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Infection of *Rhizoctonia solani* (Sheath blight of rice) through seed and soil inoculation

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Abstract
Sheath blight of rice caused by *Rhizoctonia solani* is one of the disease in all crop growing areas of the world. *R. solani* pathogen was isolated from sheath blight diseased samples collected from Agriculture research station, Sakoli (Bhandara). Pathogenicity test of isolate was proved by artificial inoculation method. Studied the mode of infection through sick soil method which shows seed rot (43.0%) and seedling infection (57.0%). and the mode of infection through seed inoculation recorded seed rot (62.0%) and seedling infection (38.0%).

Keywords: Sheath blight, seed, soil, inoculation, rice, *Rhizoctonia*, isolation

Introduction
Rice is an important crop worldwide, serving as the staple food for half of humanity and additionally being used in industry and for animal feed. Rice is grown in various agro-ecological zones in tropical and subtropical areas, especially in Asia, the continent accounting for 90% of the world production (IRRI, 2015a). India is the world's second largest rice producer and consumer next to China. About 90 per cent of rice grown in the world is produced and consumed in Asian continent. The production of rice to be achieved by 2020 is 128 Mt to feed the growing population in India. To meet the global demand, it is estimated that about 114 Mt of additional milled rice needs to be produced by 2035 with an increase of 26% in next 25 years. (Anonymous, 2016) [1]. In Maharashtra, rice is cultivated on 15.13 lakh hectares in regions *viz*. Vidarbha (7.95 lakh ha.), Konkan (3.83 lakh ha.), Western Maharashtra (3.23 lakh ha.) and Marathwada (0.12 lakh ha.) with annual production of 41.71 lakh tones. Rice production worldwide is affected by various biotic and abiotic stresses. Sheath blight of rice caused by a soil-borne fungal pathogen, *Rhizoctonia solani* (Kuhn) is a destructive disease in all crop growing areas of the world. Sheath blight of rice caused by *Rhizoctonia solani* is one of the major biotic constraints in India and reduce rice yield ranging from 20-50% depending on the severity of the disease and stages of infection. The disease has spread widely in terms of both occurrence and intensity over past 20 years.

Materials and Methods
Collection of diseased samples
The infected samples showing typical symptoms of sheath blight of rice were collected in the paper bags from the rice crop grown in the Agriculture Research Station, Sakoli (Bhandara), and brought to the laboratory for further studies.

Collection of seeds
Seeds were collected from Krishi Vidyan Kendra, Gadchiroli.

Isolation of fungal pathogen by tissue isolation method
Fresh samples of diseased leaves, showing sheath blight symptoms were brought to the laboratory in paper bags. These samples were washed with running tap water to remove inert material. Small bits of desired size were cut by taking care that each bit contained half infected and half healthy portion. Such bits were then disinfected with 0.1 per cent sodium hypo chloride solution for 1 minute followed by three washings in distilled sterile water to remove the traces of mercuric chloride. These bits were then placed on sterilized blotters for drying.

Properly dried bits were transferred aseptically in sterilized Petri plates containing sterilized, solidified PDA medium. This fungal growth around the bits was transferred to PDA slants and maintained as stock culture for further studies.

Identification, Purification and maintenance of fungal culture

The fungal culture identified based on morphological characters and published literature and was purified by following hyphal tip method and culture obtained was maintained on potato dextrose agar (PDA) medium slants by adopting subsequent sub culturing at periodical, regular intervals. Seven days old culture was used for further studies.

Pathogenicity test

The Pathogenicity of the isolates was determined by injection method on 40-day-old susceptible rice cultivar Swarna. The culture with fungal mycelia (and sclerotia) was taken in a disposable syringe of 0.5ml capacity then placed in between the tillers in the central region of the hill, 5-10 cm above the water line. Care was taken that no air bubble was trapped in the suspension. Bigger wounds were avoided at the point of injection. Healthy uninoculated plants served as control. The pathogen was reisolated on the PDA medium from the symptomatic plants and microscopic observations made were found similar to that of the organism isolated from naturally diseased rice plants. Thus, the test pathogen was confirmed as *Rhizoctonia solani* and Pathogenicity of *Rhizoctonia solani* was proved.

