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Study on the management of potato black scurf disease by using biocontrol agent and phytobiocides

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Abstract
In this study three phytobiocides (turmeric, chilli and pepper) were used *in vitro* and two in screen house along with biocontrol agent *Paecilomyces sp.* against *Rhizoctonia. solani.* All the three phytobiocides, effectively restricted the fungal growth, when pre-amended in PDA medium. After 21 days of incubation the minimum colony diameter (32mm) was recorded in the plates that were treated with turmeric followed by chilli (45mm) and pepper (47mm) respectively when compared with control (56.50mm). A second experiment, to check the efficacy of phytobiocides and biocontrol agent was carried out in the screen house condition. Two most effective phytobiocides (turmeric and chilli) and biocontrol agent (*Paecilomyces sp.*) were used at the rate of 10 and 20 g/pot. Turmeric was found to be the most effective, decreasing disease severity to 10.50% when compared with diseased control. Similarly, *Paecilomyces sp.* was also effective in reducing disease severity to 14.75%. Conversely, chilli was the least effective phyto biocide.

Keywords: Phytobiocides, Biocontrol agent, *Paecilomyces spp*

1. Introduction
Potato (*Solanum tuberosum*) ranks fifth in total crop production and third among food crops after wheat and rice in Pakistan [30]. Pakistan is a major potato producing country, contributing an area of 159000 ha with a total annual production of 3491000 tonnes. While KPK shares an area of 8900 ha with a total production of 118000 tonnes [1]. Potato is one of the richest sources of carbohydrate i.e. 10-30% while other components including proteins, fats and vitamins are 1-1.5%, 1-2% and 0.45% respectively [29].
Potato is one of the important crops which have major pathological problems. In our country, more than fifteen diseases so far have been reported on potato [32]. Their importance, however, varies considerably in different potato production zones [5]. Seed and soil -emerging diseases of potato are a huge threat to potato growth and production in all the 8 agro potato ecological zone in Pakistan [2, 3, 24, 34] and almost in all the potato growing zones in the entire world [39].
Rhizoctonia canker means ("roots death" from Greek) ordinary called black scurf. It is one of the oldest and the most ordinary infection of potato stems and stolons beneath the soil surface. It attacks the potato plants all the time, from planting to harvest but early stage is the most dangerous one because it causes eye germination inhibition, sprout death [25]. In later phase it causes the sunken black to brown zones on stolon and stem. Other symptoms are malformation, cracking, pitting and necrosis on stem end which results in poor quality tubers [11, 21].
Weedy plants namely *Lantana camara* and *Capparis decidua* have been used to manage *R. solani* [36]. Due to safe and non phytotoxicity many higher plants natural products have been successfully used in plant disease control. Now researchers are looking for other management approaches like use of different levels of soil moisture and different biocontrol agents and phytobiocides. Biological control and use of environment friendly phytobiocides can be more effective in control of this soil borne disease. The objective of this study was to determine the effect of phytobiocides against *R. solani in vitro* conditions and to determine the combine effect of biocontrol agent (*Paecilomyces sp.*) and phytobiocides for the control of black scurf of potato in screen house conditions.

2. Materials and Methods
The study was carried out at Malakandher Research Farm and laboratory of Plant Pathology Department, The University of Agriculture, Peshawar during 2013.

2.1 Collection of *Rhizoctonia solani* from infected potato tubers

The infected potato tubers were collected from a local market. White skin potato tubers contain more numbers of sclerotia. Sclerotia were cut out along with some potato skin with help of a scalpel. After surface sterilization to eliminate the contaminants with 0.1 percent mercuric chloride solution for 20 to 30 seconds, sclerotia were rinsed three times in sterile distilled water. Sclerotia were allowed to dry on sterile filter papers. Those pieces were then plated on the Potato Dextrose Agar (PDA) medium under aseptic conditions. The plates were incubated at 27 ºC for 7 to 10 days.

2.2 Mass multiplication of inoculum of *R. solani*

Mass multiplication of inoculums was done. For this 5mm plugs were cut and placed in 20 ml petri plates containing Potato Dextrose Agar media. The plates were then incubated at 27 ºC [10]

2.3 Identification of pathogen and biocontrol agent

R. solani and *Paecilomyces sp* was identified by morphological examination and its characteristics e.g culture color, observation of spore and mycelial structure under microscope, using Simplified Identification Fungi Key [22].

