Pak. J. Bot., 56(1): 377-387, 2024. DOI: http://dx.doi.org/10.30848/PJB2024-1(19)

POPULATION DYNAMICS AND AGGRESSIVENESS OF FUNGAL PATHOGENS ASSOCIATED WITH CHILLI ROOT ROT

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Abstract

Chilli (*Capsicum annum* L.) is one of the most important vegetable crops worldwide and root rot disease is a significant threat to its production. This study documented the disease prevalence, incidence, and severity of root rot, as well as identified the aggressiveness of fungal and oomycetes microbial communities associated with this disease in chilli growing districts of Punjab, Pakistan. In 2020-21, a field survey was conducted in nine different districts and disease samples were collected from symptomatic chilli fields. Of the 112 fields visited, 73 were found with symptomatic root rot infection. The risk of disease prevalence, severity, and incidence was highest in Lahore 87.5%, 69%, and 64.14% respectively, while the lowest disease prevalence, incidence, and severity were recorded in district Bahawalnagar 16.67%, 14.8%, and 17% respectively. It is concluded that, the disease prevalence, incidence, and severity level of the disease is higher in Lahore compared to other chilli growing areas of Punjab, Pakistan. A total of fifteen fungal colonies were isolated and categorized into two different groups: A=Pathogenic, and B=Non-Pathogenic based on the frequency of detection and pathogenic status. The pathogenicity and aggressiveness of group-A isolates were tested on the highly susceptible chilli variety 'Desi', and it was concluded that *P. capsici* and *R. solani* cause the highest incidence and severity of root rot. Three different culture media (PDA, MEA, and PARP) were used for isolation, and it was observed that all fungal and oomycetes were easily isolated on PDA media, and the percentage frequency on PDA media was also maximum as compared to MEA and PARP media. However, to our knowledge, this is the first study to explore and measure the aggressiveness of fungal and oomycete pathogens for root rot of chilli in the Punjab region of Pakistan and the first to systematically evaluate various cultural media for detection of pathogens associated with this problem.

Key words: *Capsicum annum*, Incidence, Severity, Microbial, Communities.

Introduction

Chilli pepper (*Capsicum annum* L.), member of the family Solanaceae, is an economically important and essential vegetable crop, grown throughout the world (Tariq *et al*., 2014; Al-Snafi, 2015; Mejía-Teniente *et al*., 2019). New Mexico is the native place of origin for chilli. It was brought into the Indo-Pak sub-continent by the Portuguese from Brazil in 1500 AD (Hussain & Abid, 2011). Continental Asia contributes about 65% of total global chilli fruit production while America, Europe, and Africa produce about 13.3%, 11.9%, and 10.1% respectively. The estimated global value of fresh and dried chilli fruit is about $30 billion and $3.8 billion respectively (Zhongming *et al*., 2019). In Pakistan, during 2019-20 it was cultivated on an area of 57.8 thousand hectares with a production of 136.8 million tons (GOP, 2020).

Chilli is a vegetable that is consumed in fresh green and red dried form as a spice (Welbaum, 2015). Its spiciness is due to compound capsaicin, present in the internal membrane, carp, placental tissues of the chilli fruit (Meghvansi *et al*., 2010). It contains many essential nutrients and bioactive compounds which act as anti-microbial, antioxidant, anti-inflammatory, anti-obesity, anti-cancer, pain therapy, body temperature regulation, and anti-viral activities (Khan, 2014). Furthermore, chilli fruit is not only cholesterol free, but it is also considered a useful source of vitamins A, B, C, carotenoids, potassium, phenolics, and folic acid (Perucka & Materska, 2005).

Chilli flourishes in tropical areas of the world because it requires a warm, humid climate to survive. Chilli plants can grow up to one meter with an extensively branched stem. Its fruits are considered as vegetables and botanically it is known as berries (Saxena *et al*., 2016). Seeds of *Capsicum annum* are 3 to 5 mm in size, pale or bright yellow in color, having reniform or discoid shape (Li, 2000).

Quality fruit production of chilli deals with various constraints of fungal, viral, and bacterial infections. Among these problems, chilli root rot caused by fungal pathogen is one of the key factors responsible for low production, deterioration of both fruit quality and quantity, and ultimately death of the plant. Characteristics symptoms of root rot appear at all growth stages of the plant. Yellowing, wilting, and death of the whole plant occur due to this infection (Fig. 1). Infected roots also express decaying symptoms, their outer layer can be easily removed and the whole plant pulled out easily (Mannai *et al*., 2018). Wilting of plants due to the attack of the pathogen has different morphological characteristics related to abiotic factors because the involvement of the pathogen in the vascular system of plants, blocks the xylem and phloem tissues of the host plants (El-Kazzaz *et al*., 2008).

Root rot disease is a potential threat to the successful production of chilli pepper and soil-borne pathogenic fungal agents associated with that disease can cause huge economic losses up to 36-56% every year in the country (Parveen *et al*., 2020). For successful management of this potential threat in chilli, it is necessary to record the incidence, severity and

prevalence of root rot that will be helpful for policymakers and researchers to develop a better management strategy against root rot disease of chilli pepper. For this purpose, a survey of different chilli growing areas is a prerequisite. That is why in present study survey was conducted, to document the disease incidence, severity, and prevalence of root rot disease and detection of fungal population dynamics associated with roots of infected chilli plants in major chilli growing areas of Punjab, Pakistan.

Material and Methods

Survey and assessment of disease prevalence: A survey was conducted during 2020-2021 for assessment of natural disease incidence, severity, prevalence, and detection of the fungal population associated with root rot of chilli in main chilli producing districts of Punjab including Faisalabad, Chiniot, Okara, Lahore, Kasur, Sahiwal, Multan, and Bahawal Nagar. In district, Chiniot 18 fields were visited while in district Lahore, Rahim Yar Khan, Muzaffargarh, Faisalabad, Bahawal Nagar, Okara, Kasur and Sahiwal, 16, 15, 14, 12, 12, 10, 8, 7 fields were visited, respectively and average field area was about 43560 square feet, and all fields were more or less the same size. A global positioning system (GPS) was used to note down the location of each chilli field. Longitude and Latitude were also considered for each field. Disease assessment was done based on typical symptoms of chilli root rot. The prevalence percentage of chilli root rot was recorded by using the following formula (Daunde *et al*., 2018).

$$\text{Disease prevalence (\% age)} = \frac{\text{No. of infected fields}}{\text{Total no. of observed fields}} \times 100$$

The incidence of chilli root rot was also recorded by the following formula (Teng & James, 2002).

$$\text{Disease incidence (\% age)} = \frac{\text{No. of diseased plants at a location}}{\text{Total no. of observed plants at a location}} \times 100$$

