RESEARCH ARTICLE

Biological management of root rot disease of wheat (*Triticum aestivum* Linn.) caused by *Rhizoctonia solani*

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The present paper reveals the biological management of root rots disease of wheat caused by the pathogen *Rhizoctonia solani* Kuhn. The bio-control agents like *Trichoderma harzianum* and *T. viride* were found to be the active inhibitors in mycelial growth of *R. solani*. *Trichoderma* spp. behaves as a safe, low-cost, effective, eco-friendly bio-control agent for different crop species. Substrates like talc formulation, farmyard manure and wheat bran have been reported to support the maximum growth of test antagonists. During the study work the bio-control agents (*T. harzianum*, *T. viride*, *Gliocladium. virens* and *G. roseum*) when applied individually reported to have significant improvement in the growth and biomass of wheat plants in addition to the reduction of root rot disease by 30 to 50 percent in comparison to the seedlings inoculated with *Fusarium oxysporum* alone, with maximum reduction by *T. harzianum* and minimum by *Trichothecium*.

Key Words: *Wheat*; *Bio-management*; *Root rot*; *F. oxysporum*; *R. solani*; *Bio-agents*

INTRODUCTION

Wheat (*Triticum aestivum* Linn.) is the second staple food crop of India after rice, with about 29.8 million hectares of land under the crop and is mainly grown in the Northern and Central part of the India. Biological control is mainly used to harmful organisms and their products to control plant diseases and effectively reduce the application of chemical fertilizers and pesticides [1]. *Trichoderma*, a biological fungus widely used for plant pest control, mainly exists in the soil, air, plant surface and other ecological environments and can effectively control a variety of plant, leaf and spike diseases [2-6].

Many studies in the past have proved the potential of Trichoderma spp. as biocontrol agents for several soil-borne plant pathogens such as *Rhizoctonia solani*, *Sclerotium rolfsii*, *Pythium* spp., *Drechslera tritici* and *Fusarium* spp. [7-9]. Few other studies have revealed that wheat bran has been proved as a suitable food base for introduction of *Trichoderma* spp. into soil [10,11].

MATERIALS AND METHODS

Studies were conducted on the etiology and bio-management of root rot of wheat (*Triticum aestivum* Linn) seedlings during the Rabi season of 2011 at rice research and regional station Khudwani, Anantnag (Jammu and Kashmir, India) situated in the temperate zone at an elevation of 1650 m amsl. The climate in general is temperate and is characterized by mild summer. The pathogen associated with the disease isolated, morphologically characterized and identified as *Rhizoctonia solani* Kuhn.

Isolation, purification and identification of pathogen

Wheat plant showing the typical symptoms of root rot disease were surface sterilized in 0.1% mercuric chloride for one minute followed by three rinses with sterilized distilled water. The pieces were then aseptically placed on Potato Dextrose Agar (PDA) medium in 90 mm petri dishes and incubated at 25°C ± 2°C for 72 hours. The fungal growth was examined and cultured on PDA. The purification of pathogen was done by single hyphal tip method and maintained in PDA slants. The identification was done by comparing with standard laboratory manual and got identified up to species level through the pathogenicity test [12].

Pathogenicity test

A soil-sand (2:1 w/w) mixture was prepared and put in earthen pots of eight kg capacity at five kg per pot. Fungal inoculation, multiplied on wheat grains was added at 50 g/kg to pot mixture separately just before seed sowing. The inoculation density of the fungi used was 8×10^4 cfu per gram of *Rhizoctonia solani*.

Viable wheat seeds were surface sterilized in 1% sodium hypochlorite for 15 minutes and washed thrice in distilled water. The seeds were then sown in pots infested with test fungus. The pots were incubated in a glasshouse at 26°C ± 3°C. The plants were critically examined and typical root rot symptoms on test plants, if any, were recorded. The causal pathogens were re-isolated and compared with mother isolate. The pathogenesis by these pathogens was thus confirmed by following the Koch's postulates.

Maintenance of Biological Control Agents (BCA)

BCAs such as *Trichoderma harzianum*, *T. viride*, *Gliocladium roseum*, *G. virens* and *Trichothecium* spp. obtained from division of plant pathology (SKUAST-K), were maintained on PDA and periodically sub-cultured. Mass culture of BCAs was prepared on coarse grinded maize grains already soaked in water and filled two-thirds portion of 250 ml conical flacks and autoclaved at 1.04 kg cm^{-2} for 20 minutes.

In vitro evaluation of Bio-Control Agents (BCAs)

Antagonistic activity of various fungal antagonists against root rot pathogen (*R. solani*) of wheat was assessed by dual culture technique [13]. Five mm discs of seven day old cultures of test pathogen as well as bio-control agent were taken with the help of a cork borer and placed on the fresh PDA containing petri-plate at the corner near the periphery about 60 mm apart in 90 mm petri-plate and incubated at 25°C ± 2°C in BOD incubator. The petri-plates in which only pathogen disc was inoculated on PDA served as control. Each treatment was replicated four times. The experiment was laid out in a completely randomized design. The mycelial growth inhibition of the pathogen over control was calculated using the formula given by Vincent [14].

