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OCCURRENCE OF DAMPING OFF OF CHILI CAUSED BY *PYTHIUM* SPP. IN THE POTHWAR REGION OF PAKISTAN

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ABSTRACT

The per acre yield of chili in Pakistan is quite low as compared to large producers of this vegetable in the world and can be attributed to an assortment of factors both biotic as well as abiotic. Among biotic factors, chili is susceptible to damping off caused by *Pythium* spp. and causes lots of damage in Pakistan. There is scantly information on the incidence and severity of *Pythium* associated with chili. Therefore, the current studies were carried out to determine the incidence and severity of *Pythium* in the Pothwar region of Pakistan. An overall incidence of 14.08% of *Pythium* damping off was recorded on chili in Pothwar. Attock showed the maximum incidence of 19.86% followed by Rawalpindi and Chakwal districts having 16.22% and 10.22% disease incidences respectively. Jhelum had the minimum disease incidence of 10.05%. Disease severity was the maximum in district Attock (11.03%) followed by Rawalpindi (9.11%), and was the minimum in Chakwal (5.42%) followed by Jhelum (5.67%). The finding of these studies will be helpful for the farmers in designing control strategies for this pathogen accordingly.

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INTRODUCTION

Chili (*Capsicum annuum* L.) enjoys an important position in the world and is considered among the most favorite vegetables after potato and tomato. The vegetable belongs to the family *Solanaceae* (Berke, 2002). Chili is one of the most common condiments used in Pakistan and other South Asian countries. Chili is rich in vitamins, especially pro-vitamin A, B_1 (thiamine), B_2 (riboflavin), B_3 (niacine), vitamins C, E, P (citrin). It also contains appreciable amounts of Phosphorus, Calcium, and Iron (MacGillivary, 1961; Macrae *et al.*, 1993; Bosland and Votova, 1999).

Chili is an herbaceous plant and is extremely sensitive to cold (Wein, 1999). It requires hot environment for its optimal growth and flourishes well on well-drained, silt loam or sandy soils. Chili plants have fibrous taproots which extend vertically 30-60 cm deep and horizontally 30-50 cm. Flowering occurs three months after planting. Flowers are hypogynous and hermaphrodite. Chili crop matures after 4 months of planting in dry and hot weathers. Chili can be cultivated under a wide range of environmental conditions; both subtropical and tropical but in general it requires temperature of 24-30 °C. In Pakistan, chili nursery is prepared during August for autumn/winter crop and in February to April for summer crop. In hilly areas, nursery is prepared in March to April. Thirty to fifty days old seedlings from the nursery are transplanted into the fields. Fully grown fresh green chilies are used as vegetable and for pickles. Red fully ripe chilies are picked for dried chili powder.

The per acre yield of chili in Pakistan is quite low as compared to large producers of this vegetable in the

world and can be attributed to an assortment of factors both biotic as well as abiotic (Ahmad *et al.*, 2017; Asghar *et al.*, 2020; Shahbaz *et al.*, 2015). Chili becomes more susceptible to a wide range of fungal, bacterial, nematodal and viral diseases under natural environmental conditions of South East Asia (Ashfaq *et al.*, 2014; Tazeem *et al.*, 2018; Tariq-Khan *et al.*, 2017; Tariq-Khan *et al.*, 2020a; Green, 1991; Hameed *et al.*, 1995; Tariq-Khan *et al.*, 2020b). Fungi and bacteria cause foliar (leaf), fruit, stem or root diseases (Kamal and Mughal, 1968; Hafiz, 1986; Majid *et al.*, 1992; Mushtaq and Hashmi, 1997; Aslam *et al.*, 2017). For farmers, these diseases cause considerable yield losses which result in huge economic loss. Among fungal diseases, damping off caused by *Pythium* spp., late blight caused by *Phytophthora* spp. (Hyder *et al.*, 2020) and *Fusarium* wilt are some of the most important diseases causing lots of damage to tomato and chili in Pakistan.

Pythium damping off is a major nursery problem in chili fields (Sherf and MacNab, 1986; Alhussaen *et al.*, 2011) where the organism kills newly emerged seedlings (Jarvis and Jarvis, 1992; Agrios, 2005; Redekar *et al.*, 2019). *Pythium* spp. have a broad host range and causes pre-emergence and post-emergence damping-off and root rots followed by considerable yield losses in chili crop. *Pythium* species are rarely host specific (Rangaswamy, 1989).

