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MANAGEMENT OF SHEATH BLIGHT OF RICE CAUSED BY *RHIZOCTONIA SOLANI* KÜHN

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A B S T R A C T

Sheath blight (ShB) of rice caused by *Rhizoctonia solani* Kühn is the most vital disease this is a serious issue in high-yielding rice production systems. It is a significant issue with the rice producing system's high yielding types. The pathogen has a very wide host range and due to non-availability of resistant cultivars, the management of ShB primarily depends upon chemical control while the chemical control is not eco-friendly and is also dangerous for human health. However, the disease can be managed with botanical pesticides and biological agents. This study was planned with the objective to manage this disease with eco-friendly approaches. Different species of *Trichoderma* and plant extracts were evaluated under the laboratory conditions with dual culture technique and poisoned food technique respectively. Results showed that all bio-controls and plant extracts significantly inhibited mycelial growth of *R. solani* at different exposure intervals (5, 10 and 15 day) ($P<0.05$). In fungal bio-control, the highest percent inhibition was given by *T. harzianum* followed by *T. atroviride* on all incubation intervals. The lowest percent inhibition was 55%, 69 and 79% given by *T. viride*. All plant extracts significantly inhibited the mycelium growth of *R. solani* ($P<0.05$). Maximum percent inhibition was observed in case of neem followed by garlic, eucalyptus, and the minimum percent inhibition was exhibited by mint on 5, 10 and 15 days of incubation intervals.

Keywords: Plant extracts, inhibition, mycelial growth, *R. solani, S. oryzae*.

INTRODUCTION

Rice (*Oryza sativa* L.) is Asia's main food crop and accounts for more than half of the world's population (Qin and Zhang, 2005). In Pakistan, accounting for 10% of total cultivable land. (Shaikh *et al*., 2011). Rice is a very valuable crop and accounts for 3.1 percent of agricultural value added and 0.6 percent of Pakistan's GDP. Pakistan is best known for its Basmati rice, a type of aromatic rice. The color, fragrance and taste of the fragrant rice are the three main factors that are widely identified, with such a strong fragrance. Pakistan plays a significant global role as a rice exporter and exports over 2 million tons annually, which is 10 percent of the world's trade. Total consumption of global rice has raised to 439 million in 2010 from 350 million tons in 1991, and this is projected to be increased to 555 million tons by 2035 (IRRI, 2010). To satisfy the demands of rising global food demand, a large growth in the projected human population requires higher crop yields.

Just like other crops, the yield of rice is also affected by several biotic and abiotic factors. Several pathogens, which often place significant development constraints such as fungal, bacterial, and viral, affect rice productivity. Rice ShB is caused by *R. solani*, which causes up to 45 percent yield losses (Margani and Widadi, 2018). *R. solani* has a diverse host range and causes a variety of diseases on major crop plants around the world (Ogoshi, 1996).

The pathogen, *R. solani* can cause various disease symptoms such as leaf blights, leaf spots, seedling damping-off, root rot, shoots and fruits rot as well as canker lesions on sprouts and stolons (Chang and Chou,

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2007). In 1910, ShB was first identified in rice in Japan, which was then spread throughout the region in rice, particularly in areas where rice as extensively cultivated. The disorder is particularly obvious during the joint elongation stages in late tillering of rice panicle differentiation. Sheath lesions, which lead to softness and sheath lodging, as well as grain filling inhibition, are early disease symptoms (Wu *et al*., 2012). Communication with different parts of plant for example leaves and tillers, as well as sclerotia present in surface water, help the fungus to spread rapidly. The disease severity depends on the methods of planting, the growth phases of the plant during outbreak time, the use of nitrogen fertilizers (Norman *et al*., 2003) and the susceptibility of the rice variety (Tang *et al*., 2007).