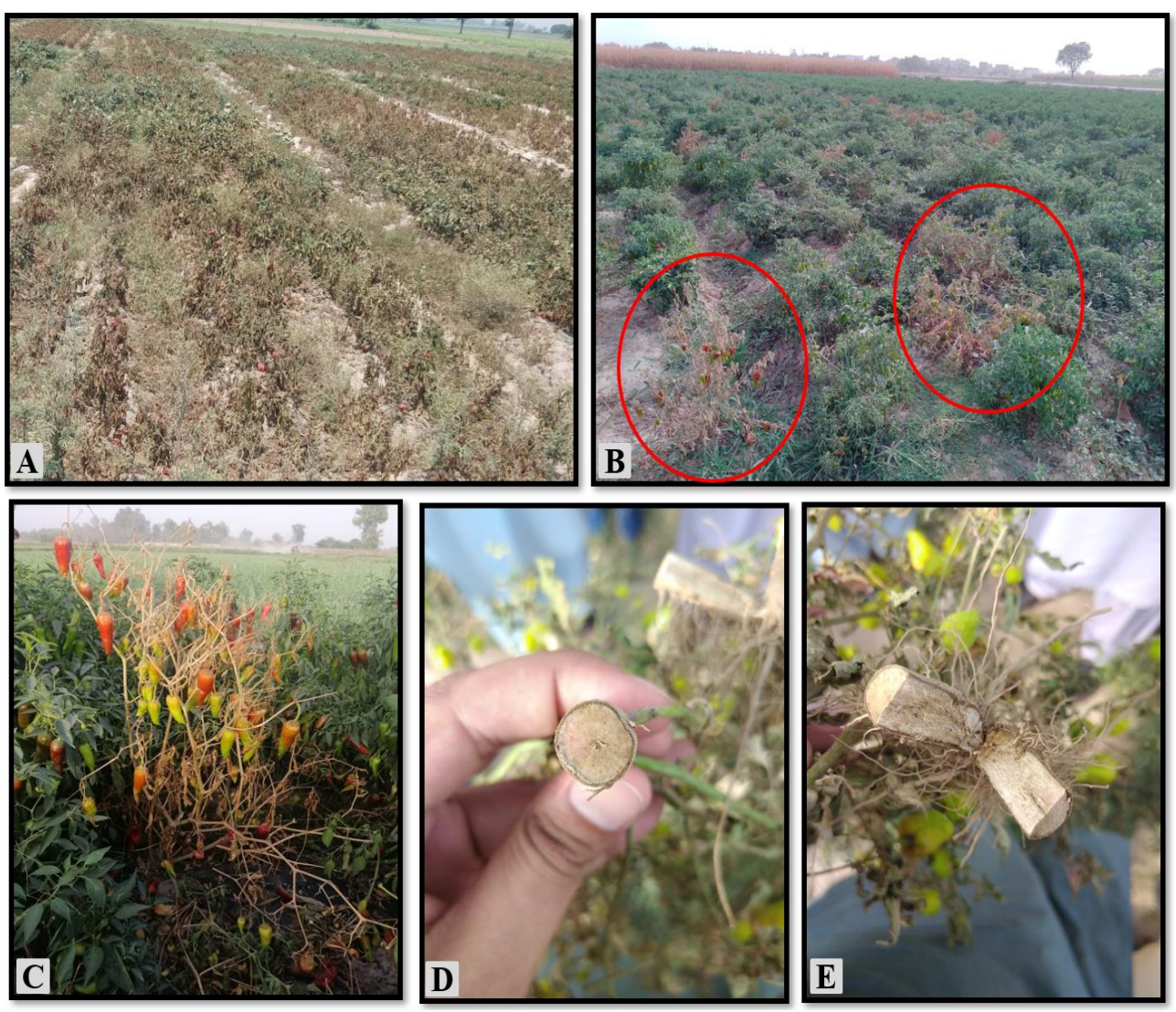

Fig. 1. Chilli pepper field and plants showing root rot symptoms; **A)** Complete death of a chilli field infected by root rot pathogen showing burning like symptoms, **B)** Showing the appearance of root rot disease in patches in chilli field, **C)** Infected plant showing permanent wilting symptoms in chilli field, **D)** The cross-section of infected chilli plant showing root rot symptoms, **E)** Splitting root of infected chilli plant showing browning vascular system.

Table 1. Rating scale for assessment of disease severity.

Severity scale	Observation
0	No. infection on roots
1	1-25% Root surface with visible lesions < 3 mm
2	26-50% Root surface with visible lesions 3 to 5 mm
3	51-75% Root surface with visible lesions > 5mm
4	> 75% Root surface with visible lesions (lesions girdling)
5	100 % Roots completely discolored and plant dead

Disease severity for root rot of chilli was calculated from each field. The following disease rating scale was used for disease severity (Table 1 and Fig. 6).

Severity percentage was calculated by using the following formula (Oljira & Berta, 2020).

$$\text{Disease severity (\% age)} = \frac{\text{Sum of numerical rates}}{\text{Number of plant scored x Maximum score on scale}} \times 100$$

Isolation, purification, and identification of fungal isolates associated with root rot of chilli: Three different standard media (PDA; Potato Dextrose Agar, PARP; Pimaricin-Ampicillin-Rifampicin-PCNB and MEA; Malt Extract Agar) were used to isolate the fungal pathogens associated with collected diseased sample of root rot of chilli from different locations. Roots of infected plants were cut into small pieces, and surface disinfestation was done with 1% sodium hypochlorite solution, following two consecutive washings with sterilized distilled water, and dried on filter paper. All three media were poured into sterilized Petri dishes (90 mm) in the laminar airflow chamber and solidified. After solidification, samples were cultured on these Petri plates and incubated at ± 25ºC. Growing fungal colonies were observed every 24, 48, and 72 hours and transferred on new Petri plates containing the same media for purification. The identification of pure fungal isolates was done under a microscope by observing distinguish morphological characters as described by Dugan (2017).

Determination of most aggressive fungal isolate causing root rot of chilli in geographical area of Punjab: Based on available literature, all 15 fungal isolates were categorized into two main groups: Group-A=Pathogenic, Group-B=Non-pathogenic. All members of Group-A multiplied, and their spore suspensions were prepared from 10 days old culture growth in autoclaved distilled water. The spore suspension was adjusted for each isolate at 1×10^6 spores/mL by using a hemocytometer. Formalin was used to disinfect the soil. The earthen pots of 30 cm diameter were filled with disinfected/sterilized soil. Three pots for each fungal isolate were taken separately and prepared spore suspensions were drenched in these pots. Thirty days old healthy chilli seedlings (6-8cm height) of highly susceptible chilli cultivar 'Desi' were taken from the seedling tray and shifted two plants per pots in each treatment. Control treatments were arranged without the accumulation of any tested fungal isolate. This experiment was conducted in greenhouse conditions under careful observation in the field area of The Department of Plant Pathology, UAF. Data for the variable degree of aggressiveness for each tested fungal isolates was taken according to the disease severity scale (Table 1), time to start of first disease symptoms, and time to complete plant death. After the appearance of severe disease symptoms, plants root samples were taken and re-isolation was carried out from this artificially inoculated disease to justify Koch's postulates (Ignjatov *et al*., 2018).

Results

Disease prevalence: Root rot disease was observed in all potentially growing chilli districts of Punjab: Faisalabad, Chiniot, Okara, Lahore, Kasur, Sahiwal, Multan, Rahim Yar Khan and Bhawal Nagar. The maximum disease prevalence was noted in district Lahore 87.5%, followed by Rahim Yar Khan, Chiniot, Muzafar garh, Faisalabad, Sahiwal, Okara, Kasur, and Bahawal Nagar with 80%, 77.78%, 71.42%, 66.67%, 57.14%, 50%, 50%, and 16.67 % respectively (Fig. 2).

Natural disease incidence and severity in farmer fields of different districts of Punjab: Sixteen chilli fields were visited in district Lahore from which 14 were attacked by root rot disease and pairwise mean comparison analysis showed that the maximum disease incidence in district Lahore was 64.14% while the minimum was 27.76% with an average of 44.17%. The highest severity index of root rot of chilli in district Lahore was observed 69% the minimum was 33% while the average disease severity in this district was recorded 41.63% (Table 2).

In district Rahim Yar Khan fifteen chilli fields were surveyed among them 12 fields were infected with root rot while three were free from disease and pairwise mean comparison test showed that the highest incidence of root rot was 46.73% and the lowest incidence was 25.7% with an average of 26.14%. While the highest severity of disease was found to be 61%, the minimum was 32% and the average disease severity was 34.8% in this district (Table 2).

In district Chiniot, root rot disease was calculated in 18 different fields whereas disease was present in 14 fields and pairwise means comparison showed that the maximum, minimum and average disease incidence was 44.73%, 27.56% and 29.93% respectively. While in this district, the maximum severity was observed 55%, the minimum was 36% and the average disease severity was 32.33% (Table 2).

Fourteen chilli fields were visited in district Muzaffargarh from which 10 were found root rot infected and pairwise mean comparison analysis showed that the maximum disease incidence in this district was calculated 41.4 %, the minimum was noted 24.81%, while the average was 23.08%. The highest severity index of root rot of chilli in district Muzaffargarh was observed 53%, the minimum was 29% with an average of 25.69% (Table 2).

