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WATER BAITING: A SOIL LESS PATHOGENICITY TEST FOR SOIL-BORNE FUNGUS

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ABSTRACT

Disease symptoms, which are characteristic features in detection and diagnosis of plant pathogens. During pathogenicity test proper symptomology helps to identify pathogen for recommendations of the management practices, the pathogenic race's differential ability of virulence under soil-based tests are difficult to observe for long run of germplasm screening. The present study confronts symptomology and pathogenicity test of three different soil borne fungus *Fusarium solani*, *Rhizoctonia solani* and *Pythium aphanidermatum under* soil less and soil-based methods to know the extent of their virulence capacity under both conditions on three different crops Chilli, Brinjal and Tomato with severe cause of damping off , *Fusarium* and *Rhizoctonia* spp are quick infective pathogens which symptoms have been initiated immediately after 3 days of inoculation and highest mortality was observed by *Phytopthora* spps.

Key words : *Fusarium* and *Rhizoctonia* spp, *Phytopthora* spps.

Introduction

Plants are being exposed by continues threatening from many plant pest and disease according to the Secretariat of the International Plant Protection Convention (IPPC) under the FAO, delay in measurement actions against these menace will have to pay a huge debt ecologically and economically, global estimated losses due pest and disease were estimated 20 to 40 percent (Kourous, 2016) and the targeting with 14 percent of damage by plants disease accounts for $220 billion dollar losses in agriculture trade (Khakimov *et al*., 2022). Plant disease are detected and diagnosed through many techniques which are cultural methods, based on symptomology of host, serology and molecular techniques (Martinelli *et al*., 2015). Present scenario 83 percent of plant infectious disease are caused by fungi ,9 per cent by viruses and phytoplasmas, and 7 percent by bacteria (Khakimov *et al*., 2022). The effective management of disease was successful priority with identification of pathogen otherwise improper identification leads to misleading management strategies with crop losses. Assessment of crop losses were done based on different disease parameters with specified damage symptoms and these symptoms are important for combating them with proper diagnosis based on its symptoms (Narayanasamy, 2011). Many types of common plant disease include rotting, withering or wilt, staining, gall formation, tumours deformity, mosaic, vein clearing different symptoms, which are specific to the pathogen. Softened plant tissues with decayed plant parts with rotten smell and easily breakdown was specified by fungal pathogens *Pythium*, *Phytopthora*, *Fusarium*, *Sclerotinia*, *Rhizoctonia* spps (Kowalska, 2021) as well as bacteria such as *Pectobacterium carotovorum*, *Xanthomonas campestris* (Slack *et al*., 2017). Soil borne pathogens are opportunistic in nature and they are highly pathogenic with complex interactions of other biotic factors (Smoliñska and Kowalska, 2018). Majority of soil bone fungal species attack the vegetable crops at very young

seedling stage caused damping off these damping occur during preemergence and postemergence in stage lead to mortality of seedings (Tziros and Karaoglanidis, 2022). Pathogenicity test can be performed by isolation the pathogen and inoculation by classic pure culture method general procedure of isolating the fungal plant pathogens. Detection of pathogenic mycelium and spores which grown on media, characteristic microscopic observations are helpful for fungal identification (Dyakov and Elansky, 2019). Different types of inoculation steps were used to detect the pathogenicity of Phytopthora species such as, leaf inoculation with two zoospore concentrations at three different temperatures and stems were inoculated using agar plugs and susceptibility was determined by reisolating and lesion length (Davis *et al*., 2021). The present research was aimed to test the pathogenicity of three different fungal pathogen under liquid substrate under after inoculation of pathogens and their effectiveness in demonstrating the pathogenicity and symptoms.

Materials and Methods

Pure culture of pathogens

Isolation of fungal pathogens from the infected seedlings sample was carried by tissue isolation method. Fungal colonies developed were examined and sub cultured. Based on morphological characters published literature the fungi were identified as *Fusarium solani*, *Rhizoctonia solani* and *Pythium aphanidermatum*. The pure culture was transferred on PDA slants and maintained for further studies

