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Utilization of Rhizobacteria and Spent Mushroom Compost for the Management of Bacterial Wilt of Potato

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ABSTRACT

Potato (*Solanum tuberosum* L.) is the fourth most important staple food in the world after wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.) and maize (*Zea mays* L.). In Pakistan, potato crop is cultivated over an area of 191.6 million hectares. On an average potato production in Pakistan is 20 tonnes per hectare. A target has set by The Government of Pakistan to produce about 4.871 million tons potatoes during Rabi Season 2020-21. (Federal Committee on Agriculture FCA) The potato production exceeds 376 million tonnes harvested from an area of 19.25 million hectares in the world (FAOSTAT 2018). *Ralstonia solanacearum* causing bacterial wilt is a major threat to potato production. Management through biocontrol agents is one of the best methods that can replace synthetic chemical-based formulations. In the current study combine effect of antagonist rhizobacteria as biocontrol agent and spent mushroom compost as biofertilizer were tested against bacterial wilt disease pathogen. Potato plant samples infected with *R. solanacearum* and rhizobacteria were collected from potato growing fields in Rawalpindi. Out of twenty tested antagonistic rhizobacterial isolates, only three viz., Rh10, Rh12 and Rh 15 showed maximum inhibitory effect against *R. solanacearum*. In another experiment different combinations of treatments containing rhizobacteria alone or combined with fresh and spent mushroom composts were also tested against the bacterial wilt pathogen under laboratory conditions. Combination of rhizobacteria along with weathered compost (T5) reduced the disease incidence to 15.92 % when compared against 77.81 % in control after six weeks. Significant increase in plant height up to 41.83 cm was also observed as compared to control viz., 35.5cm. Similarly, T2 (only fresh compost), T3 (containing fresh compost along with rhizobacteria) and T4 (rhizobacteria along with weathered compost) also showed better results as compared to against control (T0) where there was no application of rhizobacteria and compost. Application of rhizobacterial along with spent mushroom compost can significantly reduce the disease incidence along with the improvement in plant growth parameters.

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INTRODUCTION

Potato is (*Solanum tuberosum* L.) popularly known as "The king of vegetables. Potato is the most important tuber crop and has major economic importance in the world. It is the fourth most important staple food, ranking first in volume produced among root and tuber crops worldwide. It is generally a high yielding crop, but the crop is vulnerable to the attack of a number of diseases that are caused by fungi, bacteria, viruses and nematodes. In Pakistan, potato crop is cultivated over an

area of 180 thousand hectares. On an average potato production in Pakistan is 20 tons per hectare. The potato production exceeds 376 million tons harvested from an area of 19.25 million hectares in the world (FAOSTAT 2018).

Bacterial wilt caused by *Ralstonia solanacearum* is a major disease affecting potato production worldwide and is mainly categorized as both seed-borne and soil-borne disease. It is highly challenging and one of the most destructive diseases of solanaceous crops worldwide (Hayward, 2005). It causes enormous losses to many economically important crops, such as tomato, potato, eggplant, pepper, tobacco, banana, chili, and peanut (Liu *et al.,* 2005). *R. solanacearum* causes wilt in over 450 host species in 54 botanical families (Allen *et al.,* 2005). More than 90% yield losses caused by bacterial wilt in tomato, potato, and other host crops if kept uncontrolled. (Aslam *et al.,* 2017).

Management of plant diseases has become a challenge for the plant pathologist for sustainable agriculture. Various control strategies including soil fumigation (French and Sequeira, 1994), planting of resistant cultivars, transgenic resistant plants, crop rotation, soil amendments, integrated control and biological control have been developed. The application of pesticides caused huge losses and health hazard problems. Out of different organisms used as biocontrol agents, rhizosphere microorganisms provide a front-line defense against pathogen attack and are ideal for use as biocontrol agents (Siddiqui, 2006). Mushroom production is currently the biggest solid-state-fermentation industry in the world (Soccol and Vandenberghe, 2003). Spent mushroom compost (SMC) is produced by the mushroom industry as a residual byproduct. It is a good source of nutrients and acts as a beneficial soil conditioner (Aslam and Saifullah, 2013). Application of spent mushroom compost as a soil amendment reduces early dying of potato caused by *Verticillium dahliae* (Haghighi *et al.,* 2006).