Table 2. Disease incidence (maximum, minimum and average) percentage and disease severity (maximum, minimum and average) percentage due to root rot infection in farmer fields in core chilli growing districts (Faisalabad, Chiniot, Kasur, Okara, Lahore, Sahiwal, Muzafar garh, Rahim Yar Khan and Bahawal Nagar) of Punjab, Pakistan.

Districts	Disease incidence (%age)			Disease severity (%age)		
	Max.	Min.	Avg.	Max.	Min.	Avg.
Faisalabad	42.67	30.5	26.45	51	31	27.17
Chiniot	44.73	27.57	29.93	55	37	32.33
Kasur	42.17	24.43	16.65	41	21	17.38
Okara	43.16	25.03	18.41	49	23	18
Lahore	64.14	27.76	44.17	69	33	41.63
Sahiwal	33.13	20.73	15.28	47	25	17.86
Muzafar garh	41.4	24.81	23.08	53	29	25.69
Rahim Yar Khan	46.73	25.7	26.14	61	32	34.8
Bahawalnagar	27.47	14.8	3.52	32	17	4.08

In Faisalabad 12 different chilli fields were surveyed among them 8 fields were infected with root rot while 4 were not infected and pairwise mean comparison test showed that the highest incidence of root rot was observed 45.7%, the lowest was 30.5% with an average of 26.45%. In this district, the highest severity of disease was 51%, the minimum was 31% while the average was calculated as 27.17% (Table 2).

In district Sahiwal, root rot disease was calculated in seven different fields, whereas, disease was present in 4 fields and 3 were observed with no infection and pairwise means comparison showed that the maximum incidence was 33.13%, minimum was 20.74% and the average was 15.28%. In this district, the maximum, minimum and average disease severity was calculated 47%, 26% and 17.86% respectively (Table 2).

Ten different chilli fields were visited in district Okara from which 5 fields were found to be root rot infected while 5 were free from this infection and pairwise mean comparison analysis showed that the maximum disease incidence in this district was calculated 43.16%, the minimum was 25% and an average was 18.41%. The highest severity index of root rot of chilli in district Okara was observed 49%, the minimum was 23% with an average of 18% (Table 2).

In Kasur 8 chilli field was surveyed among them 4 fields were infected with root rot while others were not infected and the pairwise mean comparison test showed that the highest, lowest, and average disease incidence of root rot was observed 42.16%, 24.43% and 16.65% respectively. In this district, the highest severity of disease was 41%, the minimum was 21% with an average of 17.38% (Table 2).

Twelve chilli fields were visited in district Bahawalnagar from which only 2 were found root rot infected while the remaining were free from disease and pairwise mean comparison analysis showed that the maximum disease incidence in this district was 27.1%, the minimum was 14.8% as an average of 3.52. The highest, lowest, and average severity index of root rot of chilli in district Bahawal Nagar was observed 32%, 17% and 4.08% respectively (Table 2).

A total of fifteen fungal isolates were isolated from infected chilli plant roots collected from nine potentially chilli growing districts of Punjab, Pakistan. Identification of these isolated fungal colonies was done on the basis of colony growth shape and pigmentation as well as microscopic (mycelium & spore color, shape, and size) characteristics. These fifteen fungal isolates were identified under a microscope (BioLogVision Microscope Model H300M) as *Phytophthora capsici (*Akter *et al*., 2007)*, Pythium aphanidermatum* (Lodhi *et al*., 2013)*, Pythium debaryanum, Pythium myriotylum* (Hyder *et al*., 2018; Dubey *et al*., 2020)*, Ceratobasidium* sp. (Tariq *et al*., 2020), *Rhizoctonia solani* (Ajayi-Oyetunde, & Bradley, 2017)*, Verticillium dahlia* (Yu *et al*., 2016)*, Macrophomina phaseolina* (Siddique *et al*., 2021)*, Fusarium oxysporum, Fusarium solani* (Leslie & Summerell, 2008)*, Alternaria* spp. (Siciliano *et al*., 2017), *Aspergillus flavus, Aspergillus niger* (McClenny, 2005)*, Penicillium* spp. (Bandh *et al.,* 2011) and *Rhizopus* spp. (Gryganskyi *et al*., 2018). From these *Phytophthora capsici, Rhizoctonia solani and Pythium aphanidermatum* were isolated more frequently compared to other pathogenic isolated colonies. The maximum frequency percentage of fungal isolates in district Faisalabad, Chiniot, Okara, Lahore, Kasur, Sahiwal, Multan, Bahawalnagar and Rahim Yar Khan was *Rhizoctonia solani* (30.8%), *Pythium debaryanum* (23.5%), *Rhizoctonia solani* (28.6%), *Phytophthora capsici* (25%), *Phytophthora capsic* (29.4%), *Rhizoctonia solani* (33.3%), *Rhizoctonia solani* (33.3%), *Pythium aphanidermatum* (40%) and *Rhizoctonia solani* (20%) respectively. The frequency percentage of each fungal isolate was shown in (Fig. 3).

Three different culture media (PDA, PARP, and MEA) were evaluated for isolation of fungal and oomycetes population associated with chilli root rot in potentially growing districts of Punjab, Pakistan. During this analysis it was observed that all fungal isolates were easily isolated on PDA media (Table 3) and the percentage frequency of fungal isolates on PDA media was maximum compared to MEA and PARP media (Fig. 4).

