^{o}C for 10 days. More detectable the detachment transversely finished of the crown zone more vital was the farthest point of the bacterium to solubilized phosphate.

Hydrogen cyanide (HCN) production of bacterial isolates

All the isolates were utilized for hydrogen cyanide production by adjusting the strategy for Lorck (1948). Quickly, nutrient broth was changed with glycine (4.4gL-1) and microscopic organisms were streaked on adjusted plate of agar. Whatman channel paper number 1 absorbed sodium carbonate by 2% in 0.5% picric corrosive arrangement was set in the highest point of the plate. The plates that were fixed with parafilm and hatched at 28±2 °C for 4 days. Improvement of orange to red shading showed HCN production.

***In Vivo* evaluation of rhizobacteria for their antagonistic activity**

Soil was collected from the field was passed through 10 work sifter to get uniform estimated particles. Natural compost, sand and soil were mixed at the proportion of 1:1:3. Mixtures were autoclaved at 15lbs psi for 20 minutes at 121°C and 9 cm width pots were filled with this soil mixture. Poured the water into the pots to wet the soil.

Preparation of rhizobacterial inoculum

The isolates of unadulterated rhizobacteria were sub-cultured on nutrient broth and incubate at 32 ˚C for 70-72 hours and furthermore shaken at 120 rpm for 72 hours. Nutrient broth suspension of 1 ml containing bacterial concentration of every isolates. The suspension of 10 mm was filled to on all the pot placed near the seedling of plant.

Preparation of inoculum of the pathogen

The fungus, *Fusarium oxysporum* was extracted from the disease infected roots of lentil and was maintained PDA. Richard's liquid medium was used for the preparation of fungal inoculum by the culturing of the isolate at 25 °C for 15 days. Blotting paper was used to collect the mycelium, for the removal of extra nutrient and water we used smearing papers. Hundred gram of mycelium was macerated in one liter of sterile water and the suspension of 10 mL having fungal mycelium of 1 g was poured around the roots, as described in inoculation technique. For the inoculation of the pathogen the healthy seedlings of 7 days old were used. The soil near the root was used to inoculate for *F. oxysporum*. The suspensions of the inoculums were used in the soil around the plants. Sterile water was used on the controls.

Design for experiments

Experiment were carried in Completely Randomized Design (CRD) having three replications and 12 Treatments (T1 = Rh-6; T2 = Rh-6 + Fw (10^5); T3 = Rh-6 + Fw (10^7);T4 = Rh-18; T5 = Rh-18 + Fw (10^5); T6 = Rh-18 + Fw (10^7); T7 = Rh-6 + Rh-18; T8 = Rh-6 + Rh-18 + Fw(10^5); T9 = Rh-6 +Rh-18 + Fw(10^7); T10 = Fw (10^5); T11 = Fw (10^7); T12 = Control)

Data collection

Data was collected to check the effect of PGPR on the lentil plant growth against *F. oxysporum*. The disease incidence and growth parameters were also analyzed.

Disease incidence

Disease incidence of *F. oxysporum* was mentioned for

development of wilt symptoms on lentil plants and data was recorded after two weeks. The percentage incidence of the disease was checked by using the available formula as below:

$$Disease\ incidence\ (\%) = \frac{No.of\ infected\ plants}{Total\ no.of\ plants} \times 100$$

Plant length (cm)

Plant length was recorded after the interval of six weeks. Shoot length and root length of plant were obtained to give height of plant. Length of root and shoot of each plant were recorded in cm. Data of all treatment were compared with control treatment of PGPR.

Plant fresh weight (g)

Plant fresh weight was taken after 9 weeks towards the end of the trial new weight of the each plant. The complete plants were selected from the treatments of control and plants treated by rhizobacteria. As a result the biomass of the control and treated plants were obtained, thusly the biomass changes for rhizobacteria were kept in view. The plant weight was calculated in g (gram).

Dry weight of plant

After the test taking 63 days the plants were stove dried. Plant dry weights were taken from all treated and control plants. That results the subsequently change in the biomass of each rhizobacterial treated and control plants as a result of the rhizobacteria were checked. Each plant weight was recorded in grams.

Statistical analysis

Information was examined measurly by utilizing complete Randomized Design (CRD) with three replications for every treatment.

RESULTS AND DISSCUSION

The samples of the soil around the stem and stem parts were sampled from the infected fields showing wilt symptoms. The twenty-five samples were collected and placed in plastic bags with proper tagging. All the collected samples were showing varying degree of wilt symptoms.