Some edible mushrooms such as *Lentinus edodes*, *Boletus edulis*, *Pleurotus ostreatus* and *Agaricus bisporus* have been reported to have antimicrobial properties. (Susana *et al.,* 2009). Aslam and Saifullah, 2013 have reported that SMC has some phenolic compounds with antimicrobial activity, which exist as an effective biocontrol of root knot nematode (Meloidogyne spp.) on tomato. The present study was planned to explore the biocontrol ability of selected rhizobacterial isolates along with spent mushroom compost against *R. solanacearum*.

METHODS AND MATERIAL

Survey and sample collection

A comprehensive survey was conducted in different potato growing areas of Rawalpindi district, Pakistan for the collection of plant samples with characteristic symptoms of bacterial wilt. Soil samples from the rhizosphere area of healthy potato plants were also collected for the isolation of beneficial rhizobacteria. All the samples were collected in polythene bags and labelled properly. Samples were brought to the laboratory and were kept at -4 °C in a refrigerator till further use in experimentation.

Isolation and identification of pathogen

Stem sections with characteristic symptoms of bacterial wilt were cut into small pieces and were rinsed with distilled water to make them free from soil debris and other contaminants. Stems sections were surface sterilized by treating with 1% Chlorox and were dipped in test tube containing distilled water followed by shaking on vortex for few seconds. This will assist the bacteria to ooze out from the infected samples. Loop full of bacterial suspension was streaked on petri plates containing Nutrient agar (NA) and 2, 3, 5 – triphenyltetrazolium chloride media (TTC) followed by incubation at 30 °C for 48 hours. Reddish bacterial colonies were appeared after two days. Purification was carried out by picking single bacterial colony and streaking on TTC medium containing petri plates (Begum *et al.,* 2012).

Pathogenicity confirmation for *R. solanacearum*

Pathogenicity of the *R. solanacearum* was tested on healthy young seedlings of potato plants. Seeds of susceptible potato variety were sown in sterilized soil. Young seedlings of potato plants were incubated with 20 µl (e.g., a drop) of a bacterial suspension of *R. solanacearum* at 10-8 cells/ml with a 1 ml-propylene syringe. Plants in the control treatment were treated with distilled water only and three replications for each isolate were maintained. Plants were monitored for up 3 weeks for symptoms expression and re-isolation of pathogenic bacteria was carried out from wilted plants by streaking the bacterial suspensions on TTC medium.

Isolation of plant growth promoting rhizobacteria

Plant Growth Promoting Rhizobacteria (PGPR) were isolated from the soil and healthy root samples by serial dilution method. Roots of healthy tomato plants were

washed gently with distilled water to remove the adhered soil debris. 1 g root sections were added in a flask containing 200 ml sterile water and were shaken on rotary shaker at 150 rpm for half an hour. Serial dilutions were streaked on nutrient agar medium containing plates and incubated at 28 °C for 48 h. Purification was done by re-streaking the single bacterial colonies on nutrient agar media. Pure cultures of PGPR strains were identified using morphological and physiological characteristics (Akhtar *et al.,* 2009).

Biochemical tests for confirmation of *R. solanacearum* and antagonistic rhizobacteria

Different biochemical tests viz., Gram staining, KOH tests, catalase oxidase test, and Kovac oxidase tests were performed for the confirmation of *R. solanacearum* and antagonistic rhizobacteria.

Gram Staining

Bacterial smear was prepared on glass slides and a drop of 0.5 % crystal violet was added to it followed by rinsing with tape water for 60 seconds. One drop of iodine solution was added to it and rinsed again with tap water. For decolonization, the glass slides were treated with 95% ethanol for about 30 seconds. After washing the specimen was counter stained with safranin for about 10 seconds. Glass slide was dried for few seconds and examined under microscope at different magnification power (10X, 40X and 100X) as described by (Begum *et al.,* 2012).

Potassium hydroxide loop test

In this test, 48 hours pure culture of *R. solanacearum* was picked up by sterilized toothpick and mixed with fresh 3% potassium hydroxide solution on glass slide for 10 seconds. On continuous stirring for few seconds, loop formation was noticed for the confirmation of gram positive or gram-negative bacteria (Chaudhry and Rashid, 2011).