Pythium, a soil borne oomycete pathogen is "fungal-like" organism and a member of the family Pythiaceae. The fungus is able to spread from diseased seedlings to healthy plants through zoospores. *Pythium* species are homothallic; sexual reproduction can occur between the same hypha when the female oogonium and male antheridium fuse together as a result their nuclei unite to form a single zygote. At high temperatures (28 °C), the oospores or zygote germinate and develop into mycelium. Infectious zoospores develop at lower temperatures of 10 to 17 °C (Alexopoulos *et al.*, 1996).

There are a number of soil inhabiting pathogenic *Pythium* spp. i.e. *P. aphanidermatum, P. ultimum, P. helicoides, P. myriotylum*, and *P. splendens* which can cause significant root rot and reductions in root growth of chili crop. *P. aphanidermatum* can cause severe root rot, reductions in plant weight and 42-62% plant mortality under conducive conditions in chili plants. There are some other *Pythium* spp. which cause low incidences of root tip necrosis in chili plants. Root tip necrosis causing *Pythium* spp. are *P. arrhenomanes*, *P. catenulatum, P. graminicola,* and *P. irregulare*, but none of these species cause losses in root weight and only *P. irregulare* reduced shoot weight. The colonization of chili plants by *P. aphanidermatum* was observed to be affected by temperature. At 28 °C, reductions in growth occurred while at 34 °C plant mortality occurred in chili transplants (Chellemi *et al.*, 2000).

There is scantly information on the incidence and severity of *Pythium* species infecting chili in Pakistan especially in the Pothwar region. Therefore, the present studies were carried out to determine the incidence and prevalence of *Pythium* in the Pothwar region of Pakistan. The findings of these studies will be helpful for the farmers in designing control strategies for this pathogen accordingly.

MATERIALS AND METHODS

The studies reported in this paper were carried out in the Pothwar region of Pakistan and in the Department of Plant Pathology of Pir Mehr Ali Shah Arid Agriculture University Rawalpindi during 2017-2020.

Survey for incidence and severity

Chili fields of the Pothwar Plateau (Figure 1) were surveyed to determine the incidence and severity of *Pythium* spp. during 2017-2020. In order to determine the incidence and severity, 15 sites were selected randomly from each district and from each site, 3 fields were selected. From each field, 50 plants were selected each after every 5 steps across a diagonal following hierarchical sampling strategy (McDonald and Martinez, 1990).

Incidence of *Pythium* in each field of each site was calculated by the following formula;

$$\text{Incidence (\%)} = \frac{\text{No of infected Plants}}{\text{Total No of Plants}} \times 100$$

The incidences of three fields were averaged to determine the incidence of each site. Similarly, the incidences of 15 sites of each district were averaged for determining the incidence of that district. Individual diseased plants of chili from each field of each site were assessed for severity of *Pythium* by following severity index scale developed by Altier and Thies (1995) (Table 1).

Collection of samples

Five diseased infected plants of chilli were randomly selected on the basis of symptoms from each field following the hierarchical sampling strategy (McDonald and Martinez, 1990).

