Disease Incidence and Severity of *Pythium* spp. and *Phytophthora* spp. Affecting Chili Pepper and Tomato Crops in Punjab, Pakistan

Sajjad Hyder¹, Saadia Naseem^{2,*}, Sohaib Azhar³, M. Ashfaq¹, Zahid Ali², Azeem Khalid⁴ and M. Inam-ul-Haq¹

Devastative crop losses in Pakistan are commonly due to infections by oomycete organisms, *Pythium* spp and *Phytophthora* spp., at pre- or post-emergence growth stages. Significant research work has not been conducted to control these pathogens and there are few data reports on disease incidence and severity in Pakistan. An extensive survey was conducted over two consecutive years in nine major vegetable-growing districts of Punjab, Pakistan. Data indicated that incidence of *Pythium* damping off in chili fields was 45.4% in Okara, followed by 41.6% in Sialkot and 37.67% in Gujranwala. The lowest disease incidence (DI) (13.8%) was observed in Jhelum district. Maximum mean disease severity (DS) (3.48%) was recorded in Sialkot while minimum severity (2.16%) was recorded in Chakwal. In tomato fields, *Pythium* damping off ranged from 5.9% to 40.13% and severity was as high as 3.53 in Okara. Late blight infection due to *Phytophthora* spp. ranged between 9.68% and 66.25% in all surveyed districts. Maximum mean disease severity on the disease rating scale (DRS) was recorded from Sialkot (4.13%) and from Okara (3.83%) and the minimum mean disease severity (1.04%) was recorded from Chakwal.

Key Words: chili pepper, Phythium, Phytophthora, tomato, plant disease

Abbreviations: DI – disease incidence, DRS – disease rating scale, DS – disease severity, LAI – leaf area infection, MDI – mean disease incidence, MDS – mean disease severity

INTRODUCTION

Diseases reduce growth of crop plants by interfering with normal growth patterns. Pakistan is located between 24° and 36° North and from 61° to 76° East. It has a subtropical climate in the warm temperate zone that provides good vegetable production. However, the climate also favors the incidence of numerous plant pathogens. Irrigated plane zones in Punjab province provide greater support for the rapid perpetuation of fungal-like agents Pythium spp. and Phytophthora spp. Chili pepper (Capsicum annuum) belongs to the family Solanaceae and serves as a valuable vegetable and a spice crop (Khan et al. 2006). It occupies 20% of the total vegetable production area. The major producers in the country are Punjab and Sindh provinces, while relatively small-scale production occurs in Khyber Pakhtunkhwa (KP) and Balochistan provinces (Iqbal et al. 2012).

Tomato (Solanum lycopersicum) also belongs to the

Solanaceae family. It is grown worldwide and ranked third in terms of global vegetable production (Javaria et al. 2012). The tomato fruit serves as a rich source of vitamins A and C, minerals, dietary fiber and protein. Its fruit can be eaten raw as fruit or used in salad, or processed as juice and cooked as paste and sauces (Dairo and Akintunde 2012). The total area under tomato cultivation is ~ 52 thousand hectares with an average country yield of ~ 530 thousand tons (Anonymous 2012).

Chili and tomato crops are affected by a number of abiotic and biotic yield-limiting factors, with plant diseases being of prime importance. Chili can be infected by 83 different diseases including more than 40 fungal pathogens. Estimated yield losses in tomato due to pathogens range from 15% to 95 % (Kuku et al. 1996, unpublished). Some of the major diseases of tomato include bacterial wilt caused by *Ralstonia solanacearum*, bacterial canker by *Xanthomonas vasicaforia*, tomato leaf curl virus disease, fusarium wilt by *Fusarium*

¹Department of Plant Pathology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan

²Department of Biosciences, COMSATS University Islamabad (CUI) Park road, Chak shahzad, Islamabad, Pakistan

³Department of Geo-Informatics, PMAS Arid Agriculture University, Rawalpindi, Pakistan

⁴Department of Environmental Sciences, PMAS Arid Agriculture University, Rawalpindi, Pakistan

^{*}Author for correspondence; e-mail: saadia.naseem@comsats.edu.pk; Phone: +92 51 9049 6122

oxysporium, early blight by *Alternaria solani* and damping-off disease by different organisms such as *Pythium* spp. *Phytophthora* spp. and *Botrytis* sp. (Dairo and Akintunde 2012).