2.4 *In vitro* screening of phytobiocides against *R. solani*

The phytobiocides including turmeric, chilli and pepper were used to investigate the effect of these on *R. solani.* All the three phytobiocides were used at rate of 150 mg/ 20ml of PDA [18]. Un-amended PDA medium served as control (Fig. 1). All the petri dishes were inoculated at the center with *R. solani*, isolated from infected potato tuber. Using Complete Randomized Design (CRD) each treatment was replicated four times and the Petri dishes were incubated after sealing with parafilm at 27 ^{0}C for a period of 21 days.

2.5 Screen house assessment of bio control agent and phytobiocides

A pot experiment was conducted in screen house. The pots having a size of 8x11 inches were filled with a medium of sterilized soil consist of clay, silt and Farm Yard Manure (FYM) in the ratio of 1:1:1. The soil first put in plastic bags and then sterilized in autoclave. Each pot was filled 2/3 of its total volume from soil. Five days before plantation of sprouted potato tubers soil was inoculated with 10 and 20 g doses of phytobiocides and biocontrol agent respectively. Potato tuber having 3-4 eyes were grown having almost same size to a depth of 4-5 cm. The selected bio control agent *Paecilomyces sp*. and phytobiocides (turmeric and chilli) were used to show their effect on *R. solani.* One treatment was kept as check for comparison.

The data was collected through visual observation using the 0-5 disease rating scale described by [4].

0 = No symptoms on potato tubers,
1 = Less than 1% tuber area affected,
2 = 1-10% tuber area affected,
3 = 11-20% tuber area affected,
4 = 21-50% tuber area affected and
5 = 51% or more tuber area affected.

The following treatment were used

T_1 = Healthy control (no *R. solani*, biocontrol agent and phytobiocides)
T_2 = Disease control (10g *R. solani* only),
T_3 = Turmeric (10g),
T_4 = Turmeric (20g),
T_5 = Chilli (10g),
T_6 = Chilli (20g),
T_7 = *Paecilomyces sp.* (10g),
T_8 = *Paecilomyces sp.* (20g).

2.6 Parameters studied

Percent eye germination, plant height, black scurf disease index and yield were measured. Randomized Complete Block Design (RCBD) was used in screen house condition and Complete Randomized Design (CRD) used in *in vitro* experiment and analyses were done by using statistic software (Statistix 8.1).

2.7 Data analyses

The radial growth of the fungus *R. solani* was measured periodically as colony diameter along two perpendicular lines. Complete Randomized Design (CRD) was used. The data were subjected to analyze of variance (ANOVA). The least significant difference (LSD) test was used to separates means when ANOVA revealed significant differences among treatments.

3. Results

3.1 *In vitro* screening of phytobiocides

After seven days of inoculation, turmeric was found to be the most effective phytobiocides, allowing only 7mm growth of *R. solani* (Table 1). Chilli also inhibits the growth of *Rhizoctonia solani* significantly (10.25mm) followed by pepper (12.50mm) when compared with control (13.50mm). However, no significant difference was observed when the colony diameter of *R. solani* on pepper amended PDA (22.25mm) was compared with control (24.25mm) after 14 days of incubation. Overall, after 21 days of incubation, turmeric was found to be the most effective inhibitor reducing the growth of *R. solani* to (32mm) followed by chilli (45mm). Pepper was found the least effective in reducing the growth of *R. solani* to (47.00mm) when compared to control (56.50mm), received distilled water only.

3.2 Screen house study

3.2.1 Effect of phytobiocides and biocontrol agents on potato eye germination (%)

On the basis of *in vitro* evaluation two best phytobiocides (turmeric and chilli) and *Paecilomyces sp.* were further tested under screen house conditions. The maximum numbers of eye germination (85%) was recorded in control, received the distilled water only to get the placebo effect. However, eyes germination of 80.5% was recorded when turmeric was mixed at the rate of 20g per each pot. Similarly, *Paecilomyces* sp. was also proved to be excellent and registered a percent eye germination of 73.75 percent. Conversely, the minimum eye germination was recorded in the pots that were pre-mixed with inoculum load only. A moderate effect on percent eye germination was also shown by turmeric and biocontrol agent when applied at the rate of 10g per pot.