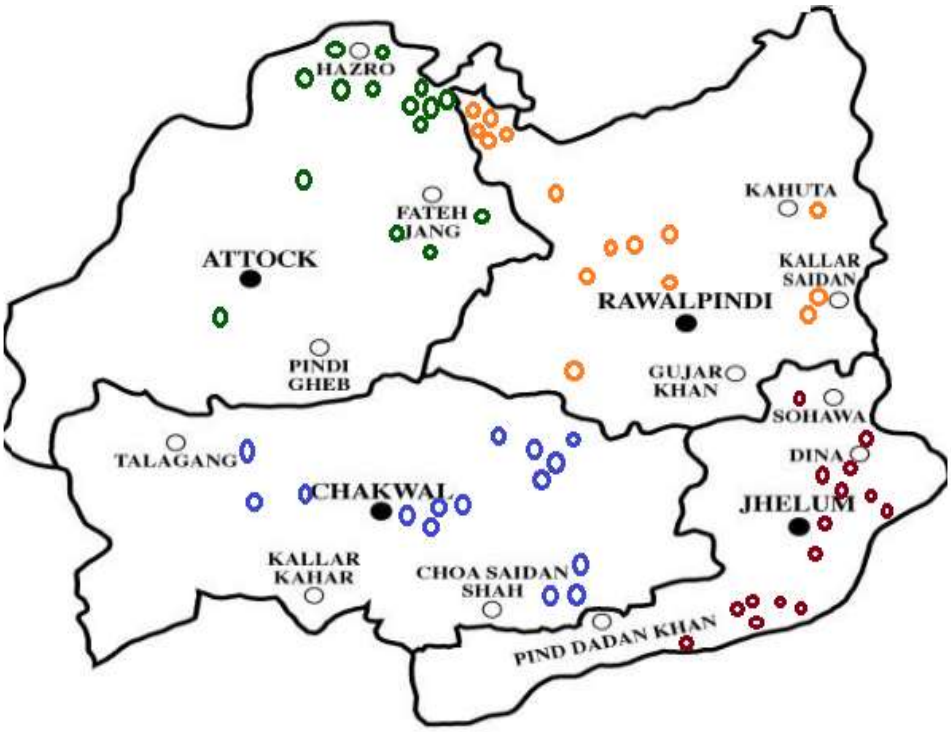

Figure 1: Individual surveyed localities in districts of Pothwar region of Pakistan.

For the isolation of *Pythium*, soil samples were taken from the rhizospheres of randomly selected plants at the soil depth of 12 cm. Samples of each field were mixed to obtain pooled sample. The collected samples were labeled properly and immediately brought to the laboratory to start isolation of *Pythium* species.

Table 1: Severity index for *Pythium* damping off.

Sr. No.	Rating	Status
1	0	Healthy
2	1	Primary root tip necrosis
3	2	Primary root tip soft and necrotic
4	3	Dead seedling
5	4	Dead seed

Isolation of *Pythium* spp.

Soil samples containing roots of *Pythium* infected chili plants were placed in plastic bags and thoroughly moistened with autoclaved water. Potato plugs of 10 mm diameter and 50 mm lengths were added into each bag. The potato tubers were washed, peeled and surface-disinfected before cutting into plugs with a sterile cork borer. After 24 hours of incubation at room temperature, the plugs were rinsed with tap water and cut into small lengthwise slices and plated on to *Pythium* V8 media amended with antibiotics.

Purification of cultures

Pure cultures of the fungal pathogens were obtained by single spore and hyphal tip method. In petriplates where *Pythium* have produced zoosporangia, pure cultures were obtained using single spore method, by picking a single zoosoprangium of fungus with the help of sterilized needle under stereoscope placed in laminar air flow hood and was transferred to petriplates and slants containing selective media. The plates and slants were incubated at 25 °C.

In petriplates where *Pythium* have not produced zoospores, pure cultures were made by hyphal tip method. To obtain pure culture, growing hyphal tips were cut along with media (using cork borer) and were placed onto the surface of petriplates containing fresh selective medium. The pletriplates were incubated for 15-24 hours at room temperature.

Identification of *Pythium* species on the basis of morphology

Water cultures of *Pythium* were prepared. Inoculum discs were added to sterile water in petriplates along with chili leaves and were incubated at 25 °C. *Pythium* species were identified based on structure of sporangia,

zoospore and sexual structures. *Pythium* isolates were identified morphologically as described by Van der Plaats-Niterink (1981) and Dick (1990). The purified and identified cultures of all the isolates of *Pythium* were maintained at 25 °C.

Pathogenicity bioassay

Chili seeds were grown in trays in the greenhouse. Four to six leaf seedlings of chili were inoculated with the already prepared inoculum. The roots of seedlings were inoculated by drenching method. Plants were covered with a plastic sheet for 16 hours to prevent the inoculum from drying. Symptoms were observed and recorded.

RESULTS

Incidence and severity of *Pythium* spp. damping off on chili in Pothwar

Pythium damping off showed an overall incidence of 14.08% in the Pothwar region. Attock showed the maximum incidence of 19.86% followed by Rawalpindi and Chakwal districts having 16.22% and 10.22% disease incidences respectively. Jhelum had the minimum disease incidence of 10.05% as shown in Figure 2. Disease severity was the maximum in district Attock (11.03%) followed by Rawalpindi (9.11%), and was the minimum in Chakwal (5.42%) followed by Jhelum (5.67%) as shown in Figure 3.