Damping off caused by *Pythium* spp. resulted in 90% plant death either as pre- or post-emergence in nurseries and under field conditions (Zagade et al. 2012). Collar rot by *Phytophthora* spp. is of great importance in chili (Hussain et al. 2013). Small-scale studies have been done on damping-off disease in Sindh province (Shahzad and Ghaffar 1993; Mushtaq and Hashmi 1997; Abdul Haq and Shahzad 1998; Inam-ul-Haq et al. 2012) but no reports appear in the literature on disease incidence (DI) and disease severity (DS) in Punjab province of Pakistan regarding *Pythium* and *Phytophthora* spp. infections. However, a report on the occurrence of *Phytophthora* root rot of peppers from Malakand division (KP province) is available (Din et al. 2012).

In the present study, a comprehensive two-year field survey was conducted in major chili and tomato-growing areas of Punjab Province, Pakistan at the district level and randomly selected subdivisions/locations level to estimate the DI and DS due to *Pythium* and *Phytophthora* spp. The information on disease incidence and severity caused by *Pythium* spp. and *Phytophthora* spp. in major vegetable-growing areas in rainfed and irrigated zones in Pakistan may help investigators to devise management strategies to cope with the major production constraints (*Pythium* and *Phytophthora* spp.) in tomato and chili crops.

MATERIALS AND METHODS

Disease Incidence and Severity of *Pythium* and *Phytophthora* spp. on Chili Pepper and Tomato

A comprehensive two-year survey (2014–15 and 2015–16) was conducted in major chili pepper and tomato-growing fields in 45 randomly selected villages in nine districts (Rawalpindi, Chakwal, Attock, Jhelum, Faisalabad, Okara, Gujranwala and Gujrat) of Punjab in Pakistan (Table 1). DI and DS of Pythium spp. and Phytophthora spp. infection on seedling and mature crop stages were evaluated in 214 fields. From each field, 30 seedlings carefully "Z" excavated in (Gnanamanickam et al. 1999) to record the infection on roots at seedling stage and leaf area infection (LAI) due to Phytophthora at late stages. The DS of Pythium spp. infection was recorded by using a 1-4 assessment scale (Lee and Hoy 1992) while disease severity due to Phytophthora spp. infection was recorded (Tables 2 and 3) according to a 1-6 assessment scale (Hartman and Huang 1995). The incidence of disease due to each pathogen was determined by a formula given as follows:

DI (%) =
$$\frac{\text{No. of infected plants}}{\text{Total No. of observed plants}} \times 100$$

The average DI recorded from each field was taken to determine the DI at the village level. Similarly, the average of each village data was taken to calculate the mean DI at the district level. The mean values obtained at the district level were compiled together to determine the overall disease scenario in the Punjab province of Pakistan.

Isolation of Pythium spp. and Phytophthora spp.

For the isolation of *Pythium* spp. and *Phytophthora* spp. from collected samples, serial dilutions (10^{-1} to 10^{-9}) were made in distilled water with the addition of 2% Tween 80 and dilutions were spread on corn meal agar - pimaricin + ampicillin + rifampicin + penta-chloronitrobenzene (CMA-PARP modified media), followed by incubation at 25 °C ± 2 for culture growth.

Purification and Preservation of Fungal Isolates

After purification by hyphal tip technique on selective CMA-PARP media and plates, the fungal isolates were incubated at 25 °C \pm 2. The isolates were cultured three to four times to get pure fungal cultures. Following purification, all the isolates were preserved by taking fungal plugs in preservation tubes containing distilled water and were stored at -20 °C.

Virulence Confirmation for the Selection of Most Pathogenic Isolates of *Phytophthora* spp. and *Pythium* spp.

Pathogenicity tests were carried out on chili (*Capsicum annuum*) and tomato plants (*Solanum lycopersicum*). The fungal isolates of *Pythium* spp. and *Phytophthora* spp. were cultured on modified CMA media at 25 °C for 7 d followed by incubation under fluorescent light for 3 d to induce sporangial formation. One day before inoculation, the colonies were covered with 20 mL of tap water, and incubated under light overnight. On the day of inoculation, petri dishes were placed for 30 min at 4 °C, followed by 60 min at room temperature to increase zoospore.

Statistical Analysis

The average mean DI and DS values from each visited field were accessed to understand the overall infection at a particular village. The averages of data obtained from each village were calculated to determine DI and DS on the district level. Standard deviation (SD) and standard error (SE) values were calculated. Error bars were applied with each value representing the DI and DS in each village in a particular district. With the disease

Table 1. Areas visited for the estimation of disease incidence and disease severity due to *Phytophthora* and *Pythium* spp. in chili and tomato crops.