Table 1: Effect of phytobiocides on colony diameter (mm) of *Rhizoctonia solani*

Phytobiocides	Colony diameter (mm)			Mean
	7 days	14 days	21 days	
Turmeric	7.00 k	15.00 h	32.00 d	18.00 d
Chilli	10.25 j	19.25 g	44.75 c	24.75 c
Pepper	12.50 i	22.25 f	47.00 b	27.25 b
Control	13.50 hi	24.25 e	56.50 a	31.41 a
Mean	10.81 c	20.18 b	45.06 a	

LSD value for Phytobiocides = 0.69, LSD value for Time = 0.83, LSD value for Phytobiocides x Time = 1.66

Table 2: Effect of phytobiocides and biocontrol agents on potato eye germination (%)

Treatments	Eye germination (%)	Increase in eye germination over disease control (%)
Control	85.00 a	148.17
Disease control	34.25 f	0
Turmeric (10g)	54.00 e	63.5
Turmeric (20g)	80.50 b	135
Chilli (10g)	54.50 e	59.12
Chilli (20g)	66.50 d	94.16
Paecilomyces sp. (10g)	67.25 d	96.35
Paecilomyces sp. (20g)	73.75 c	115.32
LSD(0.05) = 4.048		

Means in the first column followed by different letters are significantly different from one another at 5% level of probability.

3.2.2 Effect of phytobiocides and biocontrol agents on potato disease severity (%)

Significant differences (P<0.05) were observed among the different treatments. The minimum diseases severity (10.5%) was recorded when turmeric was used 20g/pot. Conversely, the maximum disease severity of 37.5% was recorded in the pots, treated with inoculum load only of 10g per each pot. The results obtained from the pots applied with *Paecilomyces sp.* at the rate of 20 g/pot was also excellent however this was par below from turmeric. Statistically similar results were obtained when *Paecilomyces* sp. and turmeric were applied at the rate of 10g per pot. Other phytobiocides and biocontrol agents also showed a reduction in disease severity when compared to control.

3.2.3 Effect of phytobiocides and biocontrol agents on potato yield/ plant

A significant increase (P<0.05) in yield of potato plants were observed when treated with phytobiocides and biocontrol agents after inoculation with *R. solani*. Maximum yield 85.25 g/pot was got when turmeric was used as 20 g/pot followed by control which was 79.25 g/pot. It shows 111.8% increase in yield over disease control which is 40.25 g/pot. Increase in the dose of the applied phyto biocide and biocontrol agent significantly enhanced the yield of potato per pot.

3.2.4 Effect of phytobiocides and biocontrol agents on plant height

A significant increase (P<0.05) in height of potato plants were observed when treated with phytobiocides and biocontrol agents after inoculation with *R. solani*. Maximum plant height (55.25cm) was recorded in pots, treated with turmeric at the rate of 20 g per pot in form of powder. Likewise, height of 51.25 cm was recorded when pots containing potato were applied with biocontrol agents (*Paecilomyces* sp.) at a rate of 20g. Conversely, the minimum (21.25 cm) plant height was recorded in the pots which received the inoculum load only. However, this was per below when compared with healthy control e.g. treated with distilled water only. The effect of chilli powder was also obvious and enhanced the plant height up to 42.5 cm. Similarly, effect of turmeric, chili and *Paecilomyces sp.* at the rate of 10 g were effective and enhanced the plant height up to 30.75, 25.75 and 29.5cm respectively

Table 3: Effect of phytobiocides and biocontrol agents on potato disease severity (%)

Treatments	Disease severity (%)	Decrease than disease control (%)
Control	3.25 f	91.33
Disease control	37.50 a	0
Turmeric (10g)	18.50 c	50
Turmeric (20g)	10.50 e	72
Chilli (10g)	23.25b	38
Chilli (20g)	16.50 cd	56
Paecilomyces sp (10g)	24.00 b	36
Paecilomyces sp. (20g)	14.75 d	60.7
LSD(%) =2.089		

Means in the first column followed by different letters are significantly different from one another at 5% level of probability.

Table 4: Effect of phytobiocides and biocontrol agents on potato yield/ plant

Treatments	Yield per plant (g)	Increase over the disease control (%)
Control	79.25 b	97
Disease control	40.25 f	0
Turmeric (10g)	57.00 de	41.61
Turmeric (20g)	85.25 a	111.8
Chilli (10g)	52.75 e	31.05
Chilli (20g)	58.25 d	44.72
Paecilomyces sp. (10g)	56.50de	40.37
Paecilomyces sp. (20g)	73.00c	81.36
LSD(0.05)=4.682		

Means followed by different letters are significantly different from one another at 5% of probability level.