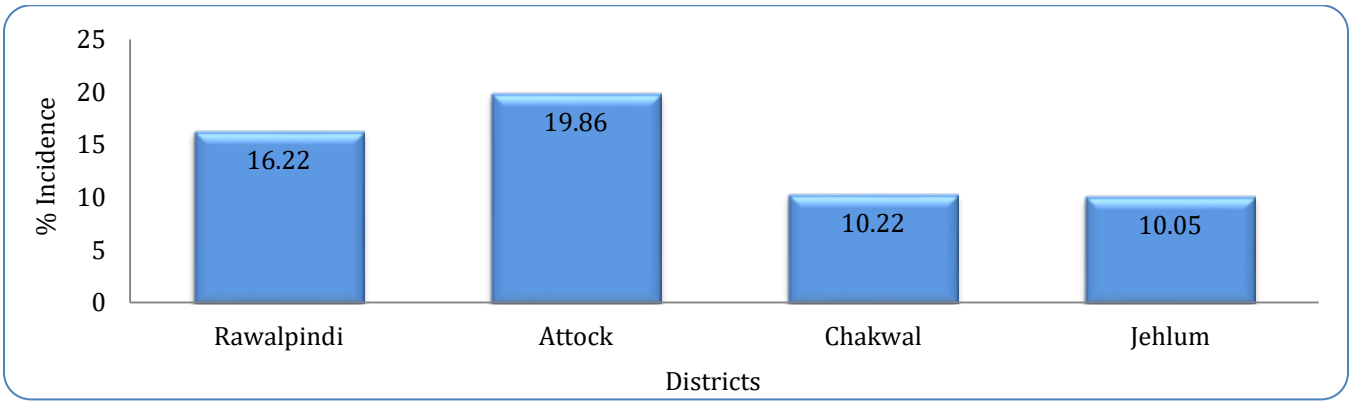

Figure 2: Incidence (%) of *Pythium* on chili in districts of Pothwar region.

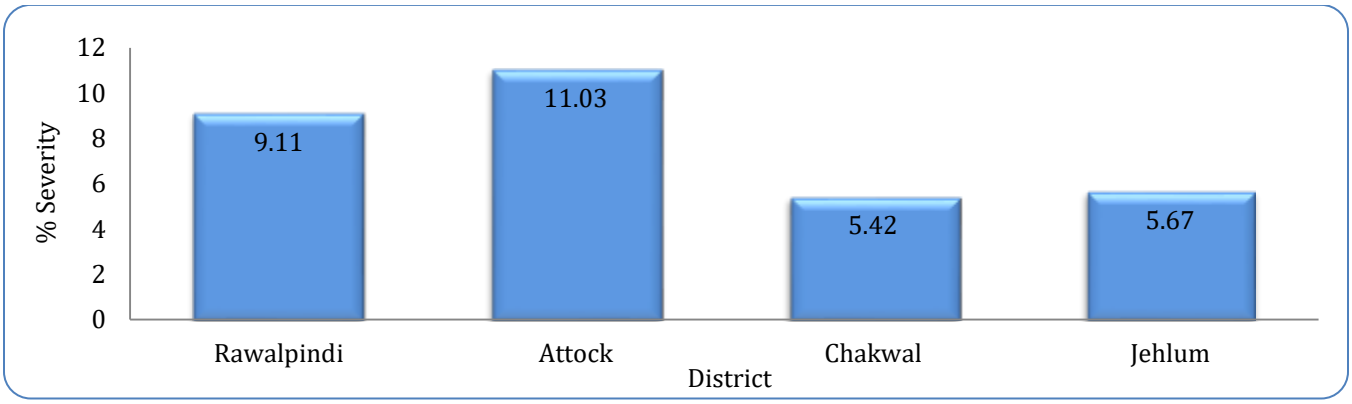

Figure 3: Severity (%) of *Pythium* on chili in districts of Pothwar region.

Tehsil wise incidences and severities of damping off in each district are given in figures 4, 5, 6 and 7. In district Attock, Fateullah had the maximum disease incidence of 24.66% while Khuda and Dhraik showed the minimum incidences of 16.00%. Similarly, Fateullah also had the maximum disease severity of 13.8% while the minimum disease severity of 7.30% was found in Walia area. Individual incidences and severities in other areas in district Attock are presented in Table 2.

In district Rawalpindi, Bhalakar had the maximum while Adiala had the minimum disease incidences of 22.00% and 6.60%. Bhalakar had the maximum severity of 12.30% and Adiala had the minimum severity of 4.50%. Individual incidences and severities of *Pythium* damping

off on chili in different localities of district Rawalpindi are given in Table 3.