The high survival rate of sclerotia, as well as its extremely broad host range and ecological behavior, make it difficult to control *R. solani*. As a result, despite the fact that some strains' polyphagous nature allows them to infect regularly rotated crop species, agronomic methods such as crop rotation are deliberately discouraged. In the fight against this disease. Fungicides with a broad spectrum of action are also available; however, they are harmful to the environment. Furthermore, chemical management approaches may not be viable or cost-effective for many soil-borne diseases. As a result, using a biocontrol technique to protect plants from these soil-borne fungi is an environmentally safe option. Biological management with the genus *Trichoderma* has been demonstrated to be successful in controlling *R. solani* in numerous experiments (Brewer *et al*., 2005) activating plant defensive responses as well as encouraging plant development (Druzhinina *et al*., 2011). *Trichoderma spp*. are filamentous fungus that are cosmopolitan, facultative, and anaerobic, and can be found in decomposing wood and agricultural soils in high quantities (Druzhinina and Kubicek, 2005). *Trichoderma* has a diverse range of lifestyles and interactions with R. solani, and it can be used to biologically control plant diseases (Anees *et al*., 2010). Plant compounds have also been reported to be effective in combating some of the most damaging plant diseases (Oloumi, 2014). Antimicrobial activity has been reported in plant extracts containing phytochemical compounds like alkaloid, tannin, flavonoid, saponin and steroid, (Yogi *et al*., 2016). The aim of this study was to create a practical and eco-friendly approach to control ShB by biocontrol methods and plant extracts in a cost-effective manner, all tested under controlled conditions.

MATERIALS AND METHODS

Collection of diseased samples: Several rice field surveys for the collection of diseased samples were conducted. The infected samples from above the ground portions were collected from diseased plants on the basis of characteristic symptoms. The samples were stored in laboratory at 4ºC for further evaluation.

Figure 1. Initial symptoms of ShB of rice in the field (A) and (B) mature symptoms of ShB in rice fields.

Isolation of pathogen and media culturing: A healthy tissue section along with diseased rice tiller was divided into tiny pieces (0.5-1.0 cm) and surface sterilized for two minutes with 1% sodium hypochlorite. Distilled water was used to wash the samples for 2-3 times, now the pieces were placed on media plates, and incubated for five days at 28°C. Potato dextrose agar (PDA) media was used for the isolation and multiplication of pathogen. The pathogen was identified based on its morphology and mycelial features under compound microscope (10x, 40x

and 100x). In order to get pure culture, a mycelial plug (5 mm) from the actively developing area of the 5-day old culture was placed on PDA plates after identification.

The mycelial plug having PDA media was transferred for multiplication and purification of pathogen *R. solani,* and incubated the petri dishes in incubator at 25˚C. The development of mycelial growth was checked after every 24 hours.

Figure 2. Microscopic view of culture.

Pathogenicity test: For pathogenicity of the isolates, detach assay was performed. Spore suspension of all isolates was prepared. The healthy plant leaves were collected from the field, surface sterilized, and washed with distilled water. Then, 5mm inoculated plug was placed on injured leaves. After that, these boxes were placed in growth room and symptoms were observed on daily basis up to one week (Forseille *et al*., 2009).

Isolation and identification of biocontrol agent (*Trichoderma* spp.): Trichoderma species were isolated from soil using two methods:

Soil dilution method: One gm of soil was taken and added in 9ml of distilled water for making 1st dilution. In the same way 4 dilutions were prepared. Few drops were taken from 4th dilution and poured in petri plates having PDA medium and plates were incubated at 25˚C (Arshi and Nasreen, 2016). *Soil plate method*: Small amount of soil (0.005-0.015 g) was taken and spread in petri plates having PDA medium. The plates were incubated at 25˚C (Arshi and Nasreen, 2016).

After growth, pathogen was identified by cultural and morphological characteristics like colony color, shape, size and hyphal morphology.

Preparation of plant extracts for in vitro evaluation: Different parts of garlic, neem, mint and eucalyptus were picked-up from the vicinity of College of Agriculture, Sargodha University, Sargodha. The samples were cleaned under running water, rinsed three times with clean purified water, and air dried for two to three hours. In a mortar and pestle, 20 gm sample were grounded, then added with 80 ml of pure distilled water and filtered through double layered muslin cloth. In a conical flask, the filtrate was collected and kept at 25-28˚C (Devi and Chhetry, 2013). This served as 100 percent stock solution of plant extract.