Isolation and culturing of pathogen

Isolation of pathogen was done by following proper isolation methods and maintained on Potato dextrose agar (PDA) slants at 4 ˚C till further use. Inoculum was prepared by culturing the fungus on PDA medium for 7 days in Petri plates. Conidial suspensions were prepared by pouring 20 ml of sterile distilled water into each Petri plate.

Purification of pathogen

Total eighteen (18) isolates were purified from the samples. Culture were multiplied on PDA media and treated with in sterilize distilled water for conformation and pathogenicity test (Table 1). Purified culture of fungus had fluffy white and pink center colored.

Table 1. Isolation of pathogen from different lentil diseased samples.

Location	Type of samples	Isolates	Color of colony
Rawalpindi	Stem	Fw-1, Fw-2	Off White
	Root	Fw-3, Fw-4	Pink Center
	Soil	Fw-5, Fw-6	Dark yellow
National Agriculture Research Council (NARC)	Soil	Fw-7, Fw-8	Pink Center
	Root	Fw-9, Fw-10	White Growth
	Stem	Fw-11, Fw-12	Fluffy white
Chakwal	Stem	Fw-13, Fw-14	White
	Soil	Fw-15, Fw-16	Dark yellow
	Root	Fw-17, Fw-18	Fluffy White

Pathogenicity test

About five week old seedlings were taken for pathogenicity test and pure isolate of the pathogen were subjected for the pathogenicity test. Lentil plants were grown under control conditions and inoculums of pathogen (10^7/ml) were applied to check their virulence. There are total 18 isolates of pathogen were isolated, out of them seven (07) isolates i.e. I-2, I-5, I-7, I-9, I-11, I-14 and I-16 when injected in the plants they shown varying degree of symptoms. Results of these isolates explain that isolates were pathogenic. All the isolates shown different degree of virulence but the Isolate I-5 cause the death of the plant within 5-7 days of inoculation whereas isolate I-11 and I-16 showed severe infection and killed the plant within 10-12 days. These experiments show that a pathogenic microbe has reduced the growth of plants and have not performed well according to its genetic potential. The other remaining isolates I-2, I-7, I-9 and I-14 showed moderate symptoms but were unable to completely overcome the plant defense. Therefore; being the most virulent, I-5 was taken as a pathogen for further experiments. Only few plants were alive but most of their parts were showing wilting symptoms. The remaining plants

were not as a healthy.

Isolation of rhizobacterial cultures

From samples of SMC (spent mushroom compost) there were 22 isolates of rhizobacteria were isolated. These rhizobacterial isolates varied in color of colony, shape and colony sized.

Characteristics of rhizobacterial colony

All isolates were fast growing Gram-negative, each strains have distinctive characteristics of colonies. The formation of the circular surface by all the colonies, and were creamy, slimy white, glossy white, white or shiny white in appearance. Colony characters of different bacterial specie diverge significantly from each other, while similar strains of bacteria showed insignificant difference. That is an important source of bacterial conformation. Color of colony, size of colony and shape of each isolates were observed and recorded (Table 3). After the observation it was supposed that mostly isolates were *Bacillus* i.e. 2, 4, 8, 9, 12, 14, 17, 18, 19, 20, 21 and 22 where as some bacterial colonies demonstrated the attributes of *Pseudomonas* i.e. 1, 3, 5, 6, 7, 10, 11, 13, 15 and 16.

Table 2. Virulence of pathogenic Isolates *Fusarium oxysporum*.

Isolates	Virulence	Isolates	Virulence
I-1	+	I-10	+
I-2	++	I-11	++
I-3	+	I-12	+
I-4	+	I-13	+
I-5	+++	I-14	++
I-6	+	I-15	+
I-7	++	I-16	++
I-8	+	I-17	+
I-9	++	I-18	+

Highly Virulent = (+++), Moderately Virulent = (++),Weakly Virulent = (+)

Table 3. Rhizobacteria colony characteristics.