Catalase oxidase test

Pure single colony of bacteria was mixed with single drop of 3 % of hydrogen peroxide (H_2O_2) with the help of an inoculating needle. Few seconds later gas bubbles were formed and observed with the naked eye (Reynolds, 2004).

Kovacs Oxidase Test

Small amount of the bacteria was spread on the filter paper impregnated with 1% oxidase reagent solution and rubbed it with the help of sterilized toothpick for few seconds. Development of deep blue/ purple color is noticed for bacterial confirmation in 10-60 seconds. (Hossain *et al.,* 2007)

In-vitro evaluation of pgpr for their antagonistic activity against *R. solanacearum*

Selected strains of rhizobacteria were tested for their antagonistic activities against *R. solanacearum* by zone inhibition method. 48 hours old culture of rhizobacterial strains were placed in center of the petri plates and *R. solanacearum* discs in the surrounding. Petri plates were incubated for two days. Efficacy of tested rhizobacterial isolates was recorded by measuring the inhibition zone against the control treatment where there was no application of rhizobacteria.

Collection of spent mushroom compost

Spent Mushroom Compost (SMC) with two degrees of decaying: fresh and six months old were taken from the National Agriculture Research Center (NARC). Islamabad, Pakistan.

In-vitro evaluation of rhizobacteria and spent mushroom compost

Different treatments of SMC were made and subjected to check their efficacy against pathogenic *R. solanacearum*. Soil mixture alone or in combination with fresh and weathered compost was filled into the pots. Suspension of *R. solanacearum* was prepared and poured into pots. Potato tubers were treated with 48 hours old culture of antagonistic rhizobacteria for 1hour and were sown into pots containing amended soil. Three replications for each treatment were maintained and control treatment contains only the application of pathogenic *R. solanacearum*. Data on diseases incidence was taken on weekly basis by using the formula given below:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Growth parameters viz., Plant height (P.H), shoot length (SL), root length (RL), fresh root weight (FRW), fresh shoot weight (FSW), dry root weight (DRW), dry shoot weight (DSW) was recorded. The data of all the treatments was compared with the control treatment. Collected data was analyzed statistically by using Complete Randomized Design (CRD) with three replications for each treatment.

RESULTS

Isolation identification and pathogenicity of *R. solanacearum*

After isolation and identification of *R. solanacearum*, pathogenicity test was performed to check the virulence of the pathogen. Out of twenty-five different tested isolates, only three isolates showed positive results to be pathogenic as given in Table 1.

Table 1. Pathogenicity confirmation of *R. solanacearum*.

R. solanacearum		
RS-4	RS-10	RS-15
+	+	+
+	+	+
+	+	+

Biochemical tests for confirmation of *R. solanacearum* and antagonistic rhizobacteria

Different biochemical tests were performed for the confirmation of *R. solanacearum* and antagonistic rhizobacteria and the results are given in Table 2. Gram staining test showed positive results for all the bacterial gents except Rh-10 and Rh-15 while KOH test results were negative for Rh-10 and Rh-12. Catalase oxidase test results were positive for all the tested bacterial isolates. Kovacs oxidase test was positive for all the tested bacterial strains except Rh-15. All the bacterial agents were observed rod shaped under microscope.

Table 2. Biochemical tests for the identification of *R. solanacearum* and PGPR isolates.

Biochemical Test	*R. solanacearum*			Antagonistic Bacteria			Cell Morphology
	RS-4	RS-10	RS-15	Rh-10	Rh-12	Rh-15	
Gram stain reaction	+	+	+	-	+	-	Rod shaped
KOH Test'	+	+	+	-	-	+	Rod shaped
Catalase Oxidase Test	+	+	+	+	+	+	Rod shaped
Kovacs Oxidase Test	+	+	+	+	+	-	Rod shaped

Determination of antagonistic activity of rhizobacteria

Zone inhibition technique was used to test the efficacy of rhizobacterial isolates against *R. solanacearum*. Results of antagonistic potential of all the test rhizobacterial isolates against pathogens were observed and recorded. Data on radial growth of pathogen isolates was measured at the end of second, fourth and sixth days of incubation period. Out of 20 tested isolates Rh-10, Rh-12, and Rh-15 showed maximum antagonistic potential against the pathogenic bacteria and were preferred for further study. Among the three selected isolates Rh-12 (15 mm) results in maximum inhibition as compared to the Rh-10 (11 mm) and Rh-15 (9 mm). The growth inhibition of the pathogen is attributed the antibiotic, antibacterial compounds and siderophore production by the rhizobacterial isolates. Data regarding zone of inhibition is given Table. 3. Most efficient rhizobacterial isolates were selected for further tests.