The mode of infection through soil inoculation

Stems of 35-40 days old rice plants were cut into small pieces of about 2 cm size and filled in to 500 ml conical flask up to one third. Flasks were autoclaved at 1.04 Kg/ cm^2 for 30 minutes. Mycelial discs of 5 mm diameter cut from the margin of 48 hours old culture of the pathogen were inoculated into the flask and incubated at 28±2 ^{0} C up to fifteen days for full growth of fungus and sclerotia formation. Meanwhile flasks were shaken to avoid clumping and to facilitate early growth of the fungus. After 15 days of incubation, the inoculums was taken out from flask and mixed thoroughly with sterilized sand plus soil mixture (1:1) at 100 g inoculums per kg soil. This potting mixture (sand +soil +inoculums) was filled in the earthen pots and watered lightly and incubated for four days. Then seeds were sown (at 40 seeds / pot) in the earthen pot. The pots with uninoculated soil served as control. All these pots were then watered lightly and kept in a greenhouse for further recording of observations on seed rot and seedling infection etc. the observation was recorded up to 20 days from the date of inoculation of the seeds.

The mode of infection through seed inoculation

The seeds of rice plant were disinfected with sodium hypo chloride solution for two minutes, followed by three subsequent washing with the sterilized distilled water. These were dried on sterile tissue paper. After that inoculums was properly mixed having mycelia and sclerotia from 9 days old culture. Seeds (40 seeds/pot) were sown in separate pots containing 1/3 rd of sterilized sand + soil mixture for raising of seedlings. Inoculated pots were kept in green house. Inoculated plants were observed for the appearance of disease symptoms up to 20 days. The pot contains seeds without culture served as control pot. All these pots were then watered lightly and observations were recorded on seed rot and seedling infection.

Results and Discussion

Isolation and purification of the fungal pathogen

The fungus associated with infected sheath was isolated on potato dextrose agar medium in the laboratory. The visible mycelial growth developed around the artificially inoculated mycelial bits within 3-4 days of inoculation. Initially, the fungal mycelium formed white, sparse mat on the surface of the medium. Later on the tips of hyphae entwined to form white, young sclerotia. As the mycelium grew old, its color changed to buff brown.

Within a week, many brown to dark brown, irregular, hard sclerotia were formed on the mycelial mat. The pure culture of the test fungus, obtained by the transferring mycelial bits was maintained by periodic transfer after every 20 days on PDA slants. The slants were stored in the refrigerator and this culture was used as stock culture for further studies.

Similar results were observed by Mughal *et al.* (2017) [4] isolated *Rhizoctonia solani* from rice sheath bits by tissue isolation method. Neha *et al*. (2016) [5] collected the diseased rice plants showing typical symptoms of sheath blight and isolated by tissue isolation method.

Identification of the fungal pathogen

The isolated fungi were identified on the basis of following morphological characteristics and published literature. The genus *R. solani* belongs to form class Deuteromycetes that does not make vegetative spores and present as mycelium and sclerotia. It produces shade of brown hypha, constriction at the point of branching and right angle branching in matured hyphae. The isolate shared typical characteristics of *R. solani* (a) branching a right angle near the distal septum of the cell in young vegetative hyphae, (b) formation of the septum in the branch near the point of origin, Sclerotia were differentiated aggregations of thick-walled cells, small (1-3-mm diameter) irregular-shaped, brown to black structures. Various earlier worker have been reported the identification of pathogen *R. solani.* Singh *et al*. (2014) [2] reported that the color of sclerotia varied from light brown to dark brown in *R. solani* from rice. Lal *et al*. (2014) [11] studies 25 isolates of *R. solani* revealed that all the isolates had hyphal branching at right angle, constriction at the point of branching of the mycelium and presence of a septum near the branching junction which is of immense taxonomical important.

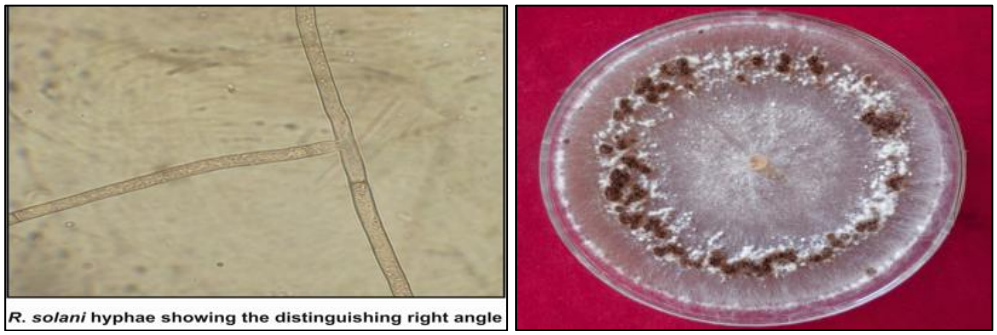

Plate 1: Mycelial growth and sclerotia of *Rhizoctonia solani* Pathogenicity test