District District	Coordinates	Location	Total No. of Fields Visited
Rawalpindi	(33.3333° N, 73.2500° E)	Adyala Jhamra, Kasala, Gorakhpur, Dhok Bawa Adyala, ZTBL Farms, NARC	32
Chakwal	(33.6772° N, 72.8558° E)	BARI Chakwal, Thoa Bhadar, Dhok Talian, Murid, Walana	20
Attock	(33.7652° N, 72.3571° E)	Hazro-Gondal Road, Hattian, Burhan and Hassan Abdal	20
Jhelum	(32.9333° N, 73.7333° E)	Kala Gujjran, Mehmoodabad, Phaliyaan and Haji Ayama	20
Faisalabad	(31.4181° N, 73.0776° E)	34 Chak Amin Pur Bangla Road, 32 Chak, Shahbazpur, JK Farms Jhumra, 72 Chak Jhang Road, 61 Chak Jhang Road, Chiniot Road	40
Okara	(30.8014° N, 73.4483° E)	Zafar Colony, Okara bypass, 35/2R and 38/2R	20
Gujranwala	(32.1667° N, 73.8333° E)	Francesabad, Kohlu Wala, Marali Wala, Tatli Wala, Ghuman Wala and Khyali bypass	20
Gujrat	(32.5833° N, 73.7500° E)	Tibi Sakhooan, Soak Kalaan, Chakkala and Hinjrah	16
Sialkot	(32.5200° N, 74.5500° E)	Thoda-Pasrur, kay-Pasrur, Dhilam-Pasrur, Guuna Kalan-Pasrur and Mallokpur-pasrur	26

Table 2. Disease rating scale (DRS) for the estimation of disease severity (DS) due to *Phytophthora* spp.

No. Value	Plant Response Leaf Area Infection (LAI)
1	1-10%
2	11-20%
3	21-40%
4	41-60%
5	61-80%
6	81-100%

distribution incidence and severity data generated, maps were produced by ESRI® ArcGIS 10.2 software for desktop with World Geodetic Survey 1984 (WGS-1984) projection system.

RESULTS

Fungal Isolates

The isolated and purified cultures of *Pythium* spp. and *Phytophthora* spp. from the infected plants are shown in Fig. 1.

Table 3. Disease rating scale for the estimation of disease severity due to *Pythium* spp.

No. Value	Response
1	Normal health appearance of
I	roots
2	Slightly infected and with discol-
2	oration
3	Moderately infected and with
S	discoloration
4	Severe symptoms

Incidence of *Pythium* spp. and *Phytophthora* spp. on Chili and Tomato Seedlings

Data were recorded from chili-growing fields of different districts in Punjab province, which showed variations in disease occurrence level within the villages and districts. In Rawalpindi, DI ranged between 10.0% and 28.6% while the overall 17.7% DI was recorded at the district level. From chili fields, 45.4% DI was recorded from Okara followed by 41.6% in Sialkot and 37.67% from Gujranwala (Table 4). The DI level ranged from 11.1% to 17.2% in Chakwal, 11.1% to 17.5 % in Attock, and 8.9% to 20.6% from Jhelum. In Sialkot, the maximum recorded DI was 48.5% from Kay-Pasur, while it was 40.8% from Thoda-Pasur and 40.4% from Mallokabad. In Faisalabad, the maximum DI (41.9%) was from 34-Chak, while the minimum average value (12.8%) was from JK Farms. From Okara district, incidence averaged between 37.2%

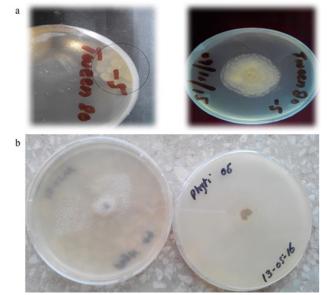


Fig. 1. Isolated and purified culture of *Phytophthora* spp. with Tween 80 (a); isolated and purified culture of *Phythium* spp. (b).

and 54.4% while 21.1% to 53.3% was from Gujranwala. In Gujrat, the incidence ranged between 5.6% and 24.4% (Table 4). The DS level and DI are graphically demonstrated in Fig. 2.

Variation in DI of Pythium and Phytophthora spp. infection of tomato seedlings was also recorded from the surveyed districts of Punjab province (Rawalpindi, Chakwal, Attock, Jehlum, Sialkot, Faisalabad, Okara, Gujranwala and Gujrat). The highest mean DI (40.13%) was recorded from Sialkot district, followed by 39.15% from Okara and 31.32% from Gujranwala district (Table 5). The difference in DI was also observed within the districts. In Rawalpindi district, the maximum incidence (24.4%) was recorded from Dhok Bawa and the minimum incidence was observed in the National Agriculture Research Center (NARC) field areas. From Chakwal district, DI ranged between 2.2% and 8.95%. From Attock district, mean DI varied between 12.2% and 17.8%. Similarly, it ranged between 8.9% and 18.9% in the fields of Jhelum districts.