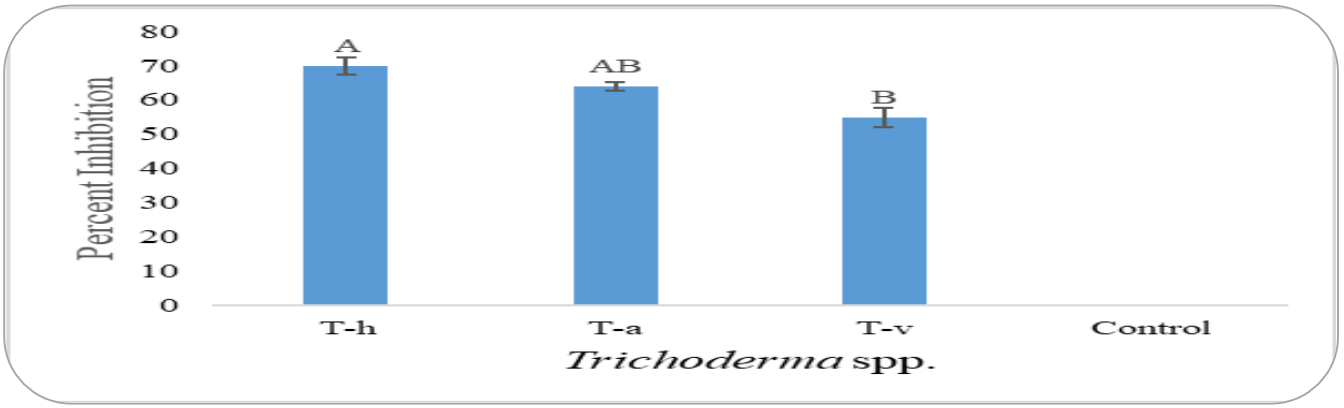
Plant extract inhibitory assay against *R. solani:* Using the food poisoning approach, plant extracts were evaluated against *R. solani* for their anti-fungal efficiency. Plant extract concentrations 15% and 20% was used in PDA. To achieve a final concentration of 15% and 20%, each plant extract was dissolved completely in 20ml slightly warm PDA. Two replications were used for each concentration. After solidifying the PDA, 5 mm mycelium plugs of 7-10 days old culture of *R. solani* were put in the middle of the petri plates. The plates were incubated at 28˚ C for 5 and 10 days to observe the radial development. PDA plates without any plant extract, served as control. Mycelium growths in treated and untreated (control) petri plates were measured.

Dual culture method: In a dual culture technique, the antagonistic activity of different *Trichoderma* isolates against *R. solani* was examined in vitro by following method of Kucuk and Kivanc (2004) with some modifications. Five-mm discs of *R. solani* were taken with cork borer and placed in plates at the periphery of petri plates having 20 ml sterile PDA. Now, another 5 mm disc of test antagonist was placed against the opposite side of each plate. In a control treatment, disc of sterilized media was placed in the plate. Plates were put in incubator at 25˚ C. Data of radial growth and inhibition was collected after 7 days of incubation. Growth of colony was measured from both side of growth. When the fungus completely

covered the plates in control treatment, inhibition percentage of fungus was calculated (Fokkema, 1973

RESULTS

Evaluation of *Trichoderma* spp. against *R.solani:* The results showed that all *Trichoderma spp*. most effectively suppressed mycelial growth of *R. solani* at 5, 10 and 15 days. Percent inhibition was higher at 15 days in case of *T. harzianum*. In case of *T. atroviride* percent inhibition was higher at 5 day which was 64% but inhibition was stopped at 15 days at 79%. Same trend was observed in *T. viride.* Higher percent inhibition 70%, 79% and 86% was given by *T. harzianum* at 5, 10 and 15 days, respectively, followed by *T. atroviride,* which inhibited mycelial growth to 64% 72% and 82%. Least percent inhibition was given by *T. viride* that was 55%, 69% and 79%, respectively (Figure 3).