Mycelial growth inhibition (%)=Mycelial growth in control-mycelial growth in treatment/mycelial growth in control ×100

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Under field conditions the disease incidence and severity were recorded at flowering stage and calculated:

Disease incidence (%)=No. of diseased units/total No. of examined plants × 100

Disease severity (%)=Sum of the individual ratings/total observed plants × Highest ratings × 100

Root rot index was calculated on the basis of percentage of root area affected according to the root rot index rating scale described by Purkayastha et al., as under:

No root rot=0

<10% root area affected=0.10

11-25% root area affected=0.25

26-50% root area affected=0.50

51-75 root area affected=0.75

<76% root area affected=1.00

RESULTS AND DISCUSSION

- Growth appeared within 36-48 hours

Colony growth: More spreading than aerial.

Colony colour: Initially white later turned brown.

Sclerotia formed: After 72 hours.

- Morphological characteristics of pathogen.

Mycelial thickness: 4-6 mm.

Colour of vegetative hyphae: White, turned brown in old culture.

Septation: Present.

Branching: Right angles (more or less).

- Sclerotial characteristics of pathogen

Shape: Irregular and flat with smooth surface.

Colour: Initially white latter turned brown.

Size: 3-4 mm (but a crust of sclerotia was more in size).

Pathogenicity

The pathogen showed the typical symptoms after 12-15 days when inoculated on wheat plants. The pathogen was re-isolated, characteristics of the pathogen (constriction at the point of formation of septa and right angled branching of mycelium) were compared and found similar with earlier one and confirmed as the cause of root rot disease of wheat [15-17].

In vitro effect of bio-control agents

The five bio-control agents were studied for their antagonistic nature against *R. solani* by dual culture method. Table below shows the average radial growth and maximum inhibition percentage of *R. solani* when treated with respective five bio-control agents (Table 1).

TABLE 1
The average radial growth and maximum inhibition percentage of *R. solani* when treated with respective five bio-control agents

Treatments	Average radial growth of *R. solani* (mm)	Inhibition percentage of *R. solani* (mm)
T. harzianum	28.71	65.87
T. viride	29.38	65.13
G. virens	35.38	54.46
G. roseum	40.1	53.22
Trichothecium spp	38	55.55
Control	90	-

Parasitism between bio-agents and *R. solani*

The parasitism between antagonists (*T. harzianum* and *T. viride*) and pathogen (*R. solani*) revealed that antagonists attacked pathogen by running along the host and penetrating pegs to suck nutrients. Coiling of *T. harzianum* around the *R. solani* caused disintegration of the host. Richardson also found that *T. harzianum* performed most drastic hyper parasitic activity to *R. solani* on PDA and in soil [18]. Parasitisms between *T. viride* and *R. solani* have shown that *T. viride* hyphae remained adhered to the host hyphae. The host hyphae also showed the stress condition due to parasitic activity of the *T. viride* which was apparent by disintegrated appearance of invaded hyphae however, no coiling was observed in case of *T. viride*. Similarly, in the hyphal interaction between *G. virens* and *R. solani*, *G. virens* hyphae also ran along the host hyphae and formed appressoria like intrusions which penetrate the host hyphae [19]. Dubey also reported that *G. virens* coiled the *R. solani* hyphae and kill the host. Study on parasitism between *Trichothecium* spp. and *R. solani* proved that the antagonist parasitized loosely as compared to *T. harzianum*, *T. viride* and *G. virens*. The parasitic hyphae were not frequently found running along the host hyphae [20]. The bio-agent produced branches which fused with the hyphal cells of the host, as similar results were observed by Elad et al., while as no interaction was observed between *G. roseum* and *R. solani*, the mycelium of both the fungi were seen intermingling with each other showing no parasitism (Tables 2 and 3).

TABLE 2
Effect of bio-control agents on root rot disease of wheat

Treatment	Disease (%)		% Suppression over control	Yield (kg/plot)
	Incidence	Severity		
T. harzianum	6.53	0.71	84.18	1.45
T. viride	7.33	0.89	80.17	1.4
G. virens	17.93	2.27	49.44	0.86

G. roseum	23.16	2.7	39.86	0.76
Trichothecium spp	18.66	2.49	45.54	0.83
Control	27.4	4.49	-	0.6
CD (P=0.05)	3.08	1.13	-	0.115

TABLE 3
Effect of duration on shelf life of *T. harzianum* in talc based formulation

Duration of sampling (days)	Avg. no. of colonies/plate	*T. harzianum*		Log (CFU/gm)
		Dilution	CFU × 10^6	
0	32	10/7	320	8.5
30	28	10/7	280	8.44
60	24.6	10/7	246	8.39
90	12	10/7	120	8.07

CONCLUSION

Though the application of synthetic pesticides at current high prices has enhanced the yield but it is a short term benefit and has increased the concern for environmentalists and public health authorities. Bio-control method of pests on the other hand is economic, long lasting and free from residual side effects and especially suppresses the inoculum load of the target pathogen to a level which would not cause potential economic loss of a crop. *Trichoderma* spp. are of the increasing interest as bio-protectants against plant diseases due to their high efficacy as bio-control agents against the soil and seed-borne pathogens and to their safe, eco-friendly and economically viable usage for the management of plant disease.

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