From canal-irrigated areas in Sialkot district, the maximum DI (55.0%) was recorded from Thoda-Pasur, followed by 40.0% from Mallokabad, while from Faisalabad district, it ranged between 17.2% and 40.8%. Minimum DI (17.2%) was recorded from JK Farms and 22.2% from 34 Chak, Faisalabad. In Okara district, DI ranged between 20.0% and 61.1%, while from Gujranwala district, the maximum DI (42.2%) was recorded in Marali Wala, and the minimum (37.8%) in Tatli Wala. In Gujrat district, DI ranged between 13.3% and 28.9% (Table 5 and Fig. 3).

The DI due to Phytophthora spp. infection of tomato

crop was also recorded, and variation in incidences was recorded at the district level and within the district (Table 6). From the surveyed districts, maximum incidence (66.25%) was recorded from Okara, followed by 55.63% from Sialkot and 34.53% from Gujranwala district, while the minimum incidence was found (9.68%, 15.68%) in Chakwal and Attock districts, respectively. The variations in the incidences within districts data indicated that in Sialkot district, incidence ranged between 53.3% and 57.5% and in Faisalabad, it ranged between 22.5% and 50.0%. From Okara district, the maximum incidence (80.0%) was recorded from 38/2R, while the minimum incidence was observed (51.1%) from Zafar colony. In Gujranwala district, the maximum incidence (40.6%) was from Francesabad, followed by 40.0% in Tatli Wala and 38.9% in Ghuman Wala, while the minimum incidence (24.4%) was from Marali Wala, and 30.0% from the fields near Khyali bypass, Gujranwala (Table 6 and Fig. 4).

Severity of *Pythium* spp. and *Phytophthora* spp. on Chili and Tomato Seedlings

The maximum mean disease severity (DS) was recorded from Sialkot district (3.48), followed by Gujranwala (3.47), Faisalabad (3.39) and Okara (3.38) while low severity was observed in Chakwal (2.16) and Rawalpindi (2.3). Variation was also found in the villages of the respective districts. In Sialkot district, the maximum disease incidence (3.8) was recorded from Mallokabad, followed by 3.6 in Thoda-Pasur. In Faisalabad, disease incidence ranged between 2.6 and 3.9, almost similar (2.7 and 3.8) to that in Okara district. In Gujranwala districts, the minimum DS ranged between 3.0 and 3.3 in Kohlu Wala, Khyali Bypass and Marali Wala, while in Gujrat district, it ranged between 1.7 and 3.3 (Table 4).

In tomato fields, the maximum DS was recorded from Sialkot (3.53), followed by Gujrat (3.44), Faisalabad (3.43) and Okara (3.38), while the minimum DS was recorded (1.70) from Chakwal and (2.18) from Jehlum. Variation in DS was also observed in district villages, as severity ranged between 3.0 and 3.7 in Faisalabad district, followed by 3.0 and 3.8 in Okara (Table 5.). In Gujranwala district, the maximum severity (3.7) was from Kohlu Wala, and the minimum (2.7) was recorded from Khyali bypass. In Gujrat district, the minimum severity (3.0) was from Soak Kalan and from Purba (3.3) as shown in Table

DISCUSSION

The GIS mapping of disease incidence and occurrence is a promising approach, which combines disease reports to indicate host distribution in the country (Naseem et al.

Table 4. Mean disease incidence (MDI) and mean disease severity (MDS) due to *Pythium* damping off in chili crop in the districts of Puniab Province, Pakistan.