Table 5: Effect of phytobiocides and biocontrol agents on mean plant height of potato

Treatments	Plant height (cm)	Increase over disease control (%)
Control	51.50 b	142
Disease control	21.25 f	0
Turmeric (10g)	30.75 d	45
Turmeric (20g)	55.25 a	160
Chilli (10g)	25.75e	21.61
Chilli (20g)	42.50 c	100
Paecilomyces sp. (10g)	29.50 d	39
Paecilomyces sp. (20g)	51.25 b	141.17
LSD (0.05)=3.657		

Means in the first column followed by different letters are significantly different from one another at 5% level of probability.

4. Discussion

R. solani is a very common fungus causing a number of diseases in different crops. The pathogen infection results in heavy losses in yield of many commercially important crops every year. Traditionally there was dependence on chemical for plant disease control, but over the time their harmful effect on human and animal health and degradation of the environment has been realized. This has necessitated the need for looking into alternative approaches in the plant disease control.

Researcher have reported fungicidal and bactericidal effects of plant extracts on specific soil borne pathogen [23, 7]. This study also found that turmeric and chilli successfully suppressed the growth of *R. solani*. Turmeric was the most effective phytobiocides reducing the radial colony diameter up to (32mm) followed by chilli (44.75mm) after 21 days of incubation when compared with control (56.50). These phytobiocides have some chemical metabolites which have inhibitory effect on the growth of *R. solani.* Several workers

have identified the suppressing effect of different chemical compounds extracted from different plants against the growth of fungi. The antifungal activity of turmeric and chilli both in powder as well as in extract form has also been confirmed by researcher previously. Hadian [20] investigated the effect of five plant extract, including turmeric against two soil borne pathogens *R. solani* and *Fusarium oxysporum in vitro*. The natural chemical compound hydroxychavicol extracted from turmeric showed fungicidal property against different fungi as reported by Ali *et al.* [6]. Significant reduction in the mycelial growth and number of spores of *R. solani* were observed when turmeric extract with different doses were incorporated in Potato Dextrose Agar medium [26, 10].

On the other hand, pepper failed to show effective inhibitory effect on *R. solani* perhaps, pepper lacks the active chemical metabolites against *R. solani.* Turmeric and chilli significantly reduced the growth of *R. solani.* Turmeric was found to be the most effective phyto biocide against *R. solani.* There is a need to study the metabolites in these phytobiocides which are active against *R. solani* and other plant pathogen. The use of phytobiocides found to be an effective and environment friendly approach to control *R. solani* both *in vitro* and screen house conditions. Plant world is a rich store house of natural chemicals. This natural products can be used as pesticides, especially against phytopathogens because it possess fungitoxicity against mycelial growth, spore germination etc [27, 37, 35]. Due to artificially produced chemicals [14, 12] different problems have been created like pathogen resistant strains development, pollution, slow biodegradation and carcinogenic etc.

Similarly many researchers have studied the antagonistic activity of biocontrol agents against *R. solani* previously [17]. Significant reduction in the colony diameter of *R. solani* was observed when *Paecilomyces lilacinus* and *Pseudomonas aeruginosa* was used. Likewise, maximum plant height and shoot weight was recorded when *P. lilacinus* was applied followed by *P. aeruginosa* at the rate of 0.1% [30]. Our findings were also parallel with Malhotra, *et al.,* [28] who studied the effect of thirteen isolates of antagonists and four strains of bacteria against *R. solani*. In this research *Paecilomyces sp* significantly reduced the disease severity, enhanced the yield per plant, plant height and eye germination as 14.75%, 73.00g, 51.25cm and 73.75% respectively at 20g/pot as compared to positive control having inoculum load only. This is because *Paecilomyces sp* suppress the growth of fungal pathogen with various mode of action such as competition for space, hyper parasitism and antibiosis. Researchers reported that it is often difficult to explore the exact mechanism of antagonism between a biocontrol agent and pathogen even *in vitro* and *in vivo* [33, 19].

5. Conclusion and Recommendation

Turmeric was found an effective phyto biocide against *R. solani in vitro* and under screen house condition. *Paecilomyces sp.* isolated from rhizosphere, was proved to be a potential biocontrol agent against *R. solani*. Phytobiocides such as turmeric and biocontrol *Paecilomyces sp.* should also be used against other soil borne plant pathogenic fungi.

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