Eco-Friendly Management of Blight of Blackgram Caused by *Rhizoctonia solani* Kuhn.

S. S. Patil [1] and S. S. Kamble [2]

Mycology and Plant Pathology Research Laboratory, Department of Botany

Shivaji University, Kolhapur

[1] *Shridharmane04@gmail.com*

Abstract

Blackgram (Vigna mungo (L.) Hepper) is one of the most highly prized pulses of India. It is important short duration legume crop and improves soil fertility by fixing atmospheric nitrogen. This important crop suffers from many viral and fungal diseases. Among the fungal diseases, Rhizoctonia blight , Macrophomina blight , Fusarium wilt , powdery mildew , Cercospora leaf spot etc. are common. Among these , Blight of Vigna mungo (L.) Hepper caused by Rhizoctonia solani Kuhn. is very serious. Different fungicides are used to manage the disease. But indiscriminate use of fungicides leads to environmental problems and also disturbs the beneficial microflora of the soil. Therefore, in the present investigation , efforts were made to manage the Rhizoctonia blight by using Trichoderma spp. (Trichoderma harzianum and Trichoderma asperellum) . Both the species gave inhibition of twelve isolates of Rhizoctonia solani Kuhn. But Trichoderma asperellum gave 68 % inhibition of the pathogen which was maximum among the two species of Trichoderma.

Keywords : *Trichoderma harzianum , Trichoderma asperellum Rhizoctonia solani* , blight , wilt

1. Introduction

Blackgram (*Vigna mungo* (L.) Hepper) belonging to fabaceae is one of the most highly prized pulses of India. Blackgram originated in India , is widely cultivated in many tropical and subtropical regions of the world. In India , it is grown in Andhra Pradesh, Bihar, Maharashtra, Gujarat, Karnataka, Madhya Pradesh, Punjab, Rajasthan etc. Crop is cultivated in all seasons but maximum area is occupied under *kharif* season. From ancient time this crop have played a very important role in human diet in India as a rich source of protein. The pulse is used in rheumatism , nervous and hepatic diseases and diabetics. Plant prevent soil erosion and conserve soil moisture and have the ability to improve soil fertility by fixing atmospheric nitrogen.

The average yield of blackgram is very low due to low inherent yield potential and susceptibility of crop to different diseases. The crop suffers from many fungal and viral diseases like *Rhizoctonia* blight , *Macrophomina* blight , anthracnose , *Fusarium* wilt , powdery mildew etc. Among these diseases , Blight of *Vigna mungo* (L.) Hepper caused by *Rhizoctonia solani* Kuhn. is one of the major constrains in blackgram cultivation causing considerable yield losses. *Rhizoctonia solani* Kuhn. is a very common soil borne pathogen with a wide host range. *Rhizoctonia solani* is a basidiomycetes fungus that does not

produce any asexual spores and only occasionally will the fungus produce sexual spores (basidiospores).This fungus can persist in the soil for extended periods by producing sclerotia .

Blight caused by *Rhizoctonia solani* Kuhn. causes huge yield losses in blackgram. Blight of blackgram caused by *Rhizoctonia solani* Kuhn. (Teleomorph: *Thanatephorus cucumeris* (Frank) Donk. is seed and soil borne disease. Disease symptoms start initially with yellowing and dropping of leaves. The fungus produce reddish brown , sunken lesions on the seedlings .These lesions may become enlarge , fuse together and ultimately the affected seedlings collapse and die.

Control of the pathogen become difficult because of its ecological behavior , its extremely broad host range and the high survival rate of sclerotia. [1] The disease is currently managed by excessive application of chemical fungicides which have drastic effect on the soil biota , pollute the atmosphere and are environmentally harmful. [2] and [3] Some fungicides are phytotoxic to blackgram. It is difficult to achieve control through host resistance or fungicides. Therefore alternative method must be followed for an efficient disease control. The biocontrol strategy offers an environmentally friendly alternative to protect plant from soil borne fungi. Biological control is an efficient and environmentally friendly way to prevent the disease [4].

Some of the most widely used biological agents in the world belongs to fungal genus *Trichoderma* . *Trichoderma spp*. are soil borne fungi and have significant antagonistic potential against wide range of phytopathogenic fungi. Weindling described in detail the action and mode of mycoparasitism by *Trichoderma* on *Rhizoctonia solani* .Genus *Trichoderma* display a remarkable range of lifestyle and interaction with *Rhizoctonia solani* and can be used as biological control of plant diseases . *Trichoderma* is found to be effective in control of *Rhizoctonia solani* , promoting plant growth as well as stimulating plant defense response. *Trichoderma spp*. are typically anaerobic facultative and cosmopolitan, filamentous fungi that can be found in agricultural soil and in other substrates like decaying wood. The biocontrol exercised by *Trichoderma spp.* is due to various mechanisms like competition , antibiosis , mycoparasitism , hyphal interaction and enzyme secretion .This trait is the basis for use of different *Trichoderma* strains as an alternative to the chemical fungicides.