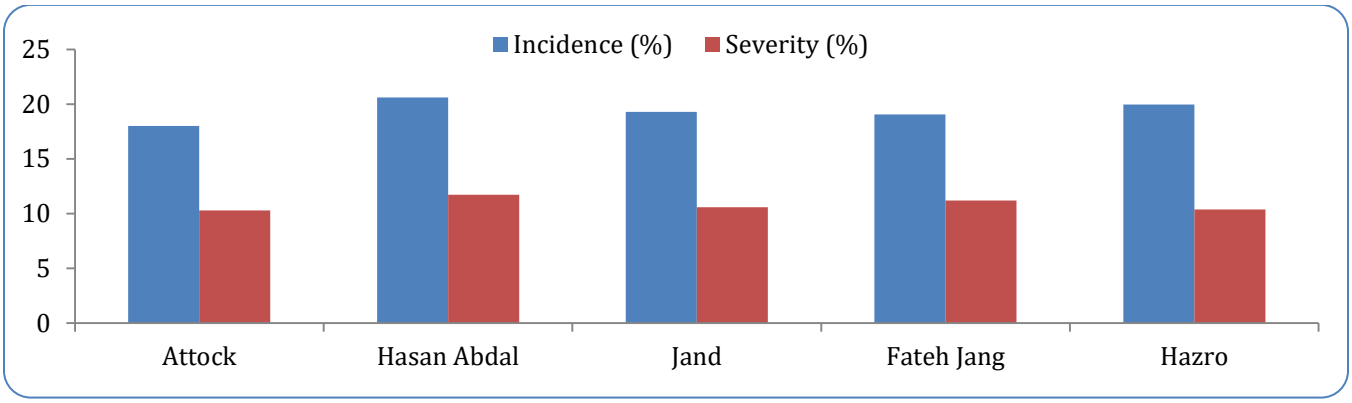

Figure 4: Incidence (%) and severity (%) of *Pythium* on chili in tehsils of district Attock of Pothwar region.

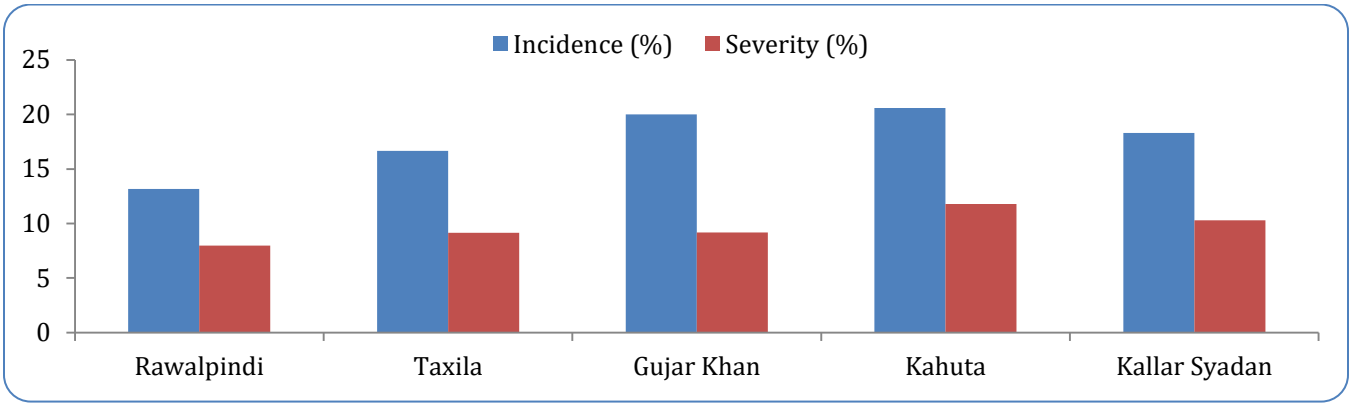

Figure 5: Incidence (%) and severity (%) of *Pythium* on chili in tehsils of district Rawalpindi of Pothwar region.

Table: 2: Incidence and severity of *Pythium* on chili in individual localities in district Attock.