S. No.	Isolates	Color of Colony	Colony Size (mm)	Shape of Colony
1	Rh-1	White colony	3	Round, small and raised
2	Rh-2	Off white	0-5	Large, flat and wavy margin.
3	Rh-3	Light brown	1-5	Circular and convex with entire margin.
4	Rh-4	Pale yellow	0-8	Medium size, flat and wavy.
5	Rh-5	Creamy white	0-5	Flat, larger, circular and margin undulated.
6	Rh-6	Creamy white	3-5	Round, medium size and slightly margin.
7	Rh-7	Reddish	3-0	Circular entire margin.
8	Rh-8	Shining pale yellow	1-0	Round, small and smooth.
9	Rh-9	Pale orange	0.5	Large, round and flattened helical.
10	Rh-10	Whitish	0.7	Flat, circular and margin undulated.
11	Rh-11	Off White	2-3	Large, flat and wavy margin.
12	Rh-12	Pale yellow shiny	1-5	Round, small and smooth.
13	Rh-13	Reddish	1-5	Circular entire margin.
14	Rh-14	Pale yellow	1-3	Wavy, medium size and flat.
15	Rh-15	Whitish	2-0	Round, medium size and slightly raised.
16	Rh-16	Off white	2	Large, wavy margin.
17	Rh-17	Pale yellow	1-0	Wavy and large flat.
18	Rh-18	Yellow	3-4	Small, round and convexly raised.
19	Rh-19	Whitish	2-5	Flat, circular and margin undulated.
20	Rh-20	Light brown	2-0	Circular, convex with entire margin.
21	Rh-21	White colony	2-3	Round, small and raised.
22	Rh-22	Whitish creamy	1-5	Flat, large circular and margin undulated.

***In vitro* evaluation of rhizobacterial antagonistic activity against pathogen**

While doing this test, it was uncovered that a large number of disengages do not having opposing capacity against the pathogen as they have not demonstrated any restraint of the development in vitro. All disengages tried and just Rh-6 and Rh-18 indicated significant opposing action against pathogen and was chosen for further test. After all the rhizobacterial isolate tried the Rh-6 delivered preferred results over Rh-18 as they separately restrained at 9.50 mm and 13.5 mm of the span respectively.

The development of pathogen face hindrance due to the anti-microbial generation. Results of all the isolates shown individuals who indicating are not demonstrating any opposing capacity against the in vitro pathogen were recorded and watched (Table 4).

Table 4. Diameter of dual culture exhibited in vitro by rhizobacterial isolates against *Fusarium oxysporum.*

S. No.	Isolates	Diameter of Dual Culture (mm)
1	Rh-1	0.00
2	Rh-2	2.00
3	Rh-3	0.00
4	Rh-4	0.00
5	Rh-5	0.00
6	Rh-6	9.50
7	Rh-7	3.00
8	Rh-8	0.00
9	Rh-9	1.50
10	Rh-10	3.50
11	Rh-11	4.00
12	Rh-12	1.00
13	Rh-13	1.50
14	Rh-14	0.00
15	Rh-15	0.00
16	Rh-16	1.00
17	Rh-17	2.00
18	Rh-18	13.5
19	Rh-19	2.50
20	Rh-20	2.50
21	Rh-21	0.00
22	Rh-22	3.00

Biochemical characterization of rhizobacteria

Gram staining

The best and easiest method is to know the bacterial isolates is Gram staining method to recognizing that either the bacterial isolates are gram positive or negative. Gram staining method was tested to perceive gram +ve and gram -ve. The results of the current test find that the restricted development by two of the organisms were due to the gram negative pathogen while the one keep responded unequivocally to the test as explain in (Table 5).

Siderophore production test

The production of the siderophores by the rhizosphere bacterial microbes resulted in the enhancement of plant development by expanding the accessibility of Fe around the root or by hindering the plant pathogens that are colonized around the stem or other harmful microscopic organisms. Period of siderophore was seen by particular yellow green fluorescent shade made continuously living things. Fluorescent shade passed on the medium having low Fe as this medium have high preference press (Fe+3) chelate. Rh-6 and Rh-18 both apparently was certain for siderophore time (Table 5). Shading was diffused into the media around the state. Rh-6 made cocoa shading orange to while Rh-18 passed on yellow to greenish shading. Above test and properties demonstrated the outcomes could recognize that Rh-18 had a spot with *Bacillus* while Rh-6 had a spot with *Psuedomonas*.

Potassium hydroxide test (Loop test)

The cell wall of Gram -ve bacteria breaks due to potassium hydroxide, viscid chromosomal material released that causes

the suspension of bacteria become thick (Table 5).

For the first 30 seconds the micro-organisms become stringy and thick, and form long strands. This was seen in Gram negative bacteria. Those microscopic organisms that leave the suspension unaltered or absence of stringing. This was seen in Gram positive bacteria.