District	Area/Village	MDI	MDS
	Adiyala Jhamra	12.6 ± 0.83	2.3 ± 0.17
	Kasala	28.6 ± 0.42	2.5 ± 0.15
	Gorakhpur	17.2 ± 0.49	2.2 ± 0.17
Rawalpindi	Dhok Bawa	25.6 ± 0.88	3.0 ± 0.00
	ZTBL Farms	10.0 ± 2.43	1.8 ± 0.17
	NARC	12.2 ± 0.56	2.0 ± 0.00
	Average	17.7 ± 3.15	2.3 ± 0.17
	BARI Chakwal	11.1 ± 0.33	2.7 ± 0.33
	Thoa Bhadur	13.3 ± 1.00	1.7 ± 0.67
O	Dhok Talian	16.1 ± 0.48	2.2 ± 0.31
Chakwal	Murid	17.2 ± 0.87	2.2 ±0.17
	Walana	13.0 ± 0.54	2.0 ± 0.29
	Average	14.1 ± 1.11	2.16 ± 0.16
	Hazro-Gondal Road	17.5 ± 0.46	2.2 ±0.27
	Hattian	12.6 ± 0.49	1.8 ± 0.15
Attock	Burhan	14.4 ± 1.33	2.7 ± 0.33
	Hassanabdal	11.1 ± 0.67	2.8 ± 0.17
	Average	13.9 ± 1.38	2.38 ± 0.23
	Kala Gujjran	11.1 ± 0.67	2.3 ± 0.33
	Mehmoodabad	14.4 ± 1.45	2.3 ± 0.67
Jhelum	Phaliyaan	8.9 ± 0.33	3.0 ± 0.00
	Hiji Ayama	20.6 ± 0.75	2.7 ± 0.33
	Average	13.8 ± 2.55	2.58 ± 0.17
	Thoda-Pasur	40.8 ± 0.69	3.6 ± 0.15
	Kay-Pasur	48.5 ± 0.67	3.3 ± 0.24
Sialkot	Gunna Kalan	36.7 ± 1.13	3.2 ± 0.17
	Mallokpur	40.4 ± 1.14	3.8 ± 0.15
	Average	41.6 ± 2.48	3.48 ± 0.14
	34 Chak	41.9 ± 0.77	3.8 ± 0.15
	32 Chak	18.9 ± 0.57	3.4 ± 0.15
	Shahbazpur	38.1 ± 0.98	3.9 ± 0.08
	JK Farms	12.8 ± 0.31	2.8 ± 0.17
Faisalabad	72 Chak	36.1 ± 1.51	3.5 ± 0.22
	61 Chak	30.6 ± 0.84	3.7 ± 0.14
	Chiniot Road	21.5 ± 0.63	2.6 ± 0.29
	Average	28.56 ± 4.14	3.39 ± 0.19
	Zafar Colony	43.3 ± 1.53	3.7 ± 0.33
	35/2R	54.4 ± 1.76	2.7 ± 0.33
Okara	38/2R	46.7 ± 2.00	3.3 ± 0.67
Chara	Okara Bypass	37.2 ± 1.28	3.8 ± 0.17
	Average	45.4 ± 3.59	3.38 ± 0.25

Continue on next page...

Continuation of Table 4

District	Area/Village	MDI	MDS
	Francesabad	49.4 ± 0.91	3.5 ± 0.22
	Kohlu Wala	24.4 ± 1.20	3.0 ± 1.00
	Marali Wala	45.6 ± 1.76	3.3 ± 0.67
Gujranwala	Tatli Wala	53.3 ± 1.15	3.7 ± 0.33
	Ghuman Wala	32.2 ± 0.67	4.0 ± 0.00
	Khyali Bypass	21.1 ± 1.20	3.3 ± 0.33
	Average	37.67 ± 5.55	3.47 ± 0.14
	Tibi Sakhoan	24.4 ± 0.67	2.7 ± 0.42
	Chakkala	15.6 ± 0.67	3.3 ± 0.67
Gujrat	Soak Kalan	18.9 ± 0.88	1.7 ± 0.33
Gujiat	Purba	5.6 ± 0.88	3.0 ± 0.58
	Hinjrah	21.1± 0.67	1.7 ± 0.33
	Average	17.12 ± 3.22	2.48 ± 0.33

Disease Incidence & Severity due to Pythium Damping off in Chili Crop in various Districts of Punjab Province, Pakistan

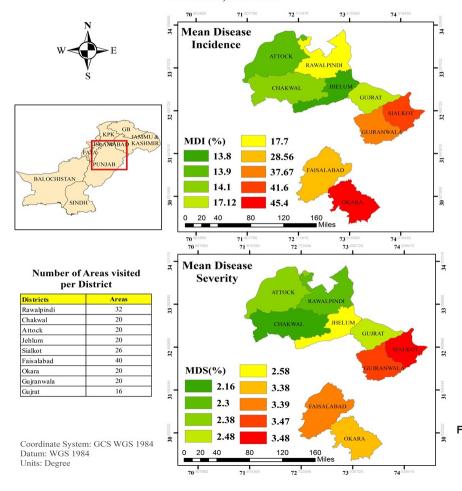


Fig. 2. Mean disease incidence and mean disease severity due to *Pythium* damping off in chili crop in various districts of Punjab Province, Pakistan.

Table 5. Mean disease incidence (MDI) and mean disease severity (MDS) due to *Pythium* damping off in tomato

