

Pathogenicity test of *Rhizoctonia solani* isolates collected from naturally infected soybean plants in different geographical locations of Chhattisgarh

ABSTRACT

Rhizoctonia solani Kuhn is a soil-borne pathogen causing diseases such as aerial blight in soybean, it has been recognized as a serious problem in soybean-growing areas resulting in considerable yield losses. The present investigation was conducted to assess the pathogenicity of *R. solani* isolates collected from naturally infected soybean plants across various geographical locations in Chhattisgarh. A total of ten isolates were obtained and identified based on morphological characteristics. Pathogenicity tests were conducted under controlled conditions using soybean seedlings as hosts. The isolates showed various levels of virulence, ranging from mild to highly aggressive. Pathogenicity tests were tested by the detached leaf method and soil inoculation technique. In the detached leaf method, the symptom appearance was early noticed in RS-1, RS-2, RS-3, RS-5 and RS-10 at 72 hours while delay symptom appeared in RS-4 and RS-9 at 120 hours. In soil inoculation technique, the germination percent and percent mortality was observed and the maximum mortality percent was observed in RS-1 (88.83%) with germination percent (53.33%) whereas minimum in RS-4 (57.50%) with germination percent (80.00%). The fungus spreads close to plant canopy through its mycelia that grow aggressively. Based on pathogenicity test, the fungus caused both leaves to blight by detached leaf assay and soil inoculation method. These results suggest that *R. solani* could be a destructive pathogen being yield losses, so it must be controlled seriously.

Keywords: Pathogenicity test, Soybean, Geographical variation, Aerial blight, *Rhizoctonia solani*

INTRODUCTION

Soybean (*Glycine max* L.) Merrill) is one of the most valuable crops in the world. It is also known as the “Golden Bean” of the 20th century. Soybean is called the “Golden Gift” of nature to mankind due to its various uses. Since time immemorial, soybean has served as milk, cheese, bread as well as oil for the people of China and east Asia; and the ancient literature of these countries called it as “gold from soil”. Off late, its versatility was recognized by the west, which called it “miracle bean”. Soybean seed has a greater nutritional value, it is a major source of protein and vegetable oil. It contains 40-42% proteins, 20-22% oil, 21% starch, vitamins- A, B, C, D & K besides essential amino acids like lysine (5%) and small amounts of calcium,

phosphorous, magnesium and iron. It is the most important and least expensive protein source produced worldwide.

The present status of soybean in India indicates the cultivation of crop over an area of 113.98 lakh ha., A total production of 14.98 million tons was recorded (Anonymous, 2023). In Chhattisgarh total area of soybean is 33.93 thousand ha. and total production is 24.74 thousand tons during the year 2021-22 (Anonymous, 2022). In Chhattisgarh, major soybean-growing districts are Rajnandgaon, Durg, Mungeli, Khairagarh-Chhuikhadan-Gandai, Bemetara and Kabirdham. Soybean is mainly grown during the *Kharif* season in sandy loam to clay loam soil in Chhattisgarh.

Rhizoctonia solani is well known and widely dispersed in soil, plant dead matter and roots causing diseases in a wide range of hosts, including root rot in soybean (Surbhi *et al.*, 2020). *Rhizoctonia solani* Kuhn is a soil-borne plant pathogenic fungus causing significant yield losses in most of the agriculturally important crops (Sturrock *et al.*, 2015). The *R. solani* produces sclerotia as a survival structure which is brown to black and composed of clusters of melanin-encrusted, thick-walled cells, formed by repeated branching from short, thick, lateral hyphae, when produced on plant parts, it is difficult to separate the sclerotia from their surrounding embedded sclerotia. Temperature is a more considerable parameter for their growth and development along with sclerotia production. Under certain conditions, young plants were found heavily affected by the disease leading to premature death of plants and substantial losses in yield. Aerial blight of soybean caused by *Rhizoctonia solani* has been reported to cause an average yield loss of about (40-50)% which can reach a devastating level of 80% under favorable conditions (Joshi *et al.*, 2018; Mathpal and Singh, 2017) and Fenille *et al.* (2002) also reported 31-60% yield losses due to aerial blight of soybean. This research was addressed two main objectives to identify the causal agent of the aerial blight disease and its pathogenicity.

MATERIALS AND METHODS

Detached leaf method

The JS 97-52 soybean variety was planted in a pot containing a soil-compost mixture (2:1) in the greenhouse. For the detached leaves assay, healthy soybean trifoliolate leaves from five-week-old plants were surface sterilized with 5% of NaOCl for 5 seconds. Sterilized leaves were rinsed twice using sterile distilled water. Sterile samples were placed on a sterile dish containing sterile filter paper and then moistened with sterile distilled water. Each leaflet was artificially inoculated with a 5mm diameter mycelial disk from 7 days *R. solani* plate culture.

Other leaves were kept healthy without inoculating as a control in a separate dish. On 5-7 days after inoculation, leaflets were evaluated for leaf blight symptoms based on the time of symptom initiation.

Soil inoculation technique

For the soil inoculation technique, the soil was autoclaved at approximately 121°C, before use the soil was separated in two groups for inoculation and non-inoculated. Each pot was filled with sterile soil. Wheat substrate (100 g) in an Erlenmeyer flask, autoclaved at 121 °C for 40 min. Each flask was inoculated with 10 discs (5mm diameter), containing the pathogen, and cultured for 7 days at 25 °C under dark light conditions on PDA. After 30 days of incubation, infested wheat brans were applied to the soil (Zhang *et al.*, 2014). For inoculated treatment, the sick pots were prepared by using the inoculum of the pathogen @ 25 gm/kg soil. The inoculum of mass multiplied on wheat grain media each isolate was added to the sterilized soil. Two kg mixture was placed in a (35.6x30x28.6cm LxWxH) size plastic pot containing sterile soil. Soybean seeds of susceptible variety JS 97-52 were surface sterilized with 0.2 % Sodium hypochlorite for one minute followed by three washings with sterile water. Ten seeds were placed in one pot and three replications of each isolate were maintained. Plants were maintained under room temperature (25 – 28°C) and watered to saturation after planting. Observation to be recorded as germination percent and percent mortality.

RESULTS AND DISCUSSION

Detached leaf Method

Pathogenicity test was performed with the ten isolates of *R. solani* collected from naturally infected soybean plants, by detached method. The initial symptom was observed after 1 day of inoculation as a water-soaked greenish-brown to reddish-brown lesion on the leaf which enlarged gradually day by day. However, 5 days after inoculation lesion became enlarged, pale yellow and covered 70% area of the leaf and turned brown or black in colour. Web-like mycelial growth was also observed. The lesion in the leaf ranges in size from a single pinpoint to coverage of the entire leaf.

In this test, the symptom appearance was early noticed in RS-1, RS-2, RS-3, RS-5 and RS-10 at 72 hours followed by RS-6, RS-7 and RS-8 at 96 hours while delay symptom appeared in RS-4 and RS-9 at 120 hours.

A similar finding was reported by Rahayu (2014) isolated *R. solani* from soybean leaves and proved its pathogenicity by detached leaf assay. Lekhashree (2017) conducted the

pathogenicity test by detached leaf method in lab conditions by use of desiccators and the fungus causing blighted symptoms after three days. Abdelghany (2022) performed a leaf inoculation assay by using detached rosette leaves prepared from 4-week-old leaf.

Table 1: Pathogenicity test of different isolates of *Rhizoctonia solani* by detached leaf assays

Isolate	Symptom Appearance (Time after inoculation)
RS-1	72 hours
RS-2	72 hours
RS-3	72 hours
RS-4	120 hours
RS-5	72 hours
RS-6	96 hours
RS-7	96 hours
RS-8	96 hours
RS-9	120 hours
RS-10	72 hours
Control	No symptoms

Soil inoculation technique

The pathogenicity test of all ten isolates of *R. solani* was conducted by artificial inoculation technique. All inoculated isolates were produced initially with small dark brown necrotic lesions on leaves under severe conditions, with lesions enlarged and blighting completely on entire leaves. The pathogen was reisolated from such leaves and compared with the original culture of the pathogen which was similar in all respects. Hence, the causal agent of the disease was confirmed as *R. solani*.

The germination percent and percent mortality was observed and the maximum mortality percent was observed in RS-1 (88.83%) with germination percent (53.33%) followed by RS-10 (84.44%) with germination per cent (60.00%), RS-3 (84.16%) with germination percent (53.33%), RS-5 (78.62%) with germination percent (56.66%), RS-2 (75.50%) with germination percent (76.66%), RS-7 (68.23%) with germination percent (63.33%), RS-6 (63.33%) with germination percent (66.66%), RS-8 (63.33)% with germination percent (70.00%), RS-9 (58.33%) with germination percent (66.66%) and minimum in RS-4 (57.50%) with germination percent (80.00%). The control pot does not show any infection.

Therefore, we concluded the pathogenicity test of ten *R. solani* isolates on soybean using a detached leaf method and soil inoculation technique, initial symptoms included water-soaked lesions that progressed to large, pale yellow, and brown or black areas with web-like mycelial growth. The severity of the disease varied among isolates, with RS-1 showing the highest disease severity, while RS-4 exhibited the lowest. Lesions ranged from small pinpoint spots to extensive coverage of the entire leaf. These findings indicate significant variation in pathogenicity among the isolates, with RS-1 being the most aggressive and RS-4 the least.

The result of the present finding agrees with earlier findings by several workers Patole *et al.* (2016) conducted a pathogenicity test of *Rhizoctonia solani* by soil inoculation method. the typical rotting symptoms were drying of plants after 20 days and it resulted in to cent per cent mortality. Pre-emergence and post-emergence mortality observed in soil inoculation were 33.33 percent and 66.66, respectively. Guney (2018) tested inoculation soil inoculation techniques for assessing the pathogenicity of *Rhizoctonia solani* and found that the mortality in the plant. Abdelghany (2022) performed a soil inoculation assay for the surveillance of pathogenicity of *Rhizoctonia solani*.

Table 2: Pathogenicity test of different isolates of *Rhizoctonia solani* by soil inoculation technique

Isolates	Germination %	Percent Mortality
RS-1	53.33	88.83
RS-2	76.66	75.50
RS-3	53.33	84.16
RS-4	80.00	57.50
RS-5	56.66	78.62
RS-6	66.66	63.33
RS-7	63.33	68.23
RS-8	70.00	63.33
RS-9	66.66	58.33
RS-10	60.00	84.44
Control	83.33	0



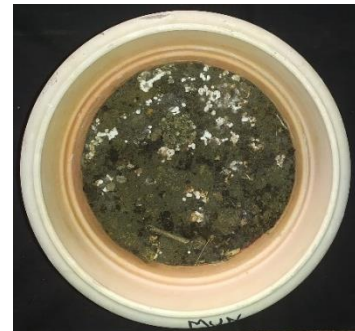
Plate 1: Pathogenicity test of different isolates of *Rhizoctonia solani* by detached leaf assays



A) Wheat grain substrate used for multiply *R. solani*



B) Mixing in soil



C) Sick pot



D) Sclerotial formation



E) Seeds sown in sick pot



F) Symptom development



G. Reisolated culture

Plate 2: Pathogenicity test of different isolates of *Rhizoctonia solani* by soil inoculation technique

CONCLUSION

The aerial blight disease of soybean caused by a soil-borne fungus *R. solani* is the most serious disease in Chhattisgarh. The fungus spreads among close plants canopy through its mycelia that grow aggressively. The pathogenicity test indicated that the isolates of *Rhizoctonia solani* collected from different geographical locations in Chhattisgarh exhibited various levels of virulence on soybean plants. This variation in pathogenicity suggests that environmental factors, genetic diversity among isolates and host resistance may influence the aggressiveness of the pathogen. The most virulent isolates caused severe symptoms and confirmed *R. solani* as a significant threat to soybean cultivation in the region.

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