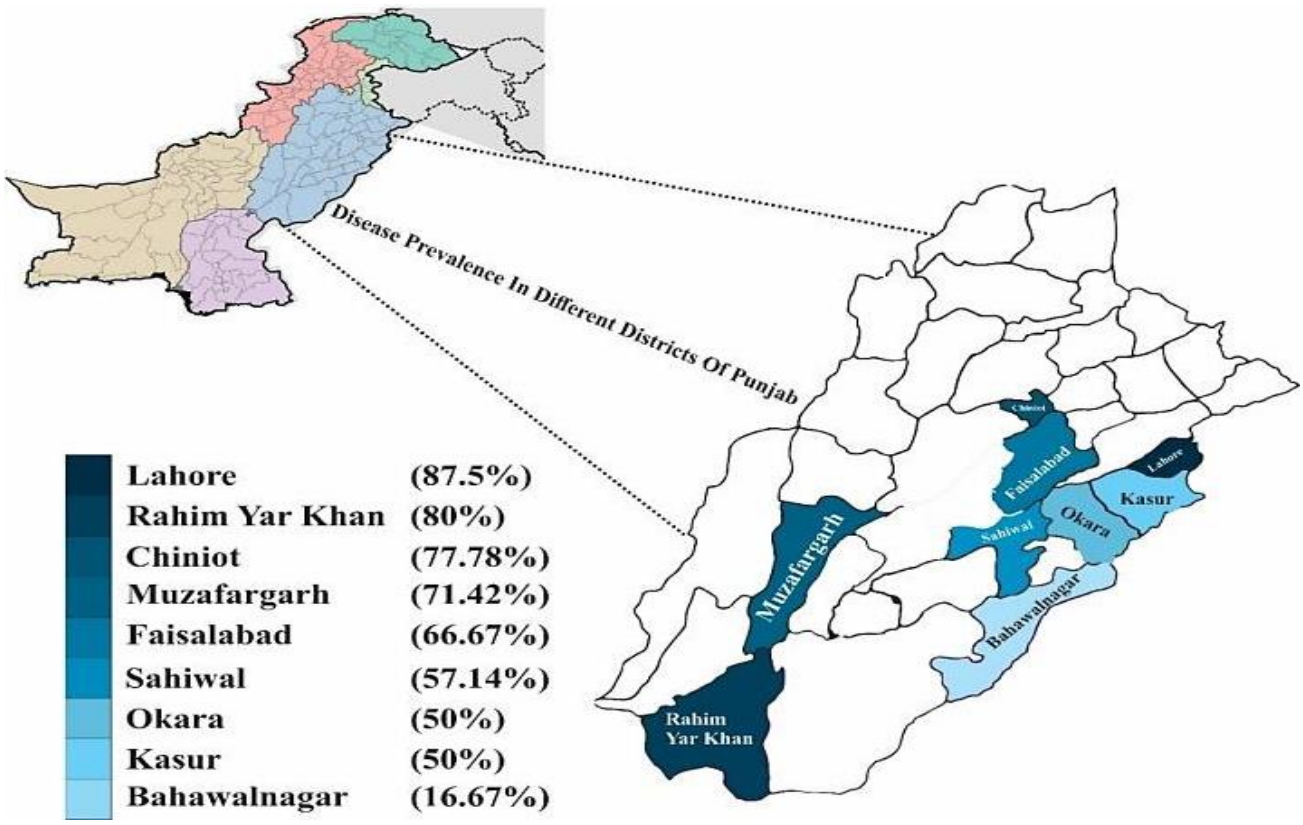

Fig. 2. Geographical map showing natural disease prevalence percentage for root rot of chilli in major chilli growing districts (Faisalabad, Chiniot, Kasur, Okara, Lahore, Sahiwal, Muzafargarh, Rahim Yar Khan, and Bahawalnagar) of Punjab, Pakistan.

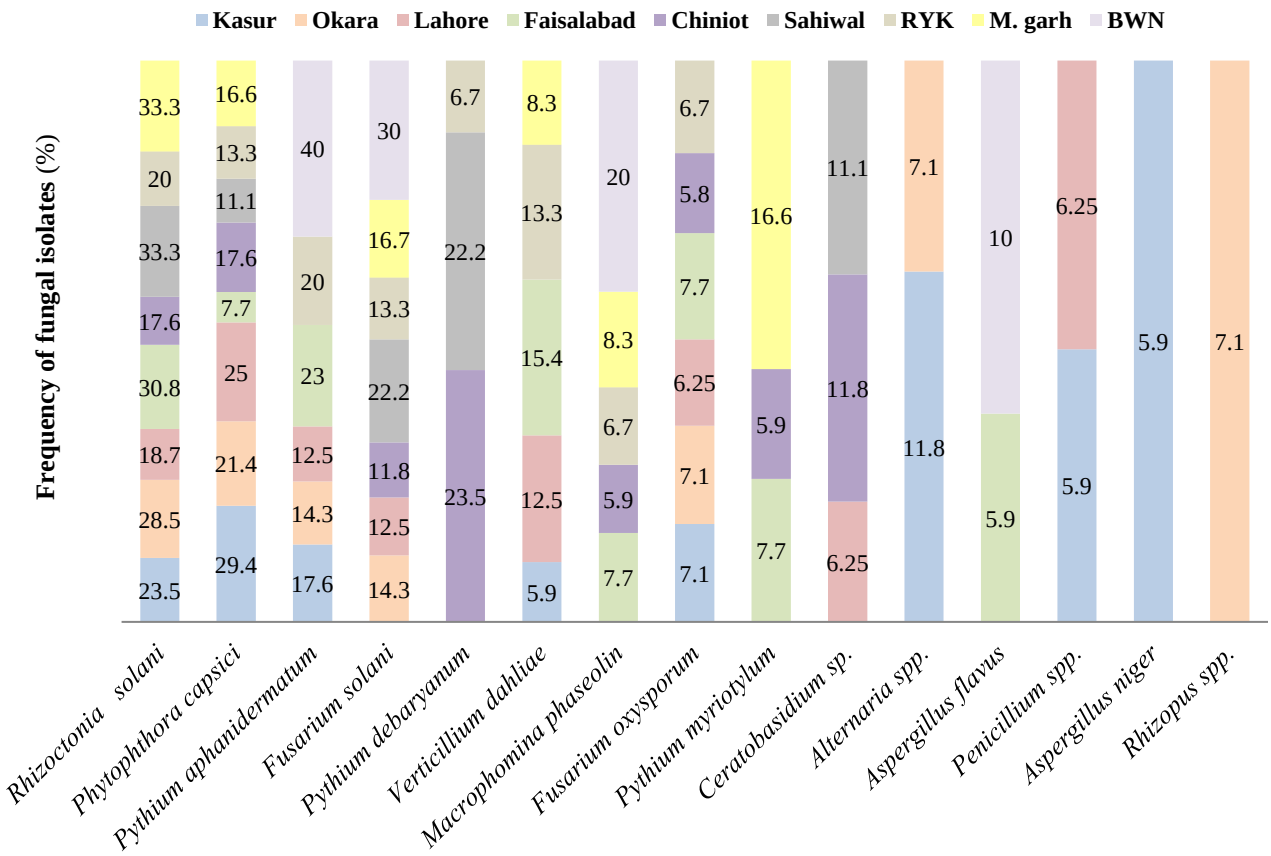

Fig. 3. Frequency Percentage of fungal isolates, Isolated from collected samples of infected chilli roots from different districts; Kasur, Okara, Lahore, Faisalabad, Chiniot, Sahiwal, Rahim Yar Khan (RYK), Muzafar garh (M. garh), and Bahawalnagar (BWN) of Punjab, Pakistan.

Table 3. Evaluation of three different culture media (PDA; Potato Dextrose Agar, PARP; Pimaricin-Ampicillin-Rifampicin-PCNB and MEA; Malt Extract Agar) for isolation of fungal population associated with root rot of chilli

Isolates	Faisalabad			Chiniot			Kasur			Okara			Multan		
	PDA	PARP	MEA	PDA	PARP	MEA	PDA	PARP	MEA	PDA	PARP	MEA	PDA	PARP	MEA
Phytophthora capsici	-	+	-	+	+	-	+	+	-	+	+	-	+	+	-
Pythium aphanidermatum	+	-	-	-	-	-	+	+	-	+	-	+	-	-	-
Pythium debaryanum	-	-	-	+	-	+	+	-	+	-	-	-	-	-	-
Pythium myriotylum	+	-	-	-	-	-	-	-	+	+	-	-	+	-	-
Ceratobasidium sp.	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-
Rhizoctonia solani	+	+	+	+	-	+	+	-	-	+	+	-	-	+	-
Verticillium dahliae	+	-	+-	-	-	-	+	-	-	-	-	-	+	-	-
Macrophomina phaseolina	+	-	+	+	+	-	-	-	+	-	-	-	+	+	-
Fusarium oxysporum	+	+	+	-	-	+	-	-	+	-	+	+	-	-	-
Fusarium solani	-	-	-	+	-	-	-	-	-	+	-	+	+		+
Alternaria spp.	-	-	-	+	-	-	+	+	-	+	+	-	+	-	-
Aspergillus flavus	-	+	+	-	+	+	-	+	+	-	-	-	-	+	+
Aspergillus niger	+	-	+	-	+	+	+	-	+	+	-	+	-	-	-
Penicillium spp.	-	+	-	+	-	+	-	+	-	-	+	-	+	-	+
Rhizopus spp.	+	+	-	+	-	-	+	-	+	+	+	-	+	+	-

Isolates	Lahore			Sahiwal			Bahawal Nagar			Rahim Yar Khan		
	PDA	PARP	MEA	PDA	PARP	MEA	PDA	PARP	MEA	PDA	PARP	MEA
Phytophthora capsici	+	+	-	+	+	-	-	-	-	+	+	-
Pythium aphanidermatum	+	-	+	-	-	-	+	-	+	+	-	+
Pythium debaryanum	-	-	-	+	-	-	+	-	-	+	-	-
Pythium myriotylum	-	-	-	-	-	+	-	-	-	-	-	-
Ceratobasidium sp.	+	-	-	+	-	-	-	-	-	-	-	-
Rhizoctonia solani	+	+	-	+	-	+	-	-	-	+	-	+
Verticillium dahliae	+	-	+	-	-	-	-	-	-	+	-	+
Macrophomina phaseolina	+	-	+	-	-	-	+	-	+	+	-	+
Fusarium oxysporum	+	-	+	-	-	-	-	-	+	-	-	+
Fusarium solani	+	-	+	+	-	-	+	-	+	+	-	+
Alternaria spp.	+	+	-	+	-	+	+	+	+	+	+	+
Aspergillus flavus	+	+	-	-	+	+	-	+	-	-	+	-
Aspergillus niger	+	-	+	-	-	-	+	-	+	+	-	+
Penicillium spp.	-	+	+	+	-	+	-	+	-	-	+	-
Rhizopus spp.	+	+	-	+	+	-	+	-	+	+	-	+