District	Area/Village	MDI	MDS
	Adiyala Jhamra	9.6 ± 0.42	2.1 ± 0.20
	Kasala	11.4 ± 0.34	2.3 ± 0.14
	Gorakhpur	9.2 ± 0.39	2.4 ± 0.15
Rawalpindi	Dhok Bawa	24.4 ± 0.88	2.7 ± 0.33
	ZTBL Farms	8.3 ± 2.06	2.3 ± 0.21
	NARC	6.7 ± 0.37	1.5 ± 0.22
	Average	11.60 ± 2.64	2.22 ± 0.16
	BARI Chakwal	2.2 ± 0.67	1.3 ± 0.33
	Thoa Bhadur	5.6 ± 0.88	1.7 ± 0.33
0	Dhok Talian	8.9 ± 0.56	2.5 ± 0.22
Chakwal	Murid	6.1 ± 0.48	1.7 ± 0.21
	Walana	6.7 ± 0.37	1.3 ± 0.17
	Average	5.90 ± 1.08	1.70 ± 0.22
	Hazro-Gondal Road	12.2 ± 0.53	2.3 ± 0.14
	Hattian	17.8 ± 0.55	2.6 ± 0.18
Attock	Burhan	14.4 ± 1.86	2.7 ± 0.33
	Hassanabdal	15.0 ± 0.76	2.3 ± 0.21
	Average	14.85 ± 1.15	2.48 ± 0.10
	Kala Gujjran	8.9 ± 0.33	2.3 ± 0.33
	Mehmoodabad	18.9 ± 0.88	3.0 ± 0.00
Jhelum	Phaliyaan	11.1 ± 0.67	1.7 ± 0.33
	Hiji Ayama	12.8 ± 0.60	1.7 ± 0.21
	Average	12.93 ± 2.15	2.18 ± 0.31
	Thoda-Pasur	55.0 ± 0.82	3.5 ± 0.15
	Kay-Pasur	36.6 ± 0.87	3.9 ± 0.11
Sialkot	Gunna Kalan	28.9 ± 0.76	a3.3 ± 0.21
	Mallokpur	40.0 ± 0.85	3.4 ± 0.18
	Average	40.13 ± 5.48	3.53 ± 0.13
	34 Chak	22.2 ± 0.71	3.4 ± 0.18
	32 Chak	30.0 ± 0.83	3.5 ± 0.15
	Shahbazpur	40.8 ± 0.54	3.7 ± 0.14
Caicalahad	JK Farms	17.2 ± 0.70	3.5 ± 0.22
Faisalabad	72 Chak	25.5 ± 0.95	3.3 ± 0.33
	61 Chak	31.4 ± 0.58	3.0 ± 0.17
	Chiniot Road	24.4 ± 0.58	3.6 ± 0.18
	Average	27.36 ± 2.87	3.43 ± 0.09
	Zafar Colony	32.2 ± 1.76	3.7 ± 0.33
	35/2R	20.0 ± 1.15	3.0 ± 0.00
Okara	38/2R	43.3 ± 1.53	3.0 ±0.00
	Okara Bypass	61.1 ± 1.23	3.8 ± 0.17
	Average	39.15 ± 8.73	3.38 ± 0.22

Continue on next page...

Continuation of Table 5

District	Area/Village	MDI	MDS
	Francesabad	36.7 ± 0.89	3.5 ± 0.22
	Kohlu Wala	32.3 ± 1.76	3.7 ± 0.33
	Marali Wala	42.2 ± 1.20	3.0 ± 0.00
Gujranwala	Tatli Wala	37.8 ± 1.20	3.3 ± 0.67
	Ghuman Wala	21.1 ± 0.67	3.7 ± 0.33
	Khyali Bypass	17.8 ± 0.88	2.7 ± 0.33
	Average	31.32 ± 3.99	3.32 ± 0.16
	Tibi Sakhoan	26.7 ± 0.77	3.5 ± 0.22
	Chakkala	16.7 ± 1.15	3.7 ± 0.33
Gujrat	Soak Kalan	13.3 ± 1.15	3.0 ± 0.00
Gujiat	Purba	14.4 ± 0.33	3.3 ± 0.67
	Hinjrah	28.9 ± 0.88	3.7 ± 0.33
	Average	20.0 ± 3.25	3.44 ± 0.13

Disease Incidence & Severity due to Pythium Damping off in Tomato Crop in various Districts of Punjab Province, Pakistan

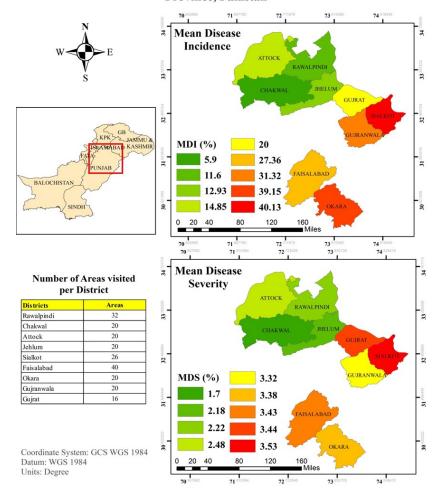


Fig. 3. Mean disease severity and mean disease incidence due to *Pythium* damping off in tomato crop in various districts of Punjab Province, Pakistan.

Table 6. Mean disease incidence (MDI) and mean disease severity (MDS) due to *Phytophthora* spp. in tomato crop production districts of Punjab Province, Pakistan.