Table 5. Response of the antagonistic rhizobacteria to gram staining, loop test and siderophore production test.

Isolates	Gram Staining	Morphology Of Cell	Siderophore Production test	KOH test Reaction
Rh-6	+	Rod Shaped	_	+
Rh-18	_	Rod Shaped	+	+

Production of hydrogen cyanide (HCN) by bacteria

Role of HCN in disease suppression has been demonstrated by several scientists in various Study on qualitative analysis of HCN indicated a strong production of HCN in Rh-18 having high ability and Rh-6 showed low ability of HCN production was estimated (Table 6).

Phosphorus solubilization test

Among the microorganism bacteria is the most important mineral phosphate solubilizer in nature. In this test corona zones were created and all the disconnects reacted decidedly. This is the affirming the phosphorus solubilizing action of the segregates (Table 6). Results of Rh-6 were superior to anything Rh-18 in this admiration in light of the fact that the radiance zone was of the more prominent breath. Rh-6 radiance zone was 14 mm in width in this manner disclosing its capacity to solubilize more Ca-P than Rh-18 separate which delivered 7mm of corona zone.

Table 6. Response of the antagonistic rhizobacteria to HCN production and phosphorus solubilization.

Isolates	HCN Production test	Phosphorus Solubilization test
Rh-6	+	+
Rh-18	+	+

***In Vivo* Evaluation of antagonistic rhizobacteria against pathogen**

After the medication there was a huge distinction found between the few prescriptions and remaining others were not showing any enormous contrast. Among each of the 12 treatment, T7 displayed the best results with 0% infection rate occurred. Treatment 4 and 1 was demonstrated that the second and 3rd best and maximum results among all the treatment i.e. T2, T3, T5, T7, T8, T9, T10, T11 and T12 as in (Table 7).

Table 7. Reduction of lentil wilt incidence of antagonistic rhizobacterial isolates against *Fusarium oxysporum*.

	Treatments	Disease incidence (%)
T1	Rh-6	0e
T2	Rh-6 + Fw (10^5)	23d
T3	Rh-6 + Fw (10^7)	46b
T4	Rh-18	0e
T5	Rh-18 + Fw (10^5)	20d
T6	Rh-18 + Fw (10^7)	34c
T7	Rh-6 + Rh-18	0e
T8	Rh-6 + Rh-18 + Fw (10^5)	18d
T9	Rh-6 + Rh-18 + Fw (10^7)	21d
T10	Fw (10^5)	48b
T11	Fw (10^7)	78a
T12	Control	0e

Alpha 0.05, Critical T Value 2.064, Standard Error for Comparison 2.6176, Critical Value for Comparison 5.4025, Among the 5 different groups like, (A, B, etc.) shows non-significantly difference in mean from each other.

Pathogen population measurement

When Plants were harvested after inoculation the wilting index of the plants were recorded. For the assessment isolation of pathogen was done from each treatment plants

in order to obtain the wilting index vaccinated with *F. oxysporum* was recorded by scoring illness seriousness on a size of 0 to 5, as 0 is the no withering and 5 shows extreme shrinking. Shrinking observed based on necrosis in the xylem as takes after: No rot is 0; 20% rot is 1; 21%-40% necrosis is 2; 41%-60% rot is 3; 61%-80% necrosis is 4 and over 80% putrefaction is 5.

Plant growth assessment parameters

Effect on plant height

After the 45 days the roots of lentil that were collected partially from inoculated with rhizobacteria. PGPR survives and proliferate in plant roots are and are good root colonizers that increase the growth of plants. After the treatments the best results for the shoot was recorded from the combination of Rh-6 and Rh-18 where the best highest length rate was obtained i.e. 39.8 cm and 33.3 cm respectively. The root length was recorded at 27.8 cm and 26.6 cm respectively. Similarly the second best results were shown by two treatments including combination of treatment 8 and 9. The results are given in ANOVA (Figure 1).