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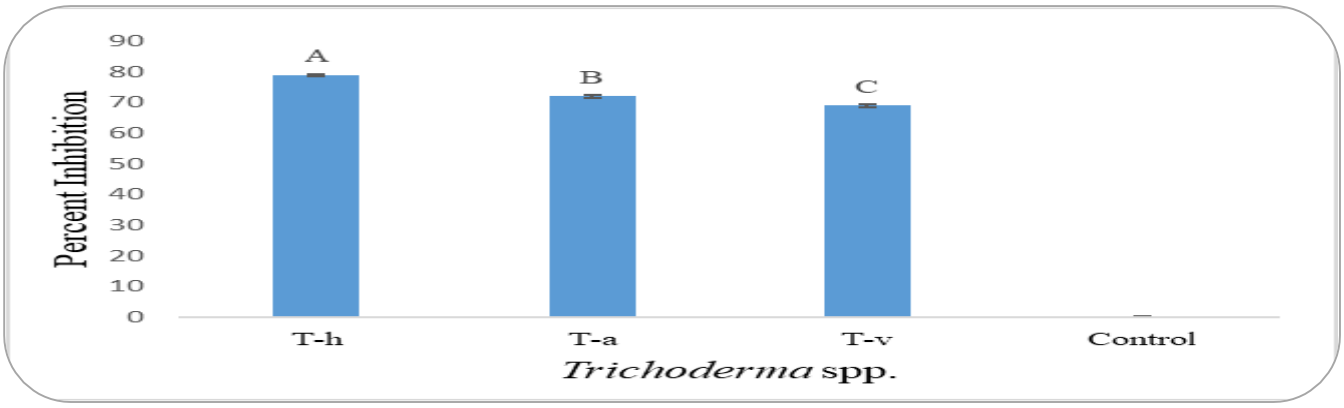

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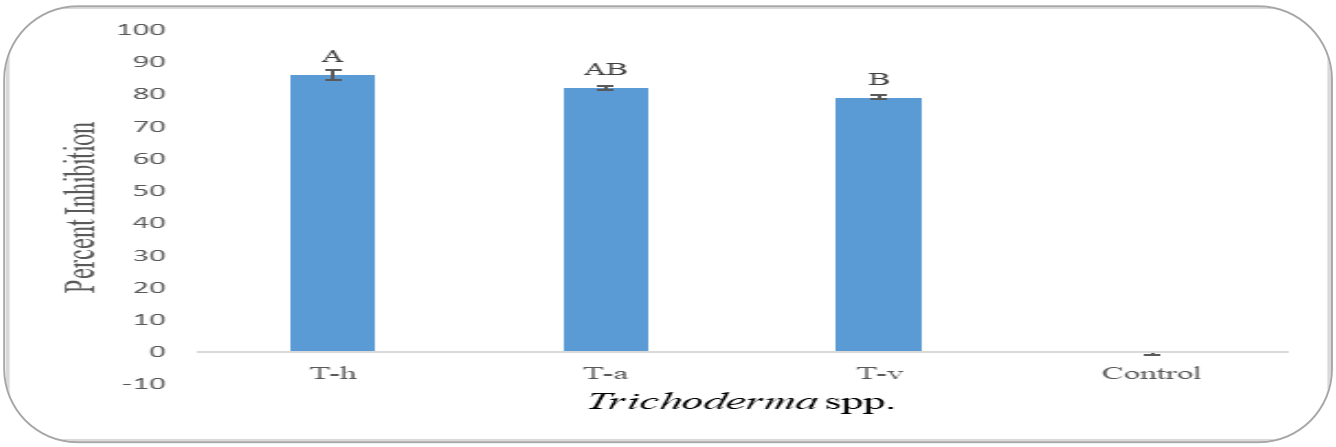
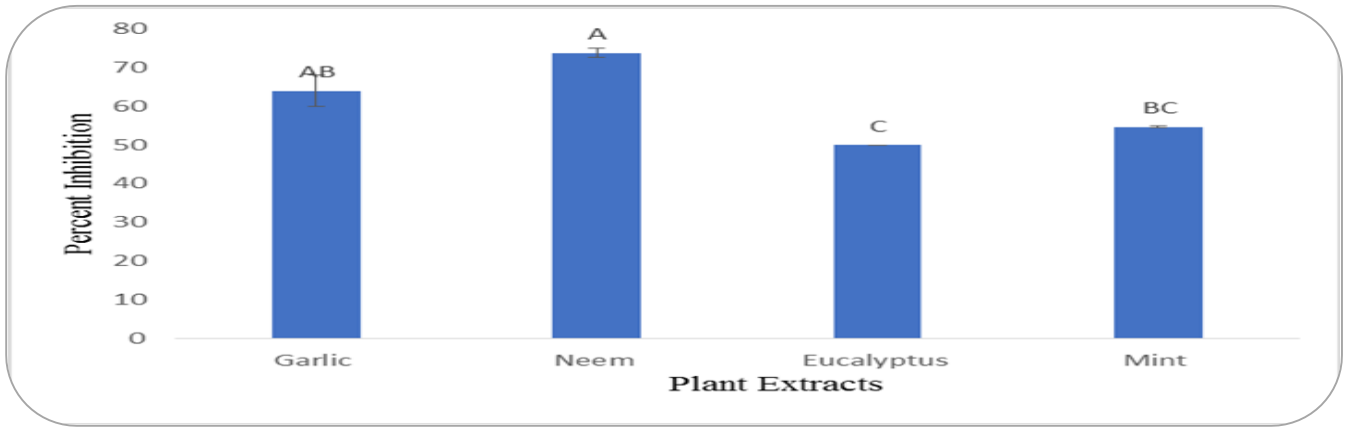