Symptoms development on artificial inoculated leaf sheaths of young rice seedlings commenced on 5th day after inoculation. Initially greenish grey to light brown, water soaked, ellipsoid to oval lesions with irregular brown margin, appeared on the leaf sheaths. Within 10-12 days after inoculation, the lesion enlarged gradually covering 60-65 per cent area of the leaf sheath. On an average, the lesions were 1.5-3 cm length. These symptoms resembled to those observed on naturally infected sheaths. Non-inoculated seedlings remained healthy. Similar results were observed by Park *et al*. (2008) [6] rice plants at late tillering stage were inoculated with *Rhizoctonia solani* by placing mycelial ball beneath the leaf sheath shows mycelial ball produced the longest lesions on rice sheath. Moni *et al*. (2016) [3] proved the Pathogenicity of *Rhizoctonia solani* causing sheath blight of rice by artificial inoculation method and confirmed all the collected isolates based on Pathogenicity test.

Re-isolation

The fungus was re-isolated from artificially inoculated leaf sheaths, on PDA and the growth of this isolate was compared with original culture which confirmed the Koch's postulate.

Control **Initial symptoms of sheath blight**

Irregular greyish white spots of sheath blight

Plate 2: Pathogenicity test

The mode of infection through soil inoculation

Soil inoculation is done by sick soil method as mentioned in methodology. The pathogen *R. solani* inoculated in earthen pots containing sterilized soil. 200 seed were used and observations were recorded on seed rot and seedling blight. From the data presented in Table-1, it is seen that the pathogen inoculated soil caused seed rot (43.0%) and seedling infection (57.0%).

Table 1: Mode of infection of *R. solani* through soil inoculation

Sr. No.	Fungi	Number of seeds		Per cent disease observed		Fungi associated	
		Sown	Germinated	Seed rot	Seedling infection	Seed rot	Seedling infection
1.	*R. solani*	200	140	43.00	57.00	*R. solani*	*R. solani*

The mode of infection through seed inoculation

Seeds of rice were inoculated with the culture of *Rhizoctonia solani* and sown in earthen pots containing sterilized soil. Observations were recorded on seed rot and seedling blight. From the data presented in Table-2, it is seen that the pathogen inoculated seeds caused seed rot (62.0%) and seedling blight (38.0%).

Table 2: Mode of infection of *R. solani* through seed inoculation:

Sr. no	Fungi	Number of seeds		Per cent disease observed		Fungi associated	
		Sown	Germinated	Seed rot	Seedling infection	Seed rot	Seedling infection
1.	*R. solani*	200	138	62.00	38.00	*R. solani*	*R. solani*

The present result similar with Sivalingan *et al*. (2006) [12] studied the transmission of *Rhizoctonia solani* causing sheath blight of rice from seed to emerging seedling of rice. Inspite of good survival of the pathogen in seed, its transmission to rice plant under field condition was very poor. Singh *et al.* (2016) [10] observed the pathogen *R. solani* is also known to cause panicle infection resulting in production of unfilled or partially filled discolored seed bearing brownish black spots or black to ashy gray patches in rice. Richa *et al.* (2016) [8] recorded that *Rhizoctonia solani* is a soil borne necrotic pathogen and it survives either as sclerotia or mycelia in the

debris of host flooding water in the rice fields and germinate on rice sheaths forming infection cushions or appressoria during the infection process.

Plate 3: Seedling infection of Rice plant due to soil inoculation

Plate 4: Seedling infection of Rice plant due to seed inoculation

Conclusions

Rhizoctonia solani was predominantly associated in collected disease samples. *Rhizoctonia solani* was pathogenic to rice crop causing sheath blight of rice. In soil and seed inoculation method, seed rot (43% and 62%) and seedling infection (57% and 38%) was observed respectively.

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