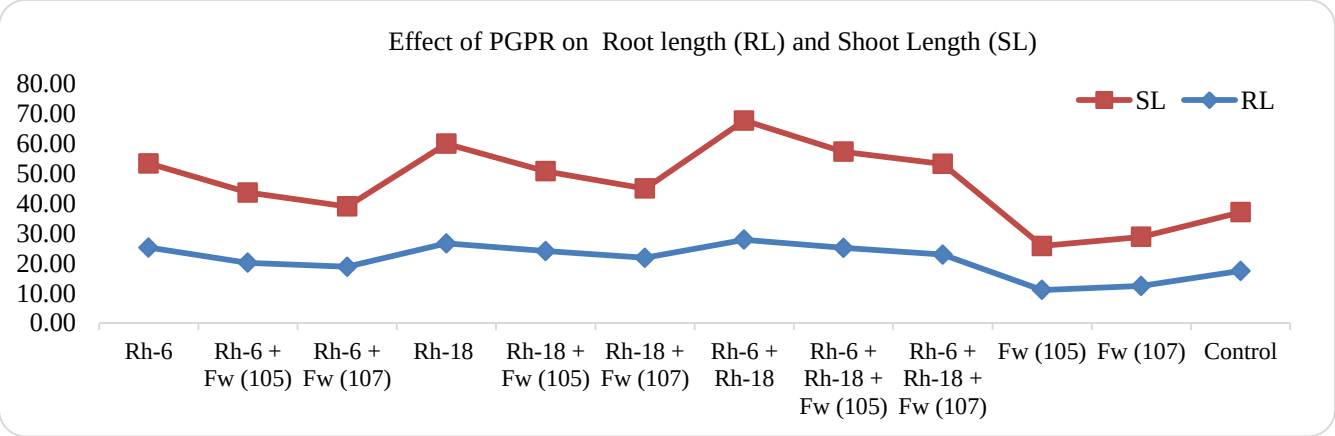

Figure 1. Effect of plant growth-promoting rhizobacteria (PGPR) on root length and shoot length of lentil plants.

Effect on fresh and dry weight of plant

Inoculation with isolates Rh-18 and Rh-6 results in the significant increase in weight of fresh shoot by 9.16 g and root fresh weight by 6.33 g of plants without fungus when compared with un-inoculated plants. When the inoculation with both caused shows much increase in fresh weight of shoot and root than that caused by inoculation. Similarly for dry weight of plant shoot and root the combination of isolates Rh-6 and Rh-18 were again gave the greater increase in the dry weight of shoot by 4.3 g and root dry weight by 1.96 g of plants, both caused a significant increase in dry weight of shoot and root as compared to plants inoculated with *F. oxysporum*.

The results also shown that the plants inoculated with only *F. oxysporum* alone caused greater significant reduction both in fresh and dry weight, as compared with non-inoculated plants. The combination Rh-6 and Rh-18 gave best results (Figure 2, 3).

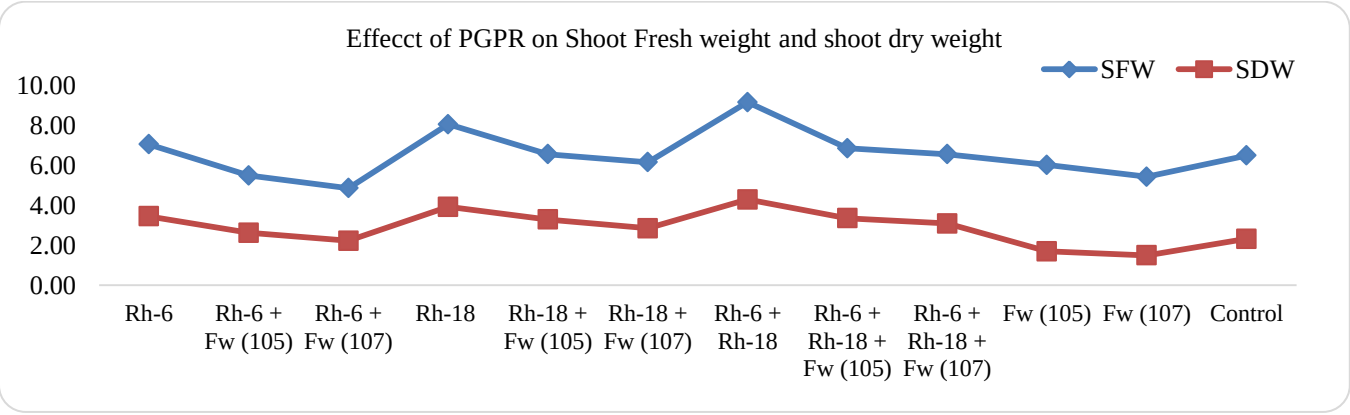

Figure 2. Effect of PGPR on shoot fresh weight and shoot dry weight of lentil plants.