Keeping in view economic importance of blackgram and yield losses due to *Rhizoctonia* blight , the present investigation was planned to evaluate biocontrol activity of *Trichoderma harzianum* and *Trichoderma asperellum* against *Rhizoctonia solani* Kuhn., causal organism of *Rhizoctonia* blight of Blackgram.

2. Material and methods :

I. Isolation and Identification of *Rhizoctonia solani* Kuhn.

Samples exhibiting blight of blackgram were collected from different localities of Maharashtra (Sangli, Kolhapur, Satara, Pune, Solapur, Latur , Aurangabad, Beed, Nagpur, Amaravati, Parbhani and Ahmadnagar). Surface sterilized pieces were placed on Czapex Dox Agar medium supplemented with Streptomycin sulphate at the rate of (50ug ml/l) in petridishes and kept at room temperature 28^0C for 7 days. Pure cultures were grown on Czapex Dox Agar Medium.

Fungal isolates were identified as *Rhizoctonia solani* Kuhn. by following available mycological literature [5]. From these samples twelve isolates of *Rhizoctonia solani* Kuhn. were obtained . (Table 1). Pure cultures of isolates were maintained on Czapex Dox Agar Medium for further study.

II. Isolation of *Trichoderma spp*.

The soil samples were collected at 5-10 cm depth from blackgram fields from different localities of Maharashtra. From these soil samples , isolation of *Trichoderma spp.* were done by dilution plate method [6] on Trichoderma Selective Medium. Isolated *Trichoderma spp*. were identified by following the manual of Bisset (1991) and Nagmani et al. (2006). Pure cultures of two *Trichoderma spp*. were maintained in BOD incubator at 28 ^{0} C for further study.

III. Evaluation of antagonistic potential of *Trichoderma spp*. by Dual Culture Method :

The antagonistic potential of *Trichoderma asperellum* and *Trichoderma harzianum* was evaluated against *Rhizoctonia solani* Kuhn. by Dual Culture Method [7].

Seven days old culture grown on medium was used. Inoculum disc of 8 mm of bioagent and pathogen was slotted with cork borer and picked up with sterile needle on sterile Czapex Dox Agar medium poured in sterilized glass petriplates (90 mm diameter) at two polar regions of the plate. Three replicates were maintained for each treatment and incubated at 28$\pm$2 ^{0}C for seven days. Individual culture of *Rhizoctonia solani* was treated as control. After seven days, radial growth of *Rhizoctonia solani* was measured. The growth inhibition of test fungus by *Trichoderma spp*. was calculated by using formula given by Vincent (1947).

I = C-T/ C X100

Where I : Inhibition of Growth

C : Radial growth of pathogen in control

T : Radial growth of pathogen in treated set

3. Result and discussion :

I. Isolation of *Rhizoctonia solani* Kuhn.

Disease samples of Blackgram were collected from different localities of Maharashtra. From these samples twelve isolates of *Rhizoctonia solani* Kuhn. were obtained.

Table 1. Shows the names and places from where collection of samples was made.

Table 1. Localities of *Rhizoctonia solani* Kuhn. causing Blight of Blackgram

Sr. No.	Locality	Symbol
1	Satara	Rs-1
2	Sangli	Rs-2
3	Kolhapur	Rs-3
4	Pune	Rs-4
5	Ahmadnagar	Rs-5
6	Aurangabad	Rs-6
7	Solapur	Rs-7
8	Parbhani	Rs-8
9	Latur	Rs-9
10	Nagpur	Rs-10
11	Beed	Rs-11
12	Amaravati	Rs-12

II. Growth Inhibition of pathogen by *Trichoderma spp.* in Dual Culture :

Antagonism of two *Trichoderma spp. (Trichoderma harzianum* and *Trichoderma asperellum*) against *Rhizoctonia solani* Kuhn. causal agent of Blight of Blackgram was tested by dual culture method.

Table 2. Antagonistic potential of *Trichoderma harzianum* and *Trichoderma asperellum* against *Rhizoctonia solani Kuhn.*

Sr. No.	*Rhizoctonia solani* Isolate	Percentage of Inhibition By	
		Trichoderma asperellum	*Trichoderma harzianum*
1	Rs-1	63	66
2	Rs-2	60	60
3	Rs-3	64	60
4	Rs-4	63	61
5	Rs-5	66	61
6	Rs-6	62	66
7	Rs-7	64	61
8	Rs-8	64	61
9	Rs-9	67	62
10	Rs-10	68	64
11	Rs-11	67	60
12	Rs-12	64	61