C

Figure 3. Mean inhibition (% ± SE) of *R.R. solani* mycelia on PDA dishes containing dual culture with different *Trichoderma* spp. (T-h: *T. harzianum*, T-a: *T. atroviridae*, T-v *T. viridae*) at 5 (a), 10 (b) and 15 (c) days after treatment. Means followed by the same letter are not significantly different, Tukey-Kramer (HSD) test at $p = 0.05$.

Evaluation of plant extracts against *Rhizoctonia solani:* Mycelial growth of *R. solani* was significantly affected by all plant extract at 5, 10 and 15 days ($P<0.05$). Percent inhibition was higher after 5th day and gradually increased at 10 and 15 days for all plant extracts with exception to garlic which gave best results at 15 days of treatment. Maximum percent inhibition was observed in neem 73%, 75% and 78%, followed by garlic, 64%, 69% and 72% at three respective days. Percent inhibition given by eucalyptus was 50%, 57% and 68% that was 54%, 55% and 59% in case of (Figure C).



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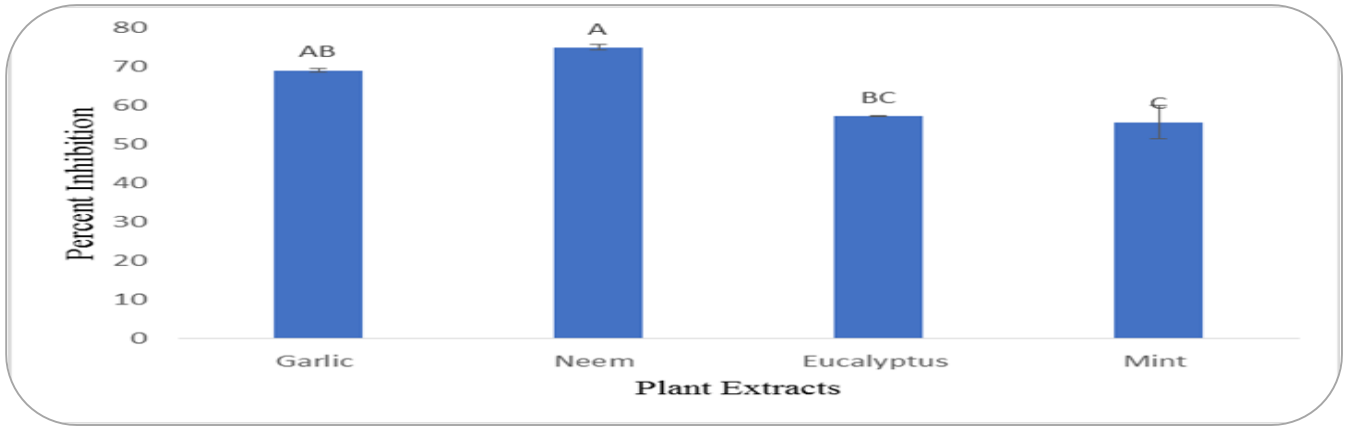

