Available online at www.jpsscientificpublications.com
Volume – 2; Issue - 1; Year – 2018; Page: 94 – 99
ISSN: 2456-7353
DOI: 10.22192/ijias.2018.2.1.1

International Journal of Innovations in Agricultural Sciences (IJIAS)

EVALUATION OF PATHOGENICITY AND METHODS OF INOCULATION OF *Rhizoctonia solani* Kuhn

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Abstract

The aim of this present research was to study the five different methods of inoculation which were followed and one which makes rice plants easily infected was identified and its pathogenicity was evaluated. Among the five methods of artificial inoculation, grain inoculation and covered with polythene bags was found to be the best in plant infection. Its recorded mean per cent infection was 61.9 per cent. The isolate *Rhizoctonia solani* 4 (Rs 4) was significantly the most virulent one which recorded the highest sheath blight intensity (64 PDI).

Key words: Rice, Pathogenicity, Methods of inoculation and Sheath blight.

1. Introduction

Rice (*Oryza sativa* L.) is an important staple food crop for majority of the world. Many biotic stresses hamper rice production and specifically, fungal diseases cause huge economic losses. Rice is cultivated in about 4.19 Million Hectares with the production of 89.09 Million tonnes with the productivity of 2125 kg/ha. Among the rice producing states of India, Tamil Nadu ranks sixth in production (5.67 Million tonnes) and second in productivity of 3070 kg/ha and area 44 Million hectares production is 106.19 million tonnes (Hane Graff, 2015). Among different fungal diseases of rice, sheath blight caused by *Rhizoctonia solani* Kuhn (*Thanetoporous cucumeris* (Frank) Donk) is emerging as a very destructive disease and it is an important one responsible for losses in grain yield (Suthin raj *et al*.,2018). The disease is both soil borne and air borne and affects seed germination and vigour to a greater extent. Rice sheath blight was considered as a minor disease in earlier days, but now it is regarded as an internationally important disease second only to rice blast (Dasgupta, 1992).

Ou (1972) studied in detail the symptoms of sheath blight under field conditions. Initial symptoms of sheath blight appear in the form of circular, oblong or ellipsoid, greenish - grey water - soaked spots of about 1cm long that occur on leaf sheath near the water level. The disease appears both on sheath and laminar portion of leaf. Disease lesions may coalesce to form bigger lesions and the disease can spread to adjacent plants in the field (Jayaprakashvel *et al.*, 2014). It is found in all rice growing areas of South Asia. The disease causes an annual yield loss of 52.5 crore tonnes in South India (Ramasamy *et al.*, 1996). The pathogenicity of *Rhizoctonia solani* varies from place to place and species to species.

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***Received*:** 07.01.2018; ***Revised*:** 28.01.2018;
***Accepted*:** 05.02.2017.

IJIAS ISSN: 2456-7353

With this background, the present investigation was conducted.

2. Materials and Methods

Isolation, maintenance and identification of pathogen

The diseased rice plants showing the typical symptoms of sheath blight disease were collected from 20 conventional rice growing areas of Nagappattinam. The pathogens were isolated on potato dextrose agar (PDA) medium (Ainsworth, 1961) from the diseased specimen showing the typical symptoms. The infected portion of the sheath was cut into small bits, surface sterilized in 0.1 per cent mercuric chloride solution for 30 sec., washed in repeated changes of sterile distilled water and plated onto PDA medium in sterilized Petri dishes. The plates were incubated for room temperature (28 ± 2 ºC) for five days and were observed the fungal growth. The fungus was purified by single spore isolation technique (Rangaswami, 1958). Identification of the isolate was confirmed (Reddy *et al.*, 2012) by comparing with the culture obtained from ITCC, IARI, New Delhi and the purified isolates were maintained on PDA slants for further studies. Totally 20 isolates were maintained and they were designated as Rs 1 to Rs 20.

Methods of inoculation of pathogen (Suthin raj *et al.*, 2016a)

Four methods were attempted on rice variety ADT-36 under pot culture. Each pot was filled with FYM and fertilizer. Thirty days old seedlings were transplanted in pots. Three replications were maintained for each treatment. The methods are as follows:

Grain inoculation method

Here, the infected seeds were kept in between the flag leaf sheath and in emerged sheath.

Sheath inoculum method

Rice sheath were collected, cut into small pieces (4 cm), transferred to open mouthed bottles and closed with a cotton wool plug. The desired quantity of water was added. The bottles were sterilized at 15 psi for 2 hrs for three successive days. The medium was used to grow *Rhizoctonia solani* pathogen. From 20 days old culture of the pathogen grown in PDA, six discs of nine mm were taken and inoculated into each bottle. The bottles were then incubated at room temperature (28 ± 2 °C) for 14 days and the inoculum thus prepared was used for subsequent studies. In this method, infected sheath were cut into small pieces and then kept in between the flag leaf sheath and in emerged sheath.

Agar method

The grown up fungus on Potato dextrose agar at room temperature were taken out in small bits with the help of a sterilized inoculum needle and inserted in a small hole in each tiller.

Spore suspension method

Rhizoctonia solani grown for ten days at room temperature on Potato dextrose agar media was scraped off from the surface and mixed in sterilized distilled water to obtain spore suspension. One drop of spore suspension was placed by sterilized plastic dropping bottle inside the flag leaf sheath enclosing the unemerged panicle. The inoculated plants were incubated in a humid chamber for 48 hrs and subsequently moved to a greenhouse maintained at 28 °C, 70 – 90 % relative humidity under a light intensity of 85 µ mol m^{-1} S^{-1} and 12 hrs photoperiod. The incidence was recorded after 30, 50 and 70 days after transplanting and the per cent disease incidence were calculated as described above. Three replications were maintained.

Assessing the pathogenicity of *Rhizoctonia solani* isolates

An amount of 30 kilograms of top soil collected from rice growing field was steam pasteurized and filled in cement pot (30 × 15 cm). Thirty days old rice seedling var. ADT 36 was transplanted 6 per pot. The spore suspension (1×10^6 ml^{-1}) of *Rhizoctonia solani* was prepared from 20 days old culture grown on PDA slants using sterile distilled water. One month old seedlings

were inoculated with a spore suspension of *Rhizoctonia solani*, one gram rice hull placed on basal leaves sheaths and covered with polythene bags and incubated in a growth chamber. After 15, 30, 45, 60 and 75 days of transplanting, they were observed periodically for the presence of disease. The intensity of sheath blight was calculated as per cent disease index (PDI) as per the grade chart proposed by (Sriram *et al.*, 2000).

0 = No infection; 1 = Less than 5 per cent of the area of leaf sheath affected; 2 = 6 - 10 per cent of the area of leaf sheath affected; 3 = 11 - 25 per cent of the area of leaf sheath affected; 4 = 26-50 per cent of the area of leaf sheath affected and 5 = More than 50 per cent of the area of leaf sheath affected.

The per cent disease index (PDI) was calculated as given by McKinney (1923).

PDI = Sum of numerical rating/Total number of tillers observed × 100/Maximum category value

Experimental design and data analysis

The experiments were conducted following Completely Randomized Design (CRD) with three replications. The significant difference, if any, among the means were compared by Duncan's Multiple Range Test (DMRT). Whenever necessary, the data were transformed before statistical analysis following appropriate method.

3. Results and Discussion

The experimental findings obtained from the present study have been discussed in following heads.

Methods of Inoculation

With a view to find out the best method of inoculation, five methods were tested in the field *viz.*, Grain inoculation method, Sheath inoculum method, Agar method, Spore suspension method and grain inoculation and covered with polythene bags on rice plants and the results are presented in Table - 1. Among the five methods of artificial inoculation, grain inoculation and covered with polythene bags was found to be the best in plant infection. Its recorded mean per cent infection was 61.9 per cent. The next best method of inoculation was the grain inoculation method which recorded 43 per cent. Grain inoculation and covered with polythene bags method of inoculation was applied for artificial inoculation in the present study.

Pathogenicity of *Rhizoctonia solani* on rice

The isolate Rs 4 was significantly the most virulent one which recorded the highest sheath blight intensity (64 PDI). This was followed by Rs 5 (58 PDI), Rs 2 (55 PDI) and Rs 1 (52 PDI) while Rs 12, Rs 16 and Rs 17 were the least virulent, which recorded 10 PDI respectively (Table - 2).

Dasgupta (1992) also found variations in the pathogenicity of the isolates of *Rhizoctonia solani* in rice. The results of the present study revealed that the most virulent isolate produced sclerotia. The same result was observed by Vidhyasekaran (1997) who reported that the most virulent isolate produced sclerotia. This result was in agreement with the results obtained by Suudi *et al.* (2013). The plant which shows disease resistance or susceptible depends on genotypes of variety. The resistance to disease should be based on knowledge of infection and pathogenicity of the fungus (Suthin raj *et al*., 2016b).

Table - 1: Infection of rice plants by different methods of inoculation with *Rhizoctonia solani*

S. No.	Methods	Days after inoculation (per cent infected leaf sheaths)					
		5	7	9	11	13	Mean
1.	Grain inoculation method	0.00	14.00	46.00	72.0	83.0	43.0[b]
2.	Sheath inoculum method	0.00	0.00	9.00	33.00	63.00	21.4[c]
3.	Agar method	0.00	4.00	10.00	20.0	25.0	11.8[d]
4.	Spore suspension method	0.00	5.00	18.00	30.0	42.0	19.0[c]
5.	Grain inoculation + covered with polythene bags	0.00	33.00	76.00	100.0	100.0	61.9[a]

*****Values in the column followed by common letters do not differ significantly by DMRT (P = 0.05)

Table - 2: Assessing the pathogenicity of *Rhizoctonia solani* isolates in pot culture condition

S.No	Isolates	PDI
1.	Rs1 – Papakovil	52
2.	Rs 2 – Orathur	55
3.	Rs 3 – Kudineyveli	48
4.	Rs 4 – Nariyankudi	64
5.	Rs 5 – Sikalpattu	58
6.	Rs 6 – Agalamkannu	42
7.	Rs 7 – Aalankudi	42
8.	Rs 8 – Pirinchumulai	28
9.	Rs 9– Karuveli	25
10.	Rs 10 – Sikkal	28
11.	Rs 11 – Poravacharry	28
12.	Rs 12 – Thanilapaddi	10
13.	Rs 13 – Aaimalai	25
14.	Rs 14 – Valivalam	49
15.	Rs 15 – Thrukkuvali	39
16.	Rs 16 – Thavur	10
17.	Rs 17 – Thirukadaiyur	10
18.	Rs 18 – Paalayur	36
19.	Rs 19 – Kizhavenmani	34
20.	Rs 20 – Keelaiyur	12

***** Values in the column followed by common letters do not differ significantly by DMRT (P = 0.05)

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DOI Number **DOI: 10.22192/ijias.2018.2.1.1**

How to Cite this Article:

S. Baskar, M. Bharathi, T. Brintha, S. Deepika, S. Vignesh and T. Suthin Raj*. 2018. Evaluation of pathogenicity and method of inoculation of *Rhizoctonia solani* Kuhn. *International Journal of Innovations in Agricultural Sciences*, 2 (1): 94 – 99.

DOI: 10.22192/ijias.2018.2.1.1