It has been observed that both *Trichoderma spp*. gave inhibition of twelve isolates of *Rhizoctonia solani* Kuhn. which was more than 50 % (Table 2). *Trichoderma asperellum* gave 68 % inhibition of pathogen which was maximum among the two species. *Trichoderma harzianum* showed maximum inhibition of pathogen collected from Satara and Aurangabad districts which is nearly 66% and least inhibition of pathogen collected from Sangli, Kolhapur and Beed which was nearly 60%. *Trichoderma asperellum* showed maximum inhibition of pathogen collected from Nagpur which is 68% and least inhibition of pathogen from Sangli district which is 60%. Both the *Trichoderma spp.* showed least inhibition of pathogen collected from Sangli district which is 60%. Both *Trichoderma spp*. overgrew the mycelium of the pathogen and sporulated abundantly on its surface which was similar to observations of Naeimi et al.(2010).

In our study *Trichoderma* strains effectively reduced the growth of *Rhizoctonia solani* under *in-vitro* conditions . In the present study, *Trichoderma asperellum* was the most effective *Trichoderma* strain with great potential to control *Rhizoctonia solani*.

There are many studies reporting that biological control with *Trichoderma* is found to be very effective (Brewer and Larkin , 2005 ; Anees et al. 2011). *Trichoderma spp.* compared to other bioagents caused maximum reduction of infection of different root rot fungi like *Fusarium spp., Macrophomina spp*. and *Rhizoctonia solani* (Dawar et al., 2008). *Trichoderma spp.* were reported to inhibit fungal infection of roots caused by pathogens like *Fusarium spp., Pythium spp*., *Rhizoctonia solani* on various crops of economic importance (Anand and Reddi , 2009). Marcello et al. (2010) proved mycoparasitic activity of *Trichoderma asperellum*. Study of Rasu et al. (2012) clearly indicated that *Trichoderma asperellum* is the superior biocontrol agent against *Sclerotium rolfsii*. Khang and Nguyen (2013) tested antifungal activity of some *Trichoderma spp.* against plant pathogens like *Rhizoctonia solani,*

Phytophthora capsici. Among tested strains DT 3.2, 2.2 and 2.3 exhibited highest antifungal activity. Asad et al. (2014) also proved biocontrol efficacy of three different isolates of *Trichoderma* (*Trichoderma harzianum* , *Trichoderma asperellum* and *Trichoderma spp*.) against *Rhizoctonia solani*. All the isolates of *Trichoderma asperellum* significantly reduced mycelial growth of *Fusarium oxysporum f sp. lycopersici* (FOL). (El-Komy et al., 2015). Amin et al. (2010), Shaikh and Sahera (2013) , Prasad and Kumar (2011), Tapwal et al. (2015) reported *Trichoderma* as potential antagonist against *Rhizoctonia solani* , *Fusarium* , *Alternaria alternata* etc.

The rice sheath blight caused by *Rhizoctonia solani* was controlled by *Trichoderma asperellum* in tropical lowland rice (De Franca et al. 2015). All the three sp. of *Trichoderma (Trichoderma harzianum* and *Trichoderma asperellum* and *Trichoderma viride*) against *Fusarium oxysporum f. sp. radicis lycopersici* (Hajji et al., 2016). Abbas et al.(2017) described biocontrol activity of different *Trichoderma* species like *Trichoderma harzianum* , *Trichoderma asperellum* and *Trichoderma viride* against various diseases caused by *Rhizoctonia solani*. Qiong et al. (2017) found and identified a novel strain of *Trichoderma asperellum* named GDFS 1009 which exhibited strong inhibitory effect against pathogen causing *Fusarium* wilt of cucumber.

4. Conclusion :

The pathogen *Rhizoctonia solani*. Kuhn. is no doubt key determinant of most of economically important crops including Blackgram by causing severe crop losses. *Trichoderma spp.* play a key role as biocontrol agents against diseases caused by *Rhizoctonia solani*. Most isolates of genus *Trichoderma* that act as BCAs have been classified as *Trichoderma harzianum*, so that *Trichoderma harzianum* is generally considered synonymous with BCA. *Trichoderma harzianum* is also the active ingredient of many commercial biological products. Compared to *Trichoderma harzianum* less work is conducted for studing antagonistic potential of *Trichoderma asperellum* . In present investigation , *Trichoderma asperellum* proved more promising antagonist than *Trichoderma harzianum* which provide another *Trichoderma spp*. as a good biocontrol agent. Information on mechanism of antagonism has been well documented . However more research is needed for the wide scale commercialization of the *Trichoderma spp*. To enhance the marketability of these fungi as BCAs feasible commercial production processes are of utmost importance. Most of the molecular interactions between *Trichoderma spp*. and *Rhizoctonia solani* have been carried out in dual culture. There is a need to consider soil microcosm having no. of *Trichoderma spp*. It would be a good approach to understand the molecular interplay of a soil microbial community in response to *Rhizoctonia solani* and *Trichoderma spp*.

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