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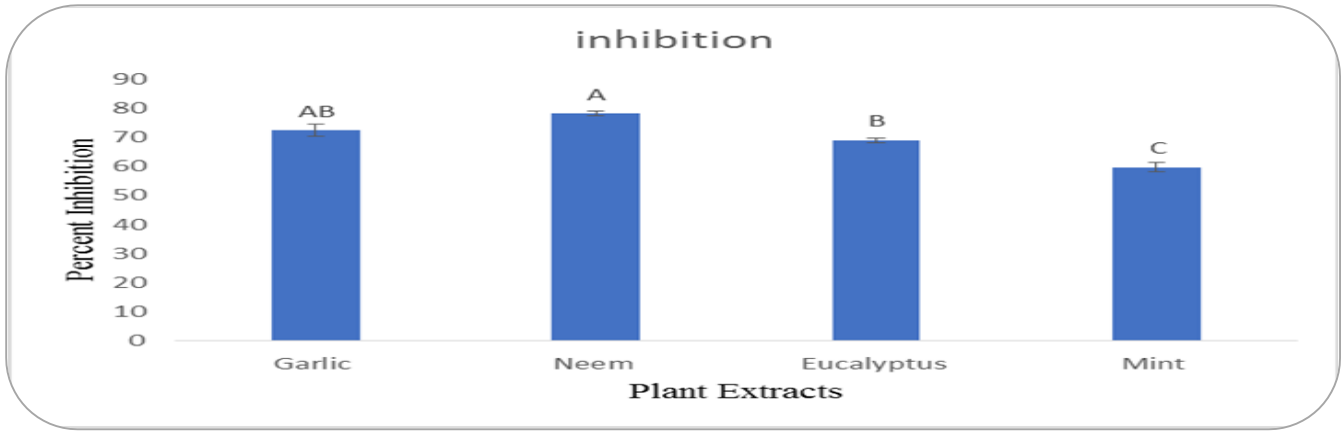

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Figure 4. Mean inhibition (% ± SE) of *Rhizoctonia solani* mycelia on PDA dishes using food poisoning technique with different plant extracts at 5 (a), 10 (b) and 15 (c) days after treatment. Means followed by the same letter are not significantly different, Tukey–Kramer (HSD) test at $p = 0.05$. The inhibition in control treatment was 0% for all treatments and intervals.

DISCUSSION

Sheath blight disease caused by *R. solani* Kuhn is a significant contagious disease of rice. The pathogen infects around 32 plant families from 188 species, indicating that it has a wide host range (Srinivasachary and Savary, 2011). Its wide host range (Zheng *et al*., 2013) makes it an extremely harmful for agriculture economy of countries around the world (Druzhinina *et al*., 2011). To variable degrees, *R. solani* can cause seed and seedling infections in zinnia, pepper, lettuce, and eggplant (Lewis and Lumsden, 2001). It produces stem canker and black scurf in potatoes (*S. tuberosum* L.), resulting in lower tuber yields and poor tuber quality (Brewer *et al*., 2005). Cotton root rot is the most severe disease caused by *R. solani* (Gajera *et al*., 2016). It was referred with various names like 'Banded rice blight', 'Oriental leaf and ShB', '*Pellicularia* ShB' and 'Sheath blight' in other Asian countries. The pathogen spreads swiftly by contact between plant elements such as leaves and tillers, as well as through sclerotia (thick compact hyphal structure) present in surface water (Tsiboe *et al*., 2017).

The goal of this research was to examine the efficacy of some antagonist bio-control treatments against *R. solani* at different time intervals. The effectiveness of test fungal antagonists was variable with respect to the incubation intervals. *T. harzianum* was the antagonist with the highest percent inhibition against *R. solani*. Previous studies have also exhibited that *T. harzianum* was extremely efficient against a variety of pathogenic-fungi (Smolinska and Kowalska, 2018).