Tehsil	Locality	Incidence (%)	Severity (%)
Attock	Dhok Gama	18±5.98	10.3±3.11
	Jhalo	20.6±3.45	12.0±1.13
	Bai	21.3±4.71	12.8±2.55
Hasan Abdal	Fatheullah	24.6±3.45	13.8±0.86
	Kacha	16±4.52	8.6±2.67
	Islamgarh	20.6±6.91	11.5±4.27
Jand	Mithal	19.3±4.71	10.6±4.39
Fateh Jang	Bahtar	22.6±10.69	12.0±5.39
	Sadkal	18.6±4.71	12.0±1.13
	Dhraikk	16.0±5.98	9.6±2.28
	Walia	20.0±5.98	7.3±2.35
	Shamsabad	16.6±5.69	10.5±3.15
Hazro	Chichian	21.3±4.71	12.2±2.55
	Kalu Khurd	20.6±4.71	9.63±5.2
	Basia	21.3±5.69	12.3±3.9
Overall		19.86	11.03

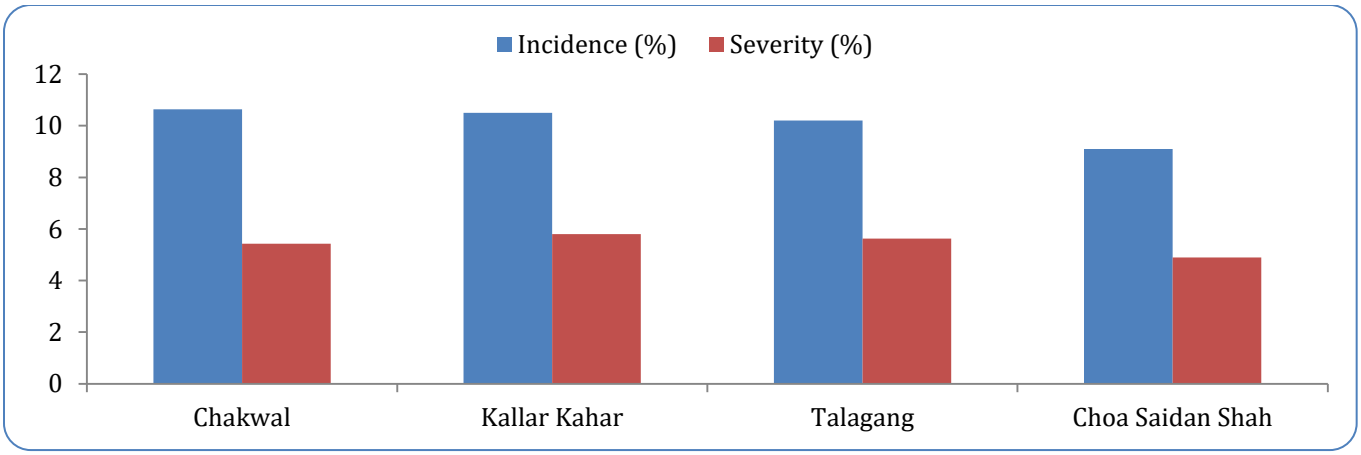

Figure 6: Incidence (%) and severity (%) of *Pythium* on chili in tehsils of district Chakwal of Pothwar region.

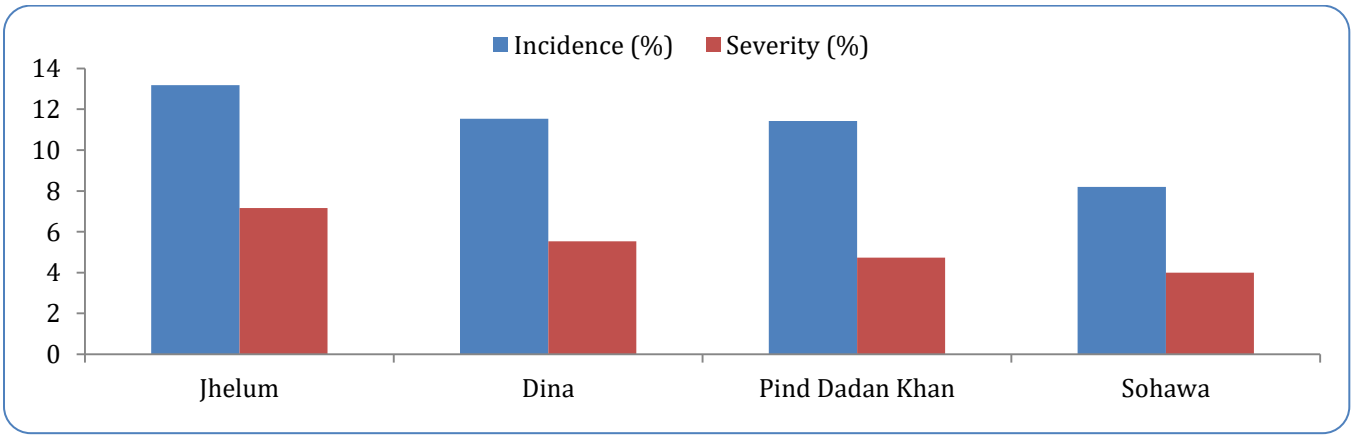

Figure 7: Incidence (%) and severity (%) of *Pythium* on chili in tehsils of district Jhelum of Pothwar region.