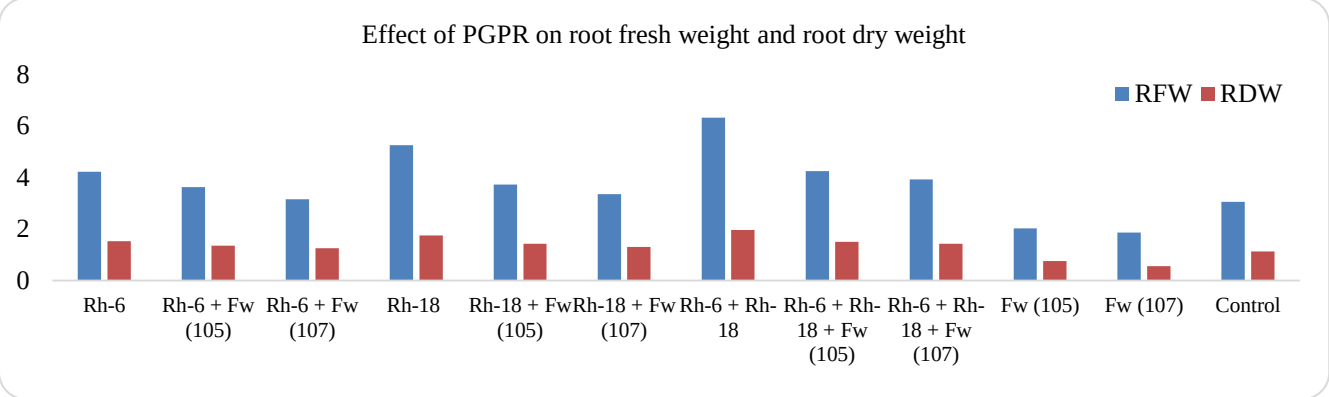

Figure 3. Effect of PGPR on root fresh weight and root dry weight of lentil plants.

Microscopic pathogen *Fusarium oxysporum* is a standout amongst the most annihilating fungal pathogen inescapable having widespread host range and almost everywhere throughout world in various environmental zones. There are no promising method has been practically available as the pathogen is multiples and diverse characteristics having multi weaponry system of regarding its control. These pathogens contribute an important role in diseases development. The control of *F. oxysporum* through plant growth promoting rhizobacteria has been worked out and several studies report many antagonist rhizobacteria resisting several strain of *F. oxysporum*, therefore different rhizobacterial isolates used against the different strains of the pathogen. After the studies, we have obtained 22 rhizobacterial isolates from spent mushroom compost and screening them for antagonists revealed that only few were antagonistic to *F. oxysporum* with varying degrees. The highest zone of inhibition was obtained with *Bacillus spp*. isolates Rh-18 followed by Rh-6 (*Pseudomonas spp*.). Inhibition of *F. oxysporum* was might be due to production of siderophore that our isolates capable. The reason for variable antagonistic activity is likely to be different concentration of antibiotic compound released by antagonists thus producing variable zone of inhibition. We found that all antagonists isolates were capable of producing HCN and Siderophore.

The current research work was conducted to check the effect of antagonistic rhizobactria on the *Fusarium* wilt development in lentil plants and *in vivo* study was initiated. According to the isolates Rh-18 and Rh-6 significantly reduced wilt incidence compare to the control. In the presence of pathogen rhizobacterial strain significantly increased plant resistance and plant growth as compared with the pathogen treated control. Corroboration with this study showed our results demonstrated rhizobacteria have potential to minimize the incidence of *Fusarium* wilt disease in lentil plants and also increased the plant height as well as the plant weight. We observed that *Bacillus spp*. isolates was more effective against *F. oxysporum* than *Psuedomonas spp.* isolates. The growth of lentil plants show positive response due to the presence of PGPR, as it is effective root colonizers that survive and proliferate along with plant roots.

The current research concluded that the growth of the lentil plants show improvement by the use of rhizobacterial isolates. The results are due to the direct antagonism of pathogens, competition with pathogens for essential nutrients or antibiotic production. The *Bacillus* spp. that reduced the wilting index in *F. oxysporum* inoculated plant. The improvement of the plant growth is due to the inhibitory effects of *Bacillus* spp. on pathogens. As concluded that by the use of *Bacillus* spp. in rapid colonization in all tissues in lentil crop, and induced resistance against *F. oxysporum*.

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AUTHOR'S CONTRIBUTION

Z. Amin**,** M. N Aslam, Y.S Khan and S. Hussain contributed in overall management of the research work, data analysis writing of the article technical inputs and Proof reading.

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