Seema and Devaki (2010) evaluated dual culture in vitro to test the effectiveness of four fungal and one bacterial biocontrol, namely *Aspergillus-spergillus niger*, *Penicillium* spp., *T. viride*, *T. harzianum*, and *B. subtilis*, against the tobacco sore shin pathogen caused by *R. solani*. *T. harzianum*, *T. viride*, *A. niger*, *B. subtilis*, and *Penicillium* spp. all inhibited *R. solani* growth by 70, 67, 57, 50, and 44%, respectively. Srinivas *et al*. (2014) observed *T, viride* was shown to be the most efficient inhibiting *R. solani* growth, followed by *P. notatum*, and *A. niger*. Johnson *et al*. (2008) revealed that soil treatment with *T. harzianum* was more viable in reducing ShB when contrasted with *T. virens* and commercial formulations of bio-agents. Abdel-Fattah *et al*. (2007) stated that the severity of brown spot-on rice leaves cultivated in a field was decreased by spraying *T. harzianum* at 15-day intervals. *Trichoderma* spp. can cause systemic and regional resistance in plants, as well as create an antagonistic relationship with pathogens by fighting them directly or limiting their growth and through colonization of plant.

T. harzianum reduced the mycelial development of *R. solani*, according to the findings of this investigation. This biological agent can function in a variety of ways, including playing an important role in the environment. To colonize different ecological niches, they employ a number of methods. Several *Trichoderma* spp. have a beneficial effect on plants by promoting growth and protecting them against fungal and bacterial diseases. They're utilized as bio-fungicides in biological plant protection and bioremediation. *Trichoderma* spp. members are also used in a variety of industries, mostly in the manufacture of enzymes, antibiotics, and other metabolites, but also in the production of biofuels (Strakowska *et al*., 2014).

The “food poisoning approach” used in present study to evaluate different plant extracts is a basic and straightforward approach for in vitro testing (Sultana and Ghaffar, 2013). The finest plant extract and its optimum concentration against fungal infection may be evaluated in a short amount of time with low resources utilizing the food poisoning approach. This method has been used efficiently to test the effectiveness of various plant extracts against fungal infections (Rather *et al*., 2012; Dar *et al*., 2013).

The utilization of different plant extracts in the management of *R. solani* was investigated in the current study. Previous research has shown that the kind of solvents used for extraction affects the effectiveness of active ingredient of extracts (Tiwari *et al*., 2005). The choice of solvents is mostly determined by the type of the bioactive ingredient contained in the plant. Methanol extracts of many Zingiberaceae family was shown to have strong antifungal and antibacterial activities. Extracts of *Inula viscosa* prepared in a range of organic solvents (n-hexane, ethanol, ethyl acetate, methanol, acetone and chloroform) were found to have antifungal action against *Cladosporium cucumerinum*, *Botrytis cinerea*, *Plasmopara viticola* and *Phytophthora infestans* (Cohen *et al*., 2002). To suppress *Fusarium sp*., plant extracts from various families were applied (Russell and Mussa, 1997). Narasimhan *et al*. (1998) investigated the efficacy of TNAU's neem and pungam

oil-based EC formulations against rice sheath rot disease in field settings and found that the formulations were effective in controlling the disease. Choudhury *et al*. (2017) also stated that 0.5 percent neem oil successfully reduced the ShB disease caused by *R. solani*.

CONCLUSION

Results of present study showed that evaluated antagonists and plant extracts were effective against *R. solani*, however, their efficacy varied with day interval. Biological antagonists *T. harzianum* found most effective against *R. solani*. In plant extracts, neem was found to be most effective against pathogen.

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Contribution of Authors:

Muhammad U. Ghazanfar	:	Conceived the idea and facilitated, guided and supervised the experiments
Asfand Y. Ali	:	Conduct experiments, Wrote and finalized the manuscript
Waqas Raza	:	Help in experiments and help to finalize the manuscript
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