District	Area/Village	MDI	MDS
	Adiyala Jhamra	36.7 ± 0.88	2.7 ± 0.17
	Kasala	32.5 ± 0.75	3.8 ± 0.13
	Gorakhpur	37.5 ± 1.18	2.8 ± 0.13
Rawalpindi	Dhok Bawa	30.0 ± 1.15	4.0 ± 0.00
	ZTBL Farms	30.0 ± 4.47	2.5 ± 0.22
	NARC	15.0 ± 0.67	1.5 ± 0.22
	Average	30.28 ± 3.33	2.88 ± 0.37
	BARI Chakwal	0.00 ± 0.00	0.0 ± 0.00
	Thoa Bhadur	0.00 ± 0.00	0.0 ± 0.00
Chaloual	Dhok Talian	25.0 ± 0.67	1.8 ± 0.17
Chakwal	Murid	16.7 ± 0.58	1.5 ± 0.22
	Walana	6.70 ± 1.00	1.9 ± 0.39
	Average	9.68 ± 4.90	1.04 ± 0.43
	Hazro-Gondal Road	14.4 ± 0.76	2.3 ± 0.33
	Hattian	23.3 ± 1.32	3.0 ± 0.29
Attock	Burhan	10.0 ± 0.58	2.0 ± 0.00
	Hassanabdal	15.0 ± 0.67	1.5 ± 0.22
	Average	15.68 ± 2.78	2.20 ± 0.31
	Kala Gujjran	20.0 ± 0.58	2.0 ± 0.00
	Mehmoodabad	12.2 ± 0.88	3.0 ± 0.00
Jhelum	Phaliyaan	17.8 ± 0.33	2.0 ± 0.00
	Hiji Ayama	20.0 ± 1.34	1.5 ± 0.22
	Average	17.50 ± 1.84	2.13 ± 0.31
	Thoda-Pasur	57.5 ± 0.99	4.3 ± 0.13
	Kay-Pasur	53.3 ± 0.50	4.0 ± 0.29
Sialkot	Gunna Kalan	55.0 ± 2.01	4.5 ± 0.22
	Mallokpur	56.7 ± 0.50	3.7 ± 0.44
	Average	55.63 ± 0.93	4.13 ± 0.18
	34 Chak	36.7 ± 0.82	3.3 ± 0.17
	32 Chak	22.5 ± 0.41	2.0 ± 0.21
	Shahbazpur	32.5 ± 0.99	2.8 ± 0.25
Caisalahad	JK Farms	50.0 ± 1.34	4.5 ± 0.22
Faisalabad	72 Chak	40.0 ± 2.68	4.0 ± 0.00
	61 Chak	27.5 ± 0.75	2.5 ± 0.26
	Chiniot Road	23.3 ± 0.50	1.7 ± 0.17
	Average	33.21 ± 3.74	2.97 ± 0.39
	Zafar Colony	51.1 ± 1.20	3.0 ± 0.00
	35/2R	58.9 ± 1.76	3.3 ± 0.33
Okara	38/2R	80.0 ± 0.58	5.0 ± 0.00
	Okara Bypass	75.0 ± 0.67	4.0 ± 0.45
	Average	66.25 ± 6.76	3.83 ± 0.44

Continue on next page...

Continuation of Table 6

District	Area/Village	MDI	MDS
	Francesabad	40.6 ± 1.28	2.8 ± 0.17
	Kohlu Wala	33.3 ± 1.15	3.7 ± 0.33
	Marali Wala	24.4 ± 0.67	3.0 ± 0.00
Gujranwala	Tatli Wala	40.0 ± 1.15	2.0 ±0.00
	Ghuman Wala	38.9 ± 1.20	3.7 ± 0.33
	Khyali Bypass	30.0 ± 1.15	3.0 ± 0.00
	Average	34.53 ± 2.65	3.03 ± 0.26
	Average Tibi Sakhoan	34.53 ± 2.65 31.1 ± 1.23	3.03 ± 0.26 2.8 ± 0.17
Guirat	Tibi Sakhoan	31.1 ± 1.23	2.8 ± 0.17
Gujrat	Tibi Sakhoan Chakkala	31.1 ± 1.23 22.2 ± 0.33	2.8 ± 0.17 2.7 ± 0.33
Gujrat	Tibi Sakhoan Chakkala Soak Kalan	31.1 ± 1.23 22.2 ± 0.33 11.1 ± 0.88	2.8 ± 0.17 2.7 ± 0.33 2.0 ± 0.00

Disease Incidence & Severity due to Phytophthora spp. in Tomato Crop in various Districts of Punjab Province,