+ = Isolated, - = Not isolated

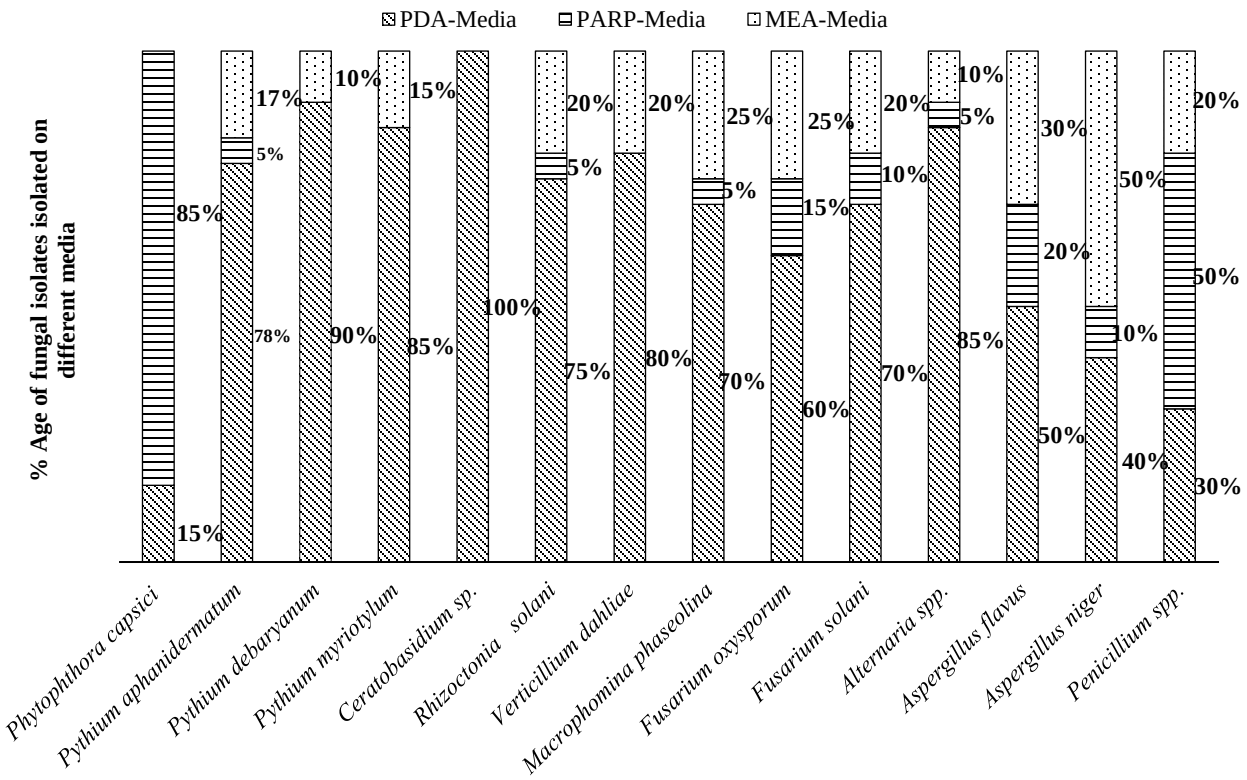

Fig. 4. Evaluation of three different growth media for detection of fungal population associated with chilli root rot.

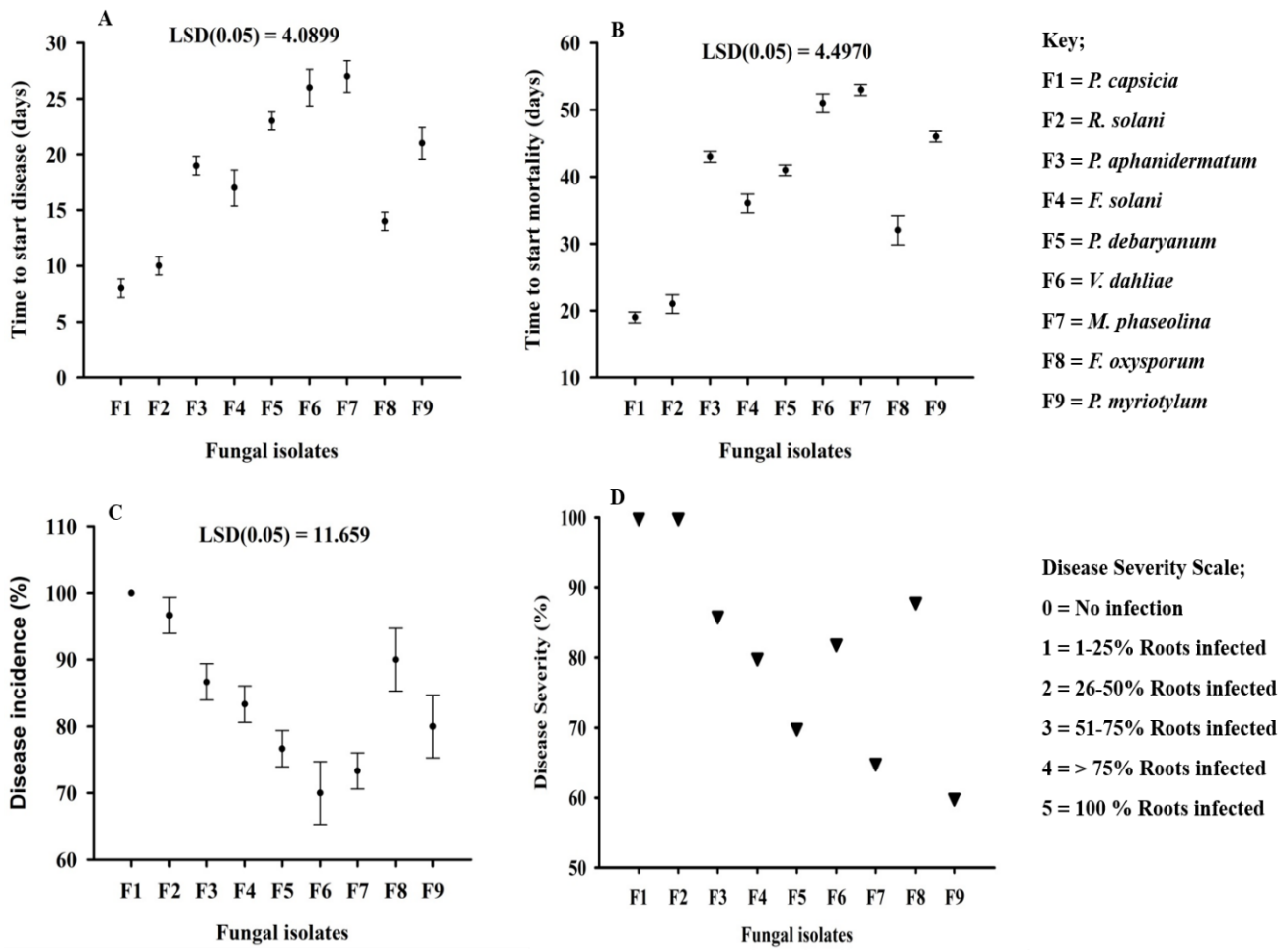

Fig. 5. Determination of most aggressive fungal isolate causing root rot of chilli in geographical area of Punjab on the basis of; a) Time to start disease (days); b) Time to start plant mortality (days); c) Disease incidence (%); d) Disease severity scale.