Table: 3: Incidence and severity of *Pythium* on chili in individual localities in district Rawalpindi.

Tehsil	Locality	Incidence (%)	Severity (%)
Rawalpindi	Adiala	6.6±3.45	4.5±2.04
	Gorkhpur	15.3±4.71	10.8±4.57
	Basali	15.3±3.45	7.5±5.39
	Sood	12.6±7.27	7.5±5.39
	Kasalan kalan	16.0±9.8	9.6±6.23
	Chak Muzaffar	16.0±8.16	8.3±4.60
Taxila	Jalala	18.0±5.98	11.0±2.99
	Brahma	13.3±3.45	7.6±2.114
	Lab Thatho	18.0±9.86	9.6±5.58
	Vani	19.3±10.2	10.2±4.8
	Mohra Murado	15.3±3.45	8.2±3.63
Gujar Khan	Koont farm	20.0±4.52	9.17±0.86
Kahuta	Batala	20.6±6.91	11.8±4.8
Kallar Syadan	Bhalakhar	22.0±5.98	12.3±2.79
	Mohra Dial	14.6±3.45	8.3±1.17
	Overall	16.22	9.11

In district Chakwal, the maximum incidence of 11.33% was recorded in Mureed and Chakral. On the other hand, Norwal area had the minimum incidence of 4.00%. In the same way, Pinwal area had the maximum disease severity of 8.10% while Norwal area showed the minimum disease severity of 2.00%. The severity and incidence of *Pythium* damping off on chili crop in each locality of district Chakwal are given in Table 4.

As regards district Jhelum, the maximum incidence was observed in Kala Gujran (16.66%) while it was the minimum in Jatipur (7.33%). Kala Gujran and Jatipur also showed the maximum and the minimum disease severity values of 9.20% and 4.00% respectively. Disease incidences and disease severities in various areas in district Jhelum are given in Table 5.

Table: 4: Incidence and severity of *Pythium* on chili in individual localities in district Chakwal.

Tehsil	Locality	Incidence (%)	Severity (%)
Chakwal	Parrtal	9.2±4.71	4.8±1.98
	Mureed	11.3±3.45	5.8±1.81
	Chakora	14.0±11.9	7.6±6.6
	Dheen	10.0±9.8	4.6±4.5
	Norwal	4.0±4.52	2.0±2.26
	Chakral	11.3±5.69	5.7±3.42
	Pinwal	16.0±8.16	8.1±4.09
	Thaneel Fatoohi	9.3±3.45	4.8±0.86
Kallar Kahar	Bhaun	10.0±5.69	5.8±2.67
	Bekhri Kallan	10.0±2.26	5.1±1.42
Talagang	Dhrabi	13.3±5.69	7.5±3.44
	Jaisal	7.3±8.56	4.3±5.07
	Choa Saidan Shah	10.0±2.26	6.1±1.72
Choa Saidan Shah	Lehri Panj garaien	7.3±2.61	3.8±1.63
	Dalwal	10±2.26	4.8±1.63
	Overall	10.22	5.42

Table: 5: Incidence and severity of *Pythium* on chili in individual localities in district Jhelum.

Tehsil	Locality	Incidence (%)	Severity (%)
Jhelum	Kala gujran	16.6±3.45	9.2±1.98
	Kotli Alayaar	13.3±4.71	7.0±2.46
	Monan	12.0±5.98	6.3±2.28
Dina	Baigpur	10.0±5.98	5.5±3.7
	Nograin	14.0±2.26	7.8±1.72
	Ghar mahal	11.3±9.42	5.5±4.49
	Chak almas	13.3±8.56	6.3±3.63
	Kalwandpur	10.0±4.52	4.8±3.11
	Chak Jani	10.0±2.26	4.8±0.86
P. D. Khan	Pinnanwal	10.0±8.16	4.8±3.45
	Chak Shadi	10.6±3.34	5.0±1.13
	Mirzapur	10.0±9.86	4.5±4.39
	Jatipur	7.3±5.69	4.0±2.59
	Haranpur	10.6±7.94	5.3±4.5
Sohawa	Sohan	8.0±2.26	4.0±1.69
	Overall	10.05	5.67

Morphological characterization of *Pythium* spp.