Water suspension of pathogens for pathogenicity of seedlings

Pure culture of *Fusarium solani*, *Rhizoctonia solani* and *Pythium aphanidermatum* were used to determine pathogenicity. Surface-sterilized (5 min in 2.5 per cent sodium hypochlorite) seeds of the susceptible brinjal were sown in autoclaved sand in 15-cm pots. Approximately 1 kg of sand was placed in each pot. The seedlings raised were used after 20 days of germination. Potato-dextrose broth (peeled and sliced potato, 200 g; dextrose, 20 g; distilled water, 1000 ml) was prepared; 100 ml of broth was added in each 250 ml conical flask and autoclaved at 15 lbs. for 20 min. The broth was then inoculated with a bit of the fungus isolates from tubes and incubated on a shaker (8 hrs. each day) at room temperature (25- 30°C) for 10 days. The entire contents of flask were diluted with sterilized distilled water to get the final inoculums dilution of 2.5% (usually about 2.5 litres of water was added to attain the desired dilution of the contents of one flask) with CCU. 6.5×10^5 spores/ ml. Then 20 ml of diluted inoculums was added into each sterilized 150 × 15 mm glass test tube. The 20-day old (from sowing) seedlings from sand were removed and the root system was washed under running tap water rinsed in sterilized distilled water and then one seedling into each tube was placed by holding it in position by a cotton plug. Sterilized distilled water was added to the tubes after every 2 days to make up the loss. Ten seedlings were used to serve as check/ absolute control. Tubes were kept in a test tube stand to hold them in position. Observations were recorded 15 days after inoculation.

Soil inoculated pathogenicity test of seedlings

Three different isolates *Fusarium solani*, *Rhizoctonia solani* and *Pythium aphanidermatum* causing damping off were multiplied on sorghum grain media. Fungal inoculums 5 percent by volume was mixed and pots were filled with soil and sand in the 3:1 ratio. These pots were kept in green house of Division of Plant Pathology, SKUAST-Jammu. After 7 days of inoculation 40 brinjal, Chilli and tomato seeds were sown per pot, pathogenicity tests of three pathogens were employed, five pots for each isolate and for absolute control (without any pathogen) were used. The pots were covered with polythene sheet for 48 hours to avoid contamination and watered at regular intervals to maintain humidity. Pathogen reisolated from experimentally diseased seedlings and grown in pure culture pathogenicity was proved.

Results and Discussion

Water culture method

Isolates of *Fusarium solani*, *Rhizoctonia solani* and *Pythium aphanidermatum* were cultured on 100 ml PDB respectively and mixed with 2.5 litres of distilled water and desired spore count of 6.5×10^5 spores/ml was achieved. The spore suspension of nearly 20 ml was poured in 10 sterile glass test tubes for each pathogen. Surface sterilized 10 days old healthy seedlings of a susceptible brinjal variety were placed inside each test tube and the observations were recorded 10 days after inoculation which showed damping off symptoms. This method gave the conformation about the ability of pathogens to cause disease. Seedlings inoculated with the *Pythium aphanidermatum* showed almost 100% symptoms of damping off when compared with the other two isolates *Fusarium solani* and *Rhizoctonia solani*, which showed 90% and 70% of disease symptoms on the healthy seedlings of brinjal respectively over the absolute control (Fig. 3).

Pot culture method

Damping off pathogens *Fusarium solani*, *Rhizoctonia solani* and *Pythium aphanidermatum* were

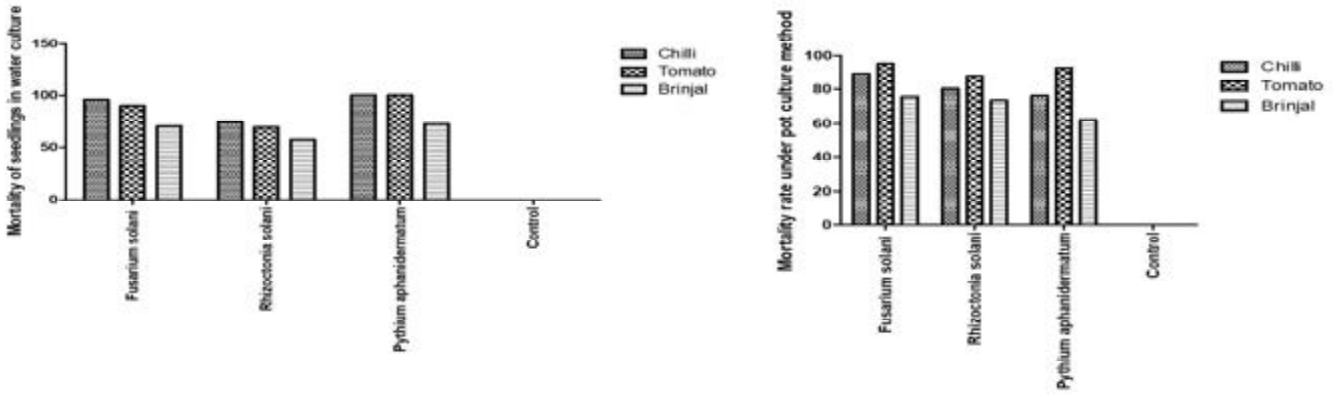


Fig. 1 : Mortality of the seedlings under water culture and pot culture methods to detect the pathogenicity.