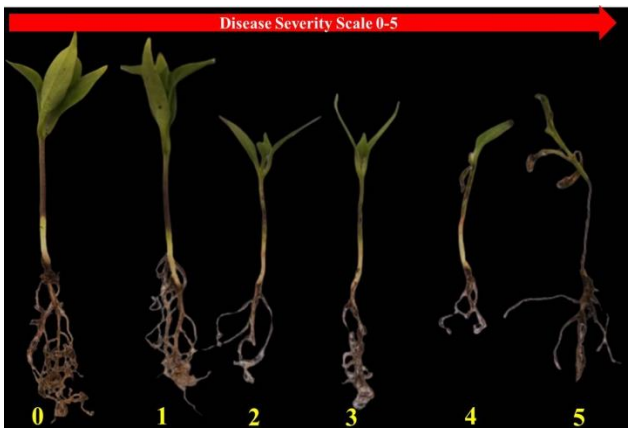

Fig. 6. Disease severity scale 0-5 showed the disease rating on the basis of symptoms on chilli plant; 0=Healthy Plant/No infection; 1= 1-25% infection on chilli plant, 2= 26-50 % infection on chilli plant, 3= 51-75 % infection on chilli plant, 4= >75% infection on chilli plant, 5= 100% roots infected.

Determination of most aggressive fungal isolate causing root rot of chilli in geographical area of Punjab: Total fifteen fungal and oomycetes colonies were identified as *P. capsici, P. aphanidermatum, P. debaryanum, P. myriotylum, Ceratobasidium* sp., *R. solani, V. dahlia, M. phaseolina, F. oxysporum, F. solani, Alternaria* spp., *A. flavus, A. niger, Penicillium* spp. and *Rhizopus* spp.. All of these isolates have been divided into two different groups. Group 'A' contains *P. capsici, P. aphanidermatum, P. debaryanum, P. myriotylum, R. solani, V. dahliae, M. phaseolina, F. oxysporum* and *F. solani,* while group 'B' contains *Alternaria* spp., *Ceratobasidium* sp., *A. flavus, A. niger, Penicillium* spp. and *Rhizopus* spp. Total 9 fungal isolates were placed in group 'A' all were common and frequently reported as pathogenic for chilli crop causing root rot infection, while in group 'B' total of 6 isolates were placed, all these isolates were less frequently isolated and considered as non-pathogenic for root rot disease in chilli plant the members of the group 'A' were taken and tested on most susceptible chilli variety 'Desi' to confirm the pathogenic and aggressiveness status of each isolate. During our experiments, data was recorded for: a) time to start disease, b) time to start mortality, c) disease incidence d) disease severity.

Time to start disease (TSD): Time to start disease appearance after inoculation of each isolate was recorded and it was found that the chilli plants inoculated with *P. capsici* and *R. solani* separately, started showing symptoms just after 8 and 10 days of post inoculation respectively, while root rot disease symptoms in *P. aphanidermatum, P. debaryanum, P. myriotylum, V. dahlia, M. phaseolina, F. oxysporum* and *F. solani,* inoculated chilli plants started showing symptoms of disease 19, 23, 21, 26, 27, 14, 17 respectively, after inoculation (Fig. 5A).

Time to start mortality (TSM): Time to start mortality after inoculation of each isolates was documented and it was observed that the chilli plants inoculated with *P. capsici and R. solani* separately, started showing the plant mortality symptoms after 19 and 21 days of post inoculation respectively while started of mortality like plant symptoms in *P. aphanidermatum, P. debaryanum, P. myriotylum, V. dahlia, M. phaseolina, F. oxysporum* and *F. solani* inoculated chilli plants were shown after 43, 41, 46, 51, 53, 32 and 36 days of post inoculation respectively (Fig. 5B).

Disease Incidence (%): Root rot disease incidence was calculated for each tested fungi and it was concluded that the highest disease incidence 100% and 96.6% was caused by *P. capsici and R. solani,* respectively, while *P. aphanidermatum, P. debaryanum, P. myriotylum, V. dahlia, M. phaseolina, F. oxysporum* and *F. solani* exhibited disease incidence 86.6, 76.6, 80, 70, 73.3, 90 and 83.3% respectively in inoculated chilli plants (Fig. 5C).

Disease severity: Disease severity was documented for each tested fungi and it was observed that the highest disease severity was caused by *P. capsici* and *R. solani,* while *P. aphanidermatum, P. debaryanum, P. myriotylum, V. dahliae, M. phaseolina, F. oxysporum* and *F. solani* were less severe as shown in (Fig. 5D).

Discussion

The root rot disease in chilli plants is a severe and important problem that damages many fields in each growing season. During this study, a total of 112 chilli fields were visited, out of which 72 fields were infected with root rot disease in all surveyed districts of Punjab. The highest disease prevalence of root rot disease in chilli was calculated in district Lahore 87.5%, followed by Rahim Yar Khan, Chiniot, Muzafar Garh, Faisalabad, Sahiwal, Okara, Kasur, and Bahawal Nagar with 80%, 77.78%, 71.42%, 66.67%, 57.14%, 50%, 50%, and 16.67 % respectively. The rate of root rot infection is highly dependent on the virulence of causal agent, host susceptibility, and environmental conditions; in case of favorability of these factors, the whole field can be lost (Bodah, 2017).

During the current survey, the maximum level of disease incidence was calculated from district Lahore 64.14% while the minimum was recorded in Bahawalnagar 14.8%. During a study the disease incidence due to *Pythium* spp. affecting the chilli crop in Punjab Pakistan was documented and maximum disease incidence was observed 45.4% in district Okara (Hayder *et al*., 2018). A highly catastrophic disease resulting in complete crop destruction due to the occurrence of Phytophthora root rot on chilli crop was also reported in Peshawar and Malakand division of KPK Province-Pakistan (Din *et al*., 2012). The highest disease severity for root rot of chilli was found in district Chiniot and Lahore 87% each followed by Rahim Yar Khan, Muzaffargarh, Faisalabad, Sahiwal, Okara, Kasur, and Bahawalnagar 84%, 81%, 78%, 71%, 69%, 83%, 52% respectively. It is concluded that, the disease incidence and severity level of root rot is higher in fields of district Lahore. But the tendency of disease severity is different than that of disease incidence. The current study also reflects the same pictures of the disease with high disease incidence and severity level.

Variations in disease incidence and severity might be due to variability in inoculum quantity and temperature of each district. The large population of plant pathogens was responsible for higher disease incidence for black root rot of cucumber (Ebben & Last, 1973). Another study conducted by Shishido *et al*., (2016) showing that disease development was dependent upon the soil inoculum quantity. The authors concluded that the disease developments in plant roots were directly proportional to the soil inoculum density. Our findings also reflected that the districts where a high fungal population was detected had high disease incidence and severity. Climatic conditions, especially temperature play a significant role in the development of plant diseases (Cheng *et al*., 2013) and the growth of pathogens (Doohan *et al*., 2003)). In Pakistan, the average temperature range is about 25-35°C during most of the year (Parveen *et al*., 2020) which is highly favorable for the occurrence of most common soil-borne fungi and oomycetes associated with root rot infection in chilli. The optimum temperature range for growth of *P. capcisi* is 26-33°C (Shelley *et al*., 2018), for *R. solani* is 26-30°C (Papavizas & Klag, 1970), for *F. oxysporum* is 25-30°C, for *F. solani* is 27-30°C (Domsch *et al*., 1980) and for *M. phaseolina* is 28-35°C (Dhingra & Sinclair, 1978).