In total, 39 isolates were recovered from chili crop in the Pothwar region. All the recovered isolates were morphologically characterized using different parameters like colony color, growth habit, hyphal swellings, size and shape of sporangia, cardinal temperature, sexuality, oogonia and anthredia. For microscopic identification, fresh slides were prepared from pure cultures of each isolate and were visualized under high power microscope.

Thirty of the recovered isolates produced loosely to compact aerial mycelium with no distinct pattern, hyphae were aseptate, hyphal swellings were absent and sporangiophores were filamentous. Isolates also responded similarly to critical temperature range (Min: 10±1 °C, Max: 40±1 °C, Opt: 28±1 °C). All the isolates were morphologically confirmed to be *Pythium aphanidermatum* as already done by various scientists. Rest of the 9 isolates showed variable morphology from colony color of hyaline, grayish with loosely to compact aerial mycelium with no distinct pattern; mycelium was aseptate, with distinct hyphal swellings and sporangia were globose. Oogoina were terminal and anthredia were monolicious. These 9 isolates were confirmed to be *Pythium ultimum*.

DISCUSSION

Pythium is a renowned soil borne Oomycete pathogen with a wide host range in warmer parts of the world. *Pythium* damping off is a very important and common problem in chili and tomato fields (Redekar *et al.*, 2019; Agrios, 2005; Alhussaen *et al.*, 2011; Sherf and MacNab, 1986), where the organism kills newly emerged seedlings (Redekar *et al.*, 2019; Jarvis and Jarvis, 1992). *Pythium* species are rarely host specific (Rangaswamy, 1989).

In the present study, incidence and severity of *Pythium* damping off on chili was observed. Incidence and severity of *Pythium* damping off on chili was the maximum in Rawalpindi (16% and 9%) and Attock (19% and11%) and the minimum in Chakwal (10% and 5%) and Jhelum (10% and 5%). This high incidence of *Pythium* in Rawalpindi and Attock can be attributed to different factors. *Pythium* is a soil borne pathogen. Its spores can survive long, harsh periods as resting spores and also have the ability to travel through water. Rawalpindi and Attock have a relatively high humid climate as compared to Chakwal and Jhelum which usually have low rainfall. High moisture contents in the soil support the motility of zoospores and increase the size of the spermosphere (Martin and Loper, 1999; Martin, 2000). It has been observed that high moisture contents of soil results in increased disease incidence and severity of *Pythium* damping-off on chili crop (Muthukumar *et al.*, 2009; Hyder *et al.*, 2018a; Hyder *et al.*, 2018b; Majeed *et al.*, 2019). Greenhouse condition of high moisture and high temperatures provides thriving conditions for *Pythium* (Kerkeni *et al.*, 2007; Manjunath *et al.*, 2010; Arora *et al.*, 2021). It is well documented that *Pythium* colonization of chili and tomato plants was observed to be affected by temperature.

Although chili and tomato plants can survive at high temperatures but when plants are infected by *Pythium* spp. growth of chili is reduced at 28 °C while at 34 °C plant dies (Chellemi *et al.*, 2000). Farmers in Rawalpindi and Attock areas also grow other solanaceous crops e.g. potato and tomato. As it is known that *Pythium* has a wide host range and are less host specific and continuous availability of other hosts increases their survival in soil (Rangaswamy, 1989). Furthermore, the farmers are incognizant about the mechanism of spread of this disease and preventive measures are not taken. Due to this main reason, the spread of the disease is increasing in the Pothwar region.

CONCLUSION

It is concluded from the present studies that Pythium damping off is fairly distributed in the Phothwar region of the Punjab province of Pakistan and warrant stringent control strategies for its management. Further, the findings of these studies will be helpful for the farmers in designing control strategies for this pathogen accordingly. The information will also be useful for the breeders to develop resistant varieties of chili against the most virulent isolates of the pathogen.

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CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

AUTHORS CONTRIBUTIONS

SS and TM designed the study, conducted surveys and performed the experiments, SS collected the data, MIH and SIM provided technical assistance, TM supervised the work, and SS and TM wrote and proofread the manuscript.