Table 3. Growth Inhibition of *R. solanacearum* by rhizobacterial isolates (mm).

Sr No.	Isolates	Zone of Inhibition (mm)
1	Rhz 1	3.00
2	Rhz 2	0.00
3	Rhz 3	1.00
4	Rhz 4	2.00
5	Rhz 5	6.5
6	Rhz 6	0.00
7	Rhz 7	1.00
8	Rhz 8	4.5
9	Rhz 9	6.00

10	Rhz 10	11
11	Rhz 11	1.00
12	Rhz 12	15
13	Rhz 13	0.00
14	Rhz 14	1.00
15	Rhz 15	9
16	Rhz 16	4.5
17	Rhz 17	2.00
18	Rhz 18	5.00
19	Rhz 19	9.00
20	Rhz 20	0.00

In-vitro evaluation of rhizobacteria and spent mushroom compost against *R. solanacearum*

In vitro evaluation of rhizobacterial isolates along with fresh and weathered compost was carried out. Disease incidence in different treatments was monitored after each week and compared the results with the control as shown in the Table 4 and 5. Results showed that among all the tested combinations of rhizobacteria and SMC, *R. solanacearum* + soil + Weathered (Six-month old compost) + Rhizobacteria showed maximum reduction in disease incidence when compared against the control treatment where there was the application of pathogenic *R. solanacearum* followed by T4 (*R. solanacearum* + soil + Weathered (Six-month old compost)) and T3 (*R. solanacearum* + soil + Fresh compost + *Rhizobacteria*). Among all the treatments, T1 (*R. solanacearum* (RS) + soil + Rhizobacteria) showed the minimum effectiveness in disease control.

Table 4. In-vitro evaluation of rhizobacteria and spent mushroom compost against *R. solanacearum*.

Treatments	Disease Incidence % (1st Year)					
	7DAYS	14DAYS	21DAYS	28DAYS	35DAYS	42DAYS
C	29.42	45.78	54.57	58.13	63.07	76.81
T1	0	16.99	20.55	23.6	26.06	27.71
T2	0	15.563	17.37	19.06	22.26	23.98
T3	0	14.26	16.05	18.74	21.54	22
T4	0	12.5	13.85	16.45	17.56	18.25
T5	0	11.396	12.99	13.86	14.97	15.92

C = Control, **T1** = *R. solanacearum* (RS) + soil + Rhizobacteria, **T2** = *R. solanacearum* + soil + Fresh compost (FC), **T3** = *R. solanacearum* + soil + Fresh compost + Rhizobacteria, **T4** = *R. solanacearum* + soil + Weathered (Six-month old compost), **T5** = *R. solanacearum* + soil + Weathered (Six-month old compost) + Rhizobacteria.

Table 5. In-vitro evaluation of rhizobacteria and spent mushroom compost against *R. solanacearum*.

Treatments	Disease Incidence % (2nd Year)					
	7DAYS	14DAYS	21DAYS	28DAYS	35DAYS	42DAYS
C	28.85	42.35	54.44	62.46	66.42	77.47
T1	0	17.06	20.6	25.21	26.73	31.71
T2	0	15.97	17.71	22.42	23.09	24.65
T3	0	14.63	16.1	19.24	21.54	22.6
T4	0	12.78	14.2	15.71	16.92	18.61
T5	0	11.61	12.99	14.05	15.03	15.94

C = Control, **T1** = *R. solanacearum* (RS) + soil + Rhizobacteria, **T2** = *R. solanacearum* + soil + Fresh compost (FC), **T3** = *R. solanacearum* + soil + Fresh compost + Rhizobacteria, **T4** = *R. solanacearum* + soil + Weathered (Six-month old compost), **T5** = *R. solanacearum* + soil + Weathered (Six-month old compost) + Rhizobacteria.