Table 1 : Number of days recorded symptoms in under water culture technique and pot culture under soil conditions for detecting pathogenicity.

Chilli	**Water culture techniques**	**Pot culture under soil conditions**
Fusarium solani	96	89
Rhizoctonia solani	75	80
Pythium aphanidermatum	100	76
Control	0	0
Tomato		
Fusarium solani	90	95
Rhizoctonia solani	70	87.5
Pythium aphanidermatum	100	92.5
Control	0	0
Brinjal		
Fusarium solani	71.1	75.5
Rhizoctonia solani	57.7	73.3
Pythium aphanidermatum	73.3	62.2
Control	0	0

Table 2 : Number of days recorded symptoms in under water culture techniques for detecting pathogenicity

Crop	**Days to symptoms**			
Chilli	Day 3	Day 5	Day 7	Total
Fusarium spp.	2	3	4	9
Rhizoctonia solani	1	3	4	8
Pythium spp.	0	4	5	9
Control	0	0	0	0
Brinjal	Day 3	Day 5	Day 7	Total
Fusarium spp.	2	3	5	10
Rhizoctonia solani	1	4	4	9
Pythium spp.	0	2	5	7
Control	0	0	0	0
Tomato	Day 3	Day 5	Day 7	Total
Fusarium spp.	1	3	4	8
Rhizoctonia solani	2	3	4	9
Pythium spp.	0	4	4	8
Control	0	0	0	0

taken to prove pathogenicity test. Pot culture experiment was conducted under greenhouse condition. Soil inoculated with pathogens and sand (3:1) was prepared and filled in pots for *Fusarium solani*, *Rhizoctonia solani* and *Pythium aphanidermatum* isolates respectively and forty seedlings were kept under observation. The inoculated and uninoculated control pots were maintained under controlled environmental conditions. *Fusarium solani* showed both pre and post emergence damping off symptoms on 38 seedlings where *Rhizoctonia solani* and *Pythium aphanidermatum* showed disease symptoms on 35 and 37 seedlings respectively form the total seedlings under observation over the control with no disease incidence. In pre-emergence damping off, seeds were rotted and in post emergence damping off, seedlings showed toppled over symptom due to infection at collar region.

Response of seedlings to virulence nature of the three pathogens in soil and water culture techniques was observed (Tables 1 and 2). Mortality rate of the seedlings inoculated with *Pythium aphanidermatum* in water culture techniques was highest 100% in chilli and tomato and 73.3% in Brinjal. *Fusarium solani* shows mortality rate of 96, 90 and 71.1 in chilli, brinjal and tomato. *Rhizoctonia solani* shows a mortality rate of 75, 70 and 57.7 in chilli brinjal and tomato, respectively.

Appearance of symptoms during the pathogenicity test shows the virulence of the pathogens during water culture *Fusarium* spp. symptoms Initiated on tender

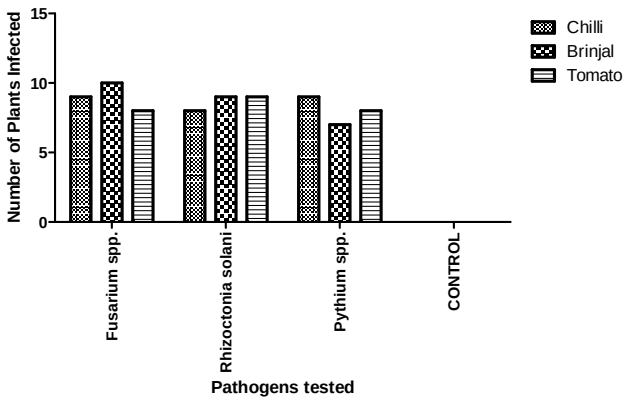
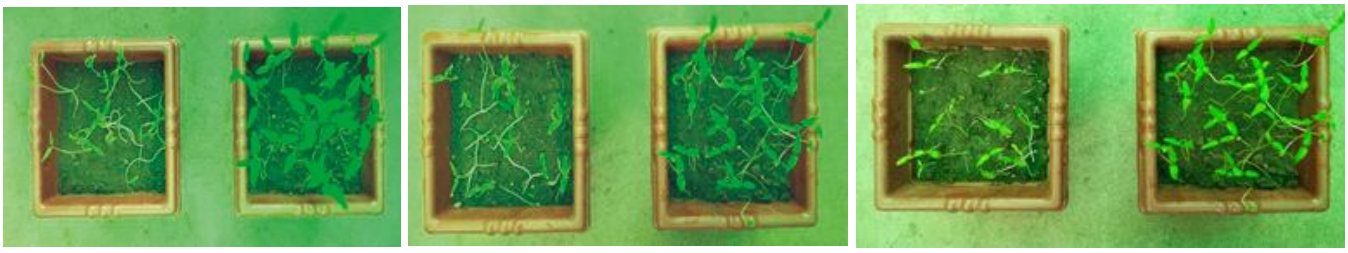


Fig. 2 : Graphical representation of days recorded symptoms in under water culture techniques for detecting pathogenicity.

symptoms were initiated at 5 days after inoculation. In tomato damping of symptoms appeared from 3 days after inoculation for both *Fusarium* spp and *Rhizoctonia solani*. However, the initial symptoms for *Pythium* spp were slow when compared to the other damping of pathogens. Screening the pathogenicity of different fungal isolates reveals the diversity of the races under different geographical conditions, these are important to assess the status of the pathogen diversity and this can be tested under quick pathogenicity test to ascertain the status. Field techniques for large-scale investigations of the pathogenicity may be complicated to carry out race identification and inheritance studies of different plant pathogens .Indirect methods of pathogen detection

Tomato **Brinjal** **Chilli**

Fig. 3 : Soil less water culture technique pathogenicity against Chilli, Tomato and Brinjal.



Tomato **Brinjal** **Chilli**

Fig. 4 : Soil based pathogenicity against Chilli, Tomato and Brinjal.

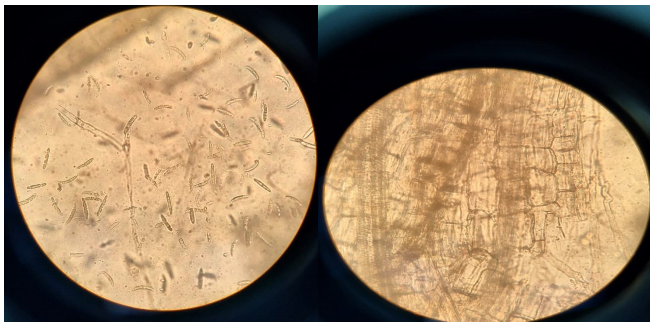


Fig. 5 : *Fusarium oxysporum*.

Fig. 6 : *Rhizoctonia solani*.

seedlings from 3 days after inoculation at end of 7 days, 9 seedlings were affected, *Pythium* spp. symptoms were initiated at 5 days after inoculation and finally 9 seedlings were infected in chilli. Brinjal crop displayed highest infection of seedlings with *Fusarium* spp and symptoms were initiated at 2 days of inoculation. In *Rhizoctonia solani* 1 day after inoculation, whereas in Pythium

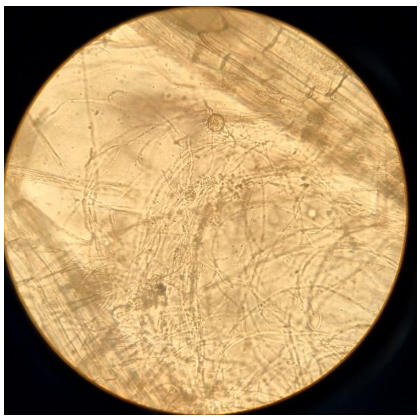


Fig. 7 : *Pythium aphanidermatum.*

identify plant diseases through different morphological changes in seedlings (Fang and Ramasamy, 2015). Soil less methods in detecting the pathogenicity always give an effective way of detecting virulence capacity in

pathogens the soil less water culture technique was used to test the pathogenicity for different isolates in chick pea plants. Screening of advanced lines of chick pea for effective virulence against *Ascochyta rabiei* and *Fusarium oxysporum* through water culture techniques (Jamil *et al*., 2002).

Conclusion

Pathogenicity test against host to detect the virulence in determining the pathogenicity. Soil less and soil-based substrates were used in detecting the pathogenicity tests but high level of screenings against wide range pathogenic races was well suited through water culture and quick assessment of virulence nature of pathogens when compare to the soil-based pathogenicity test.

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