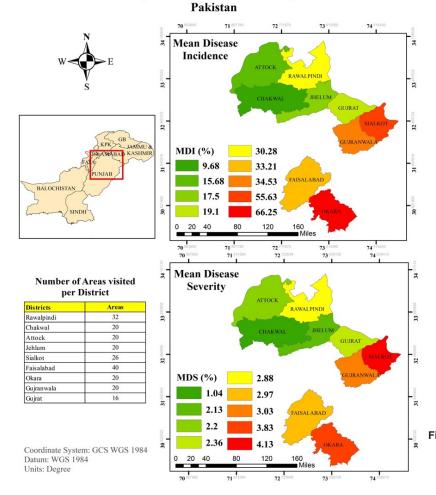


Fig. 4. Mean disease incidence and mean disease severity due to *Phytophthora* spp. in tomato crop in various districts of Punjab Province, Pakistan.

2016). In this study, a field survey in most important chiliand tomato-growing areas of nine major districts of Punjab province in Pakistan was conducted to estimate the DI and DS due to *Pythium* spp. and *Phytophthora* spp. The recorded data on selected parameters reflect variations in DI at the district level, thus variation was also observed at the subgroup level.

The genus Pythium belongs to class Oomycetes consisting of over 120 different described species that occupy terrestrial and aquatic habitats (van der Plaats-Niterink 1981; Dick 1990). Pythium spp. can survive in the soil as a saprophyte, on plant parts and can also attack young succulent plant parts (Hendrix and Campbell 1973; van der Plaats-Niterink 1981; Krober 1985). Phytophthora spp. also belongs to the same family and consists of about 60 species in the genus, which attack a wide variety of agricultural important crops around the globe. Phytophthora cinnamomi is a single species, which can attack about 1000 host plant species (Erwin and Ribeiro 1996). Numerous studies on Pythium spp. have been carried out in various parts of the world but less work has been done so far on the occurrence of Pythium spp. in Pakistan (Malik and Khan 1944; Ahmed 1956; Mira and Qureshi 1978; Shahzad and Ghaffar 1993; Abdul-Haq and Shahzad 1998; Lodhi et al. 2004).

There is no report or data available regarding *Pythium* and *Phytophthora* spp. disease incidence and severity from Punjab province-Pakistan (Lodhi et al. 2004). However, there are reports on occurrence and distribution of *Pythium aphanidermatum* in the Sindh agro-ecosystem as well as isolates that have been obtained in high frequency from different sources and locations. Susceptibility of the chili crop to the various diseases caused by *Phytophthora* was reported from Lower Sindh, Pakistan (Ahmed and Iqbal 1989; Saleem et al. 1996; 1998; Hussain et al. 1990; Hussain et al. 2013). No report is available on incidences and severity.

Our results indicated that in terms of maximum disease incidences, 45.4% was observed from chiligrowing fields at the seedling stage in Okara district, followed by 41.6% in Sialkot districts and 37.67% from Gujranwala district while from tomato fields, maximum (40.13%) disease incidence was observed at the seedling stage of tomato crop. *Pythium* spp. resulted in 90% plant death either at pre- or post-emergence stages (Sowmini 1961). In a study conducted on the occurrence of Phytophthora root rot on pepper crop in Peshawar and Malakand divisions in KP province-Pakistan, high catastrophic disease distribution to complete crop destruction has been reported (Din et al. 2012). The current research also reflects the same picture of the disease with high DI and DS level. We observed variation

in DI and DS level, which indicated that the variation in DS may be due to numerous factors, e.g. field location, adopted management strategies, irrigation, used cultivar and level of inoculum. Our results also indicated that maximum DI and DS was recorded from the plane areas of Punjab province where a canal irrigation system is available with frequent supply of water and extensive cultivation is practiced.

ACKNOWLEDGMENTS

This research was supported by the Punjab Agriculture Research Board (PARB) through research grant No. 437. We also thank our universities for providing space and facilities for conducting the research.

REFERENCES CITED

- ABDUL HAQ M, SHAHZAD S. 1998. Oomycetes from soil of Bajour Agency, FATA, Pakistan. Pak J Bot 30: 305–306.
- AHMAD S. 1956. Fungi of Pakistan: Monograph 1. Biological Society of Pakistan, Lahore, Pakistan. 126 p.
- AHMED SM, IQBAL A. 1989. Root and collar rot of chillies caused by *Phytophthora capsici* (Van Breeda As Haan) waterhouse. A new record for Pakistan. J Agr Res 27: 155–156
- ANONYMOUS. 2012. Food and Agriculture Organization. Rome, Italy.
- DAIRO KP, AKINTUNDE JK. 2012. Evaluation of plant growth-promoting rhizobacteria for the control of bacterial wilt disease of tomato. Global J Biosci Biotechnol 1: 253–256.
- