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Evaluation of phytobiocides and different culture media for growth, isolation and control of *Rhizoctonia solani in vitro*

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Abstract
Evaluation of different culture media (Corn meal agar, water meal, potato dextrose agar, malt extract agar) for isolation and growth of *Rhizoctonia solani* and phytobiocides (Neem oil, garlic, ginger, turmeric and pepper) to investigate their effect upon the inhibition of mycelial growth of *R. solani* was carried out *in vitro.* Maximum biomass (21.50 mg) was harvested from potato dextrose broth followed by malt extract broth (19.50 mg), while the lowest biomass (3.50 mg) was produced by sterilized tap water. Food poison technique was used by amendment of phytobiocides in potato dextrose agar (PDA). Neem oil was used @of 0.1 ml/20ml potato dextrose agar medium, while the rest of phytobiocides were added @of 60mg/20ml of the medium. *R. solani* was inoculated aseptically on the center of the phytobiocides amended medium. Results of the study revealed that all the phytobiocides reduced the colony diameters of the *R. solani* significantly ($P \leq 0.05$) except ginger, compared with control (Un-amended PDA medium). Garlic was found to be the most effective inhibitory phytobiocides restricting the colony diameter to (38.25mm) as compared with other tested plant products.

Keywords: *Rhizoctonia solani,* Phytobiocides, Culture media, Mycelia.

1. Introduction
Rhizoctonia solani (teleomorph *Thanatephorus cucumeris*) was initially reported as a potato pathogen more than 150 years ago [16], and has since been proven to be a widespread and destructive fungal pathogen of many plant species [18]. There are currently 13 anastomosis groups (AGs) of *R. solani* [6], with those belonging to AG 3 predominately responsible for the infection of potato plants worldwide [10, 3, 8, 9, 14, 17]. Inoculum sources of *R. solani* include seed tubers and soil, both of which can harbour mycelium and sclerotia [22]. *R. solani* infects subterranean stems and stolons and severe lesions can have a negative effect on plant growth and tuber development [4]. The development of sclerotia on the surface of progeny tubers is a significant problem for growers, with severe black scurf infestations significantly reducing the quality and value of crops destined for both the ware and seed markets [7, 21]. Black scurf is is widely spread in Pakistan, especially in northern areas of Swat, Kaghan, Dir and Hunza valley etc. A few areas of Punjab are also affected.
The vegetative mycelium of *R. solani* and other *Rhizoctonia* fungi are colorless when young but become brown colored as they grow and mature. The mycelium consists of hyphae partitioned into individual cells by a septum containing a dough-nut shaped pore. This septal pore allows for the movement of cytoplasm, mitochondria, and nuclei from cell to cell. The hyphae often branch at a 90^{o} angles and usually possess more than three nuclei per hyphal cell. The anatomies of the septal pore and the cellular nuclear number have been used extensively by researchers to differentiate *R. solani* from other *Rhizoctonia species*.
R. solani causes a wide range of commercially significant plant diseases. It is one of the fungi responsible for damping off in seedlings, as well as black scurf of potatoes, bare patch of cereals, root rot of sugar beet, belly rot of cucumber, sheath blight of rice and much other pathogenic condition.
Sclerotia consist of loosely constructed knots of melanised hyphae, with no cellular differentiation into a rind or medulla [22, 24]. Unlike many other sclerotia forming plant Pathogens, *R. solani* sclerotia only undergo direct myceliogenic germination, whereby

vegetative hyphae capable of infecting the host grow directly out of the sclerotium [11]. Mycelia and sclerotia can grow and develop on plant debris as well as tubers, allowing inoculum to survive in the soil as well as on seed from season to season [13]. Despite the negative economic impact of sclerotia on potato, both as a tuber blemish and a source of inoculums on seed and in soil, past studies on the effects of environmental and nutritional factors on *R. solani* pathogenic to potato have tended to focus on mycelia growth [1, 22]. As a result, data on factors that influence sacerdotal formation or germination by *R. solani* is limited. This study aims to compare different media for isolation and growth of *R. solani* and evaluate different Phytobiocides for the control of *R. solani in vitro.*

2. Materials and Methods

2.1 Collection of *Rhizoctonia solani* infected potato tubers

The infected potato tubers were collected from a local market in Peshawar. Black sclerotia erumpent from the tuber skin clearly indicated infection by *R. solani*, i.e. Black scurf disease. As the white skin potato tubers contained more number of sclerotia so mostly these potato were collected. The size of sclerotia was 2 to 10 mm. Infected potatoes contained sclerotia are shown in (fig 1).

Fig 1: Potato tubers showing Black scurf infection in the form of sclerotia of *Rhizoctonia solani.*

Media used in the study

The media and their respective ingredients are listed below:

a) Potato Dextrose Agar (PDA)
b) Corn Meal Agar (CMA)
c) Malt Extract Agar (MEA)
d) Water Agar (WA)

These media were prepared by adding their respective ingredients to distilled water in flasks, sealing with aluminium foil and autoclaving at 121 ^{0}C (15 psi) for 15 minutes. The media were then poured aseptically in sterile petri dishes and allowed to cool.

Isolation of *Rhizoctonia solani* on different culture media

Sclerotia were cut out along with some potato skin with help of a scalpel. After surface sterilization with 0.1 percent mercuric chloride solution for 20 to 30 seconds, sclerotia were rinsed three times in sterile distilled water to eliminate the surface contaminants. Sclerotia were allowed to dry on sterile filter papers. The pieces were then plated on the four media under aseptic condition within a laminar flow unit. Each treatment was replicated four times in a Completely Randomized (CR) design. The plates were sealed with parafilm to avoid contamination and then incubated at 28 ^{0}C for 10 days until sclerotial germination. Slides were made from the culture of *R. solani* obtained from PDA (fig 2).

Preparation of pure culture for growth

A 3mm inoculum disc was cut out with a sterile cork borer from the culture of *R. solani* and inoculated at centre of the different media in petri dishes under aseptic conditions. Each treatment was replicated four times in a CR design. The petri dishes were incubated at 28 ^{0}C and checked regularly for further growth of *R. solani* and recording data.

Comparison of biomass of *Rhizoctonia solani* on different broth media

Broth preparations of the four different media were made by exclusion of agar. The broth media were sterilized by autoclaving at 121 ^{0}C (15 psi) for 15 minutes in 200 ml flask. Each flask was inoculated with a 3mm diameter inoculum disc cut out with a sterile corn borer from a fresh culture of *R. solani*. Each treatment was replicated four times in a CR design. The flasks were sealed with parafilm to avoid contamination and then incubated at 28 ^{0}C for 30 days until *R. solani* biomass production. After this broth was filtered through filter paper and biomass was left behind. The biomass was weighed with electronic balance.

Phytobiocides used in the study

Phytobiocides including neem oil, garlic, ginger, turmeric and pepper oils were used to investigate the effect of these oil on *R. solani*. In case of neem oil, 1ml was added to 20ml PDA in each petri dish, while in case of other phytobiocides, 20ml of PDA in each petri dish was amended with 60 gm of the finely ground plant product individually. Unamended PDA medium served as control. All the petri dishes were inoculated at the center with *R. solani*, obtained from the cultural bank of the department. Each treatment was replicated four times in a complete randomized (CR) DESIGN. All the petri dishes were sealed with parafilm and incubated at 25 ^{0}C for a prolonged period of times.

3. Results

3.1 Isolation of *Rhizoctonia solani* from infected potato tubers

Among the four media tested, *R. solani* was successfully isolated only on potato dextrose agar.

3.2 Growth of *Rhizoctonia solani* on different agar media

Among the four agar media, potato dextrose agar (PDA) supported the most abundant growth of *R. solani* (24.oo mm colony diameter) after 10 days of incubation, which was significantly (P<0.05) higher than that on the rest of the media (Table-1). Growth of the fungus was similar on both malt extract agar (MEA) and corn meal agar (CMA) but significantly higher than that on water agar (WA) which supported the least growth (13.00 mm). However, after 20 days of incubation, growth of the fungus was highest on MEA (36.00 mm) followed by PDA (28.50 mm) and CMA (22.50 mm). WA allowed only a limited further growth which ranked the lowest (16.00 mm) among the four media.

After 30 days of incubation, PDA again registered the maximum growth of (49.50 mm), which was statistically at par with the growth on MEA (46.00 mm) but significantly higher than the growth on CMA and WA. After 47 days of incubation, the relative performance of the four media remained unchanged from 30 days of incubation. However, considerable further growth occurred between 30 and 47 days of incubation in all media.

3.3 Biomass production by *Rhizoctonia solani* on different 3.4 broth media

Among the four broth preparations, potato dextrose produced the highest biomass of *R. solani* (21.50 mg) followed by malt extract (19.50 mg) (Table-2). These two broth preparations did not differ significantly from one another but both of them differ significantly from corn meal and sterilized tap water.

3.5 Effect of phytobiocides on colony diameter (mm) of *Rhizoctonia solani*

After 10 days of inoculation, garlic was found to be the most effective phytobiocides, allowing only 9.25mm growth of *R. solani* (Table 3). Neem oil and turmeric also inhibited the growth of *R. solani* significantly ($P \leq 0.05$) as compared with control. However ginger and pepper did not inhibit the growth significantly but after 20 days of incubation significant reduction were noticed in the radial growth of the pathogen.

After 30 days of incubation, it was found that only ginger failed to reduce the growth of *R. solani* significantly as compared to control. Again, garlic was found to be the most effective inhibitor of *R. solani*. However, it did not differ significantly from pepper and turmeric which were also effective against *R. solani.*

Table 1: Colony diameter (mm) of *Rhizoctonia solani* over prolong period of incubation

Media	10 days	20 days	30 days	47 days
Corn meal agar	17.00B	22.50C	29.00B	54.00B
Water agar	13.00C	16.00D	30.00B	51.00B
Potato dextrose agar	24.00A	28.50B	49.50A	70.50A
Malt extract agar	17.50B	36.00A	46.00A	68.00A
$LSD_{(0.05)}$	LSD=3.876	LSD=5.967	LSD=7.029	LSD=5.937

Means in a column followed by different letters differ significantly from one another at 5% level of probability.

Table 2: Biomass (mg) production by *Rhizoctonia solani* on different broth media

Broth	Weight
Sterilized tap water	3.50B
Potato dextrose broth	21.50A
Malt extract broth	19.50A
Corn meal broth	5.50B

Means followed by different letters differ significantly from one another at 5% probability.

Table 3: Effect of phytobiocides on colony diameter (mm) of *Rhizoctonia solani*

Treatment	10 days	20 days	30 days
Control	28 AB	48.38 AB	63.5 A
Garlic	9.25 D	24 D	38.25 C
Neem Oil	20.88 C	38 BC	52 B
Ginger	30.13 A	52 A	62 BA
Turmeric	18.38 C	36 BC	47.5BC
Pepper	22.13 BC	26 CD	47.1 BC
Lsd(0.05)	6.76	12.32	10.16

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

4. Discussion

Potato (*Solanum tuberosum* L.) is the most important vegetable crop of the world, including Pakistan, ranking number one among all vegetables both in production and consumption. The climate of Pakistan, especially of Khyber Pakhtunkhwa province, is very suitable for the production of potatoes. The average per hectare production of potato in KPK is well below its potential. One of the reasons for this low yield is the occurrence of different bacterial, fungal, viral and nematode diseases. Among these diseases, *Rhizoctonia* canker commonly called black scurf, caused by the fungus *R. solani* Kühn has been a severe problem in all potato production agro-ecological zones of the country [20].

This study showed that growth of *R. solani* was best on PDA as compared to other media, which is in line with the findings of Parmeter [19]. Who reported that the fungus grew most rapidly on PDA, because PDA is supposed to be the most nutritive media for growth of *R. solani* as it use carbon mainly for its nutrition purpose.

The presence of potato starch in PDA might have stimulated the growth of *R. solani* because potato is the natural host of the pathogen. Similarly, malt extract agar contains both N and C which are required for rapid growth of the fungus. Therefore malt extract agar served as well as PDA for the growth of *R. solani* in this study. On the contrary, corn meal agar and water agar did not support rapid growth of the fungus as they were poor in these essential nutrients.

Potato dextrose broth supported the production of greatest biomass of *R. solani* in this study. This finding is mine with the observations of Kühn [15] who also reported greater biomass production of *R. solani* on potato dextrose broth. Again, the presence of potato starch in the broth might have stimulated the growth of the fungus as potato is the natural host of the pathogen. The use of phytobiocides is a cheap, environment friendly and sustainable approach to plant disease control. Researcher have reported fungicidal and bactericidal effects of plant extract on specific soil borne pathogen [15, 12, 2].

This study also found that garlic, pepper, turmeric and neem oil successfully suppressed the growth of *R. solani*. These phytobiocides might have some chemical metabolites which have inhibitory effect on the growth of *R. solani.* On the other hand, ginger failed to show any inhibitory effect on *R. solani*

perhaps, ginger lacks the active chemical metabolites against *R. solani.* Garlic, pepper, turmeric and neem oil significantly reduced the growth of *R. solani.* Garlic was found to be the most effective phyto biocide against *R. solani.* These plant products should be tested *in vivo* to find their efficacy under field conditions. There is a need to study the metabolites in these phytobiocides which are active against *R. solani* and other plant pathogen.

5. Conclusion and Recommendations

The comparison among four different media indicated the superiority of potato dextrose agar and malt extract agar over corn meal agar and water agar in enhancing the growth of *R. solani*. Biomass of *R. solani* on potato dextrose broth and malt extract broth was significantly greater than on corn meal broth and sterilized tap water. For *in vitro* studies of *R. solani*, potato dextrose agar should be preferred over other media. Garlic was found to be the most effective phytobiocides against *R. solani*. Detailed studies are needed to elaborate the result of this research.

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