Data on growth parameters

Different growth parameters viz., Plant height, fresh and dry root and shoot weight were recorded. The data of all the treatments were compared with the control is given in Table 6 and 7. In response to the application of various treatments, all the treatments showed an increase in the plant height when compared to against control treatment where there was only pathogenic bacterial suspension was applied. All the treatments showed almost same results in enhancing the plant growth parameters viz., Fresh Root Weight, Fresh Shoot Weight, Dry Root Weight and Dry Shoot Weight. Among all the treatments, T5 (R. solanacearum + soil + Weathered (Six-month old compost) + Rhizobacteria) results in maximum reduction in disease severity when compared against control treatment.

Table 6. Growth parameters in response to the applications of PGPR and SMC.

Treatments	Growth Parameters (1st year)					
	P.H	FRW	FSW	DRW	DSW	DS
C	35.5	11.83	23.66	8.14	15.17	74.49
T1	38.5	14.91	23.58	5.57	17.86	36.25
T2	40.5	17.33	23.16	8.72	18.11	25.83
T3	41.33	17.16	24.16	8.94	17.31	23.16
T4	40.5	17.36	23.14	8.51	18.36	26.33
T5	41.83	15.66	26.16	8.51	19.61	25.45

P.H = Plant Height, **FRW**= Fresh Root Weight, **FSW** = Fresh Shoot Weight, **DRW** = Dry Root Weight, **DSW** = Dry Shoot Weight, **DS** = Disease Severity.

Table 7. Growth parameters in response to the applications of PGPR and SMC.

Treatments	Growth Parameters (2nd year)					
	P.H	FRW	FSW	DRW	DSW	DS
C	35.5	11.83	23.66	8.14	14.75	74.49
T1	38.5	14.91	23.58	5.57	17.86	36.25
T2	40.5	17.33	23.16	8.72	18.11	25.83
T3	41.33	17.16	24.16	8.94	17.31	23.16
T4	40.5	17.36	23.14	8.51	18.36	26.33
T5	41.83	15.66	26.16	8.51	19.61	25.45

P.H = Plant Height, **FRW**= Fresh Root Weight, **FSW** = Fresh Shoot Weight, **DRW** = Dry Root Weight, **DSW** = Dry Shoot Weight, **DS** = Disease Severity.

DISCUSSION

Management of plant diseases has become a challenge for the plant pathologists for sustainable agriculture. The application of pesticides damaged the crops and caused health hazard problems. Increasing awareness and health issues associated with the applications of pesticides, an alternative method has been adopted by plant protection to reduce that risk. Biocontrol is one of the best strategies used by plant pathologists for disease management. Among various organisms which serve as a biocontrol agent, rhizosphere microorganisms (PGPR) which supply a front-line protection against pathogenic attack and are ideal for use (Siddiqui, 2006). PGPR, which not only exerts a beneficial effect on the plant they colonize but also interacts with the plant roots as well as with other microorganisms in the rhizosphere. Some of the PGPR are antagonists to recognized root pathogens and result in prevention of disease development. Introduction of biocontrol agents from outside in the rhizosphere to achieve disease suppression was also described by Maji and Chakrabartty (2014).

Many studies have shown that the biological control of bacterial wilt can be done by using various BMs (biological microorganisms) including Bacillus (Wei *et al.,* 2011), Pseudomonas spp. (Ramesh *et al.,* 2009), Streptomyces spp. (Boukaew *et al.,* 2011), Acinetobacter spp. and Enterobacter spp. (Xue *et al.,* 2009), *Stenotrophomonas maltophilia* (Messiha *et al.,* 2007) and Actinomycetes spp. (Tan *et al.,* 2006). Sarkar and

Chaudhuri 2013 have reported the suppressive effect of some antagonistic bacteria on *R. solanacearum*. Some naturally occurring antagonistic rhizobacteria such as Bacillus spp., Pseudomonas spp. have been reported to control this disease. *B. subtillus* have been reported to be the best application in controlling bacterial wilt of tomato in both in-vitro and in greenhouse conditions.