Different soil-borne fungi and oomycetes attacking roots of crop plants have been reported in Pakistan (Parveen *et al*., 2020). During this study 15 different fungal colonies were isolated and identified as *Phytophthora capsici, Pythium aphanidermatum, Pythium debaryanum, Pythium myriotylum, Ceratobasidium* sp., *Rhizoctonia solani, Verticillium dahlia, Macrophomina phaseolina, Fusarium oxysporum, Fusarium solani, Alternaria* spp., *Aspergillus flavus, Aspergillus niger, Penicillium* spp., and *Rhizopus* spp. from which *Phytophthora capsici, Rhizoctonia solani,* and *Pythium aphanidermatum* were found more frequent compared to other pathogenic isolated fungal isolates. Due to the involvement of more than one causal agent, this disease is known as root rot complex. Some typical examples of complex plant diseases are the root rot complex of pea attributed to *Phytophthora* spp., *A. euteiches*, *R. solani* and *Pythium* spp., *F. oxysporum*, *F. avenaceum*, *F. solani*, *Mycosphaerella pinodes* (Xue, 2003; Hosseini *et al*., 2015); Zitnick-Anderson *et al*., 2018) and the black root rot disease of strawberry caused by *Rhizoctonia, Pythium* and *Fusarium* pathogens (Manici *et al*., 2005; Louws *et al*., 2012). Our findings clearly support the previous studies that; chilli crop is attacked by several soil-borne pathogenic fungal species which are responsible for high losses in quality and production of chilli fruit in various parts of the world (Lu *et al*., 1984; Abada, 1994; Mushtaq & Hashmi, 1997). *R. solani, M. phaseolina, F. solani, F. oxysporum* and *Pythium* sp. were detected from root rot infected specimens collected from different locations of Sindh province of Pakistan and concluded that *R. solani* and *Pythium* sp. were more pathogenic compared to others (Hussain *et al*., 2013). The occurrence of these root rot associated soil-borne fungal pathogens in Pakistan is due to a favorable temperature range between 25 to 35°C which prevails in most parts of the year (Parveen *et al*., 2020).

All these isolates were classified into two different groups. Group 'A' contains nine fungal isolates *P. capsici, P. aphanidermatum, P. debaryanum, P. myriotylum, R. solani, V. dahlia, M. phaseolina, F. oxysporum* and *F. solani,* all were reported as more frequent pathogens for chilli crop causing root rot infection. In this study all these isolates were tested for their pathogenic and aggressive behavior for chilli plants on the basis of different parameters: time to start disease symptoms, time to start plant mortality, disease incidence percentage, and disease severity scale. Our results showed that *P. capsici* and *R. solani* were the most aggressive fungal pathogens compared to other tested isolates for root rot disease in chilli crop.

During this study, three different growth media (PDA, PARP and MEA) were used for isolation of all possible fungal populations associated with disease samples collected from each district. During this evaluation, it was observed that all fungal isolates were easily isolated on PDA media and the percentage frequency of fungal isolates on PDA media was highest compared to MEA and PARP media. Our finding indicates that PDA is one of the best growth media for detecting all possible fungal isolates associated with root infection in chilli plants.

Conclusion

The present study concluded that the root rot of chilli is a highly prevalent disease in current and potentially chilli-growing areas of Punjab, Pakistan. The disease prevalence was most severe in district Lahore, Rahim Yar Khan and Chiniot while the minimum was recorded in district Bahawalnagar. A total of 15 different fungal isolates were detected from which *Phytophthora capsici* and *Rhizoctonia solani* were found to be more frequent and aggressive pathogens compared to other isolates. The assessment of this study delivers valuable information to the researchers to conduct an advanced study for the development of suitable strategies for effective management of root rot of chilli when multiple pathogenic agents are present. This may increase the production and quality of chilli crops by minimizing the root rot infection in chilli fields.

Acknowledgments

This article is extracted from the Ph. D dissertation of Muhammad Usman Ali. We are highly grateful to the Endowment Fund Secretariat University of Agriculture, Faisalabad, Pakistan (EFS #77129/20) and the Higher Education Commission Pakistan for granting monetary support for this work.

References

Abada, K.A. 1994. Fungi associated with root rot of pepper and some factors affecting disease incidence. *7th Congress of Phytopathology, Giza,* 220-226.

Al-Snafi, A.E. 2015. The pharmacological importance of Capsicum species (*Capsicum annuum* and *Capsicum frutescens*) grown in Iraq. *J. Pharma. Biol*., 5(3): 124-142.

Akter, M.K., M.D. Hossain, K. Nahar, M.B. Meah and M.A. Hossain. 2007. Isolation and identification of *Phytophthora capsici* and its mating type determination. *J. Agrofor. Environ.,* 2: 89-92.

Ajayi-Oyetunde, O.O. and C.A. Bradley. 2017. Identification and characterization of *Rhizoctonia* species associated with soybean seedling disease. *Plant Dis*., 101(4): 520-533.

Bodah, E.T. 2017. Root rots diseases in plants: a review of common causal agents and management strategies. *Agri. Res. Tech.,* 5(3): 555661.

Daunde, A.T., K.T. Apet, A.P. Suryawanshi and V.S. Khandare. 2018. Prevalence of collar rots of chilli caused by *Sclerotium rolfsii* Sacc. Under the agro-climatic zones of Marathwada region of Maharashtra. *J. Pharm. Phytochem*., 7(4): 1905-1908.

Din, U.I., F. Raziq, S. Hussain and Saifullah. 2012. Occurrence of Phytophthora root rot (*Phytophthora capsici* L.) of pepper in Peshawar and Malakand divisions. *Sarhad J. Agric.*, 28: 443-450.

Dubey, M.K., A. Zehra, M. Aamir, M. Yadav, S. Samal and R.S. Upadhyay. 2020. Isolation, identification, carbon utilization profile and control of *Pythium graminicola*, the causal agent of chilli damping off. *J. Phytopathol.*, 168(2): 88-102.

Dugan, F.M. 2017. The Identification of Fungi: An Illustrated Introduction with Keys, Glossary, and Guide to Literature. Mycology. APS Publication https://doi.org/10.1094/9780890545041

El-Kazzaz, M.K., G.B. El-Fadly, M.A.A. Hassan and G.A.N. El-Kot. 2008. Identification of some *Fusarium* spp. using molecular biology techniques. *Egypt. J. Phytopath*., 36(2): 57-69.

GOP, G. 2020. Pakistan Economic Survey 2019-20. Finance Division, Economic Advisor's Wing: Islamabad, Pakistan.

Hussain, F. and M. Abid. 2011. Pest and diseases of chilli crop in Pakistan: A review. *Int. J. Biol. Biotech*., 8(2): 325-332.

Hussain, F., S.S. Shaukat, M. Abid, F. Usman and M. Akbar. 2013. Pathogenicity of some important root rots fungi to the chilli crop and their biological control. *Int. J. Biol. Biotechnol*, 10: 101-108.

Hyder, S., S. Naseem, S. Azhar, M. Ashfaq, Z. Ali, A. Khalid and M. Inam-ul-Haq. 2018. Disease incidence and severity of *Pythium* spp. and *Phytophthora* spp. affecting chilli pepper and tomato crops in Punjab, Pakistan. *Phillipp. Agric. Sci.*, 101: 36-44.

Khan, F.A., T. Mahmood, M. Ali, A. Saeed and A. Maalik. 2014. Pharmacological importance of an ethnobotanical plant: *Capsicum annuum* L. *Nat. Prod. Res.*, 28(16): 1267-1274.

Li, T.S.C. 2000. Medicinal plants: Culture, utilisation and phytopharmacology. CRC press.

Lu, J.Y., C.F. Yu and L.H. Ju. 1984. Studies on differentiation of the virulence of *Verticillium dahlia* on pepper. *Acta Phytopathologia Scientia.*, 14: 213-18.

Leslie, J.F. and A.B. Summerell. 2008. The Fusarium laboratory manual. John Wiley & Sons.

Lodhi, A.M., M.A. Khanzada, S. Shahzad, A. Ghaffar and C.A. Lévesque. 2013. Prevalence of *Pythium aphanidermatum* in agro-ecosystem of Sindh province of Pakistan. *Pak. J. Bot*, 45(2): 635-642.

Mannai, S., H.J. Khiareddine, B. Nasraoui and M. Daami-Remadi. 2018. Rhizoctonia root rot of pepper (*Capsicum annuum*): comparative pathogenicity of causal agent and biocontrol attempt using fungal and bacterial agents. *J. Plant Pathol. Microbiol.*, 9(431): 2.

Meghvansi, M.K., S. Siddiqui, M.H. Khan, V.K. Gupta, M.G. Vairale, H.K. Gogoi and L. Singh. 2010. Naga chilli: A potential source of capsaicinoids with broad-spectrum ethnopharmacological applications. *J. Ethnopharmacol*., 132(1): 1-14.

Mejía-Teniente, L., A. Blanca, A.B. Durán-Flores, T.P. Irineo, M.G.C. Mario, Rivera-Bustamante, F. Rafael, A.F.P. Ana and R.G. Guevara-González. 2019. Hydrogen peroxide protects pepper (*Capsicum annuum* L.) against pepper golden mosaic geminivirus (PepGMV) infections. *Physiol. Mol. Plant Path*., 106: 23-29.

McClenny, N. 2005. Laboratory detection and identification of Aspergillus species by microscopic observation and culture: The traditional approach. *Med. Mycol*., 43: S125-S128.

Mushtaq, M. and M.H. Hashmi. 1997. Fungi associated with wilt disease of Capsicum in Sindh, Pakistan. *Pak. J. Bot.*, 29: 217-222.

Oljira, T. and S. Berta. 2020. Isolation and characterization of wilt-causing pathogens of local growing pepper (*Capsicum annuum* L.) in Gurage Zone, Ethiopia. *Int. J. Agron.,* 2020: 1-8.

Parveen, G., F. Urooj, S. Moin, H. Farhat, M.F. Fahim and S. Ehteshamul-Haque. 2020. Estimation of losses caused by root rotting fungi and root knot nematodes infecting some important crops in Lower Sindh and Hub, Balochistan of Pakistan. *Pak. J. Bot.*, 52(2): 673-678.

Pawar, S.S., N. V. Bharude, S.S. Sonone, R.S. Deshmukh, A.K. Raut and A.R. Umarkar. 2011. Chillies as food, spice, and medicine: A perspective. *Int. J. Pharm. Biol. Sci*., 1(3): 311-318.

Perucka, I. and M. Materska. 2003. Antioxidant activity and content of capsaicinoids isolated from paprika fruits. *Pol. J. Food Nutr. Sci.*, 12(2): 15-18.

Siddique, S., A. Shoaib, S.N. Khan and A. Mohy-Ud-Din. 2021. Screening and histopathological characterization of sunflower germplasm for resistance to *Macrophomina phaseolina*. *Mycologia*, 113(1): 92-107.

Siciliano, I., G. Gilardi, G. Ortu, U. Gisi, M.L. Gullino and A. Garibaldi. 2017. Identification and characterization of *Alternaria* species causing leaf spot on cabbage, cauliflower, wild and cultivated rocket by using molecular and morphological features and mycotoxin production. *Eur. J. Plant Pathol.*, 149(2): 401-413.

Saxena, A., R. Raghuwanshi, V.K. Gupta and H.B. Singh. 2016. Chilli anthracnose: The epidemiology and management. *Front. Microbiol.*, 7: 1527.

Tariq, M., Q. Ali, A. Khan, G.A. Khan, B. Rashid, M.S. Rahi, A. Ali, I.A. Nasir and T. Husnain. 2014. Yield potential study of *Capsicum annuum* L. under the application of PGPR. *Adv. Life Sci.*, 1(4): 202-207.

Tariq, A., F. Naz, M. Shahid, A.S. Gondle, A. Sattar, M. Nabeel and I. Hassan. 2020. First report of root rot caused by *Ceratobasidium* sp. AG-Fa on *Capsicum annuum* in Pakistan. *J. Plant Pathol.*, 102(4): 1323-1324.

Welbaum, G.E. 2015. Vegetable Production and Practices. CAB International Publisher.

Yu, J.M., I.H. Cafarov and M. Babadoost. 2016. Morphology, molecular identity, and pathogenicity of *Verticillium dahliae* and *V. longisporum* associated with internally discolored horseradish roots. *Plant Dis.*, 100(4): 749-757.

Cheng, C., X. Gao, B. Feng, J. Sheen, L. Shan and P. He. 2013. Plant immune response to pathogens differs with changing temperatures. *Nat. Comm.*, 4(1): 1-9.

Shelley, B.A., D.G. Luster, W.M. Garrett, M.B. McMahon and T.L. Widmer. 2018. Effects of temperature on germination of sporangia, infection and protein secretion by *Phytophthora kernoviae*. *Plant Pathol.*, 67(3): 719-728.

Doohan, F.M., J. Brennan and B.M. Cooke. 2003. Influence of climatic factors on Fusarium species pathogenic to cereals. In *Epidemiology of mycotoxin producing fungi* (755-768). Springer, Dordrecht.

Dhingra, O.D. and J.B. Sinclair. 1978. Biology and Pathology of *Macrophomina phaseolina*.

Ebben, M.H. and F.T. Last. 1973. Cucumber black root rot caused by *Phomopsis sclerotioides*. *Ann. Appl. Biol.*, 73(3): 259-267.

Papavizas, G.C. and N.G. Klag. 1970. Colonization and growth of *Rhizoctonia solani* in soil. *Rhizoctonia solani, Biology & Pathol.*, 108-122.

Domsch, K.H., W. Gams and T.H. Anderson. 1980. *Compendium of Soil Fungi*. Vol. 1, Academic Press New York: 859.

Shishido, K., H. Murakami, D. Kanda, S.I. Fuji, T. Toda and H. Furuya. 2016. Effect of soil inoculum density and temperature on the incidence of cucumber black root rot. *Plant Dis.*, 100(1): 125-130.

Zitnick-Anderson, K., K. Simons and J.S. Pasche. 2018. Detection and qPCR quantification of seven Fusarium species associated with the root rot complex in field pea. *Can. J. Plant Patho.*, 40(2): 261-271.

Hosseini, S., M. Elfstrand, F. Heyman, D.F. Jensen and M. Karlsson. 2015. Deciphering common and specific transcriptional immune responses in pea towards the oomycete pathogens *Aphanomyces euteiches* and *Phytophthora pisi*. *BMC genom.*, 16(1): 1-18.

Louws, F., J. Sun, H. Whittington, J. Driver, K. Peeden and B. Liu. 2012. Evaluation of fungicides and mustard meal to manage black root rot of strawberry and analysis of *Pythium*, *Fusarium*, and *Rhizoctonia* on strawberry roots. *Phytopathol.,* 102: 72.

Manici, L.M., F. Caputo and G. Baruzzi. 2005. Additional experiences to elucidate the microbial component of soil suppressiveness towards strawberry black root rot complex. *Ann. Appl. Biol.*, 146(4): 421-431.

Xue, A.G. 2003. Biological control of pathogens causing root rot complex in field pea using *Clonostachys rosea* strain ACM941. *Phytopathol.*, 93(3): 329-335.

Zhongming, Z., L. Linong, Y. Xiaona, Z. Wangqiang and L. Wei. 2019. Understanding peppers and chillis from around the world. *Qual. Quant.,* 49(6): 2269-2290.

Teng, P.S. and W.C. James. 2002. Disease and yield loss assessment. In Plant Pathologist's pocketbook (3rd ed., pp. 25–38). Wallingford: CABI Publishing.

Bandh, S.A., A.N. Kamili and B.A. Ganai. 2011. Identification of some *Penicillium* species by traditional approach of morphological observation and culture. *Afr. J. Microbiol.,* 5(21): 3493-3496.

Gryganskyi, A.P., J. Golan, S. Dolatabadi, S. Mondo, S. Robb, A. Idnurm, A. Muszewska, K. Steczkiewicz, S. Masonjones, H.L. Liao and M.T. Gajdeczka, 2018. Phylogenetic and phylogenomic definition of *Rhizopus* species. *G3: Genes, Genomes, Genetics*, 8(6): 2007-2018.

(Received for publication 7 April 2022)