Lian *et al.,* 2011 reported that rhizobacteria are the most effective over other antagonistic. In the greenhouse experiment inhibition had been observed when the antagonistic was applied before or simultaneously with the pathogen, while antagonistic was less effective when applied after pathogen inoculation. This indicated that the biocontrol effects of the antagonistic rhizobacterial strains are more likely to be a preventive effect rather than a therapeutic effect on the disease. Spent mushroom compost (SMC) is produced by the mushroom industry as a residual byproduct. It is a good source of nutrients and acts as a beneficial soil conditioner. The occurrence of phenolic compounds in SMC has antimicrobial activity on tomato (Aslam *et al.,* 2013). Application of spent mushroom compost (SMC) as a soil amendment reduces early dying of potato caused by *Verticillium dahliae* (Haghighi *et al.,* 2006).

Keeping in view the environmental issues and importance of biological control, the present study was planned to explore the biocontrol of *R. solanacearum* by using rhizobacteria and spent mushroom compost. Recent studies have proved that biological control of bacterial wilt disease could be achieved by using spent mushroom compost and antagonistic rhizobacteria. Different biochemical tests i-e Gram staining, KOH tests, catalase oxidase test, Kovac oxidase test were used for the confirmation of *R. solanacearum* and antagonistic rhizobacteria in this study were similar to the result reported by Begum *et al.,* 2012 and Kuarabachew *et al.,* 2007. In the in vitro antibiosis, the inhibition zone of Rh-10, Rh-12 and Rh15 showed 11, 15 and 9mm, respectively. Similarly, in the in vitro antibiosis, the smallest and largest inhibition zone of 1.2 and 2.4 cm diameter were caused by Pfri (Indian isolates) and Pfw1 (Wolayta isolates) respectively. Pfw1 have 200% efficiency being the most efficient isolates followed by Pfs2 and Pfwt3 with 125% and 111% efficiency, respectively (Kuarabachew *et al.,* 2007).

Spent mushroom composts fresh and weathered showed lesser severity than PGPR. These results indicate that the two kinds of composts had suppressive characteristics to bacterial wilt with respect to the reference growth media. No significant difference in the reduction of bacterial wilt incidence in potato was observed in the first week. Furthermore, two consecutive years of pot experiments showed that bacterial wilt incidence was lower down in the treatment T5 (weathered + PGPR) i.e 11.39%, 12.99%, 13.86%, 14.97% and 14.97% respectively (Singh *et al.,* 2012).

This work demonstrates that the activity in the compost mixed with PGPR was biological in nature. Beneficial rhizobacteria, as well as SMC, can induce systemic resistance in plants (Zhang *et al.,* 1998). The microflora in the compost along with PGPR that induced SAR remains unknown. These results suggest, however, that significant opportunities exist for the development of improved inoculants for composts yielding novel disease control strategies. Compost along with PGPR also enhances plant growth parameters i.e Plant height, FRW, FSW, DRW and DSW. T5 (T5= *R. solanacearum* + soil + Weathered (Six-month old compost) + Rhizobacteria] showed better growth as compared to control (C). In the same way, bacterization of tuber with selected efficient of an antagonistic rhizobacteria along with weathered compost T5 significantly increased the plant height, fresh and dry weight of root and shoot, disease severity by 41.83%, 15.66%, 26.16%, 8.51%, 19.61%, 25.45% respectively as compared to T0 (control).

This result agreed with Kuarabachew 2007 where they found an increased plant height and dry weight by 76.89% and 28.44%, respectively as compared to T1. Potato tuber planted after bacterization in treatments T3, T4 and T5 [T3= *R. solanacearum* + soil + Fresh compost + Rhizobacteria, T4= *R. solanacearum* + soil + Weathered (Six-month old compost), T5= *R. solanacearum* + soil + Weathered (Six-month old compost) + Rhizobacteria] showed the most significant growth enhancement (Plant height and dry weight) when compared to potato plants in other treatments C-T1 [C = Control, T1 = *R. solanacearum* (RS) + soil + Rhizobacteria]. This could be explained on the basis of the possibility of production of growth stimulating substance (hormone), increased nutrient availability in presence of isolates. The results presented in this study suggest that the antagonistic rhizobacteria isolated from the soils, when mixed with fresh and weathered compost according to Kuarabachew 2007.

CONCLUSIONS

It was concluded that the application of rhizobacterial

along with spent mushroom compost can reduce the disease incidence along with the improvement in the plant growth parameters without a significant reduction in plant growth.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS CONTRIBUTIONS

All the authors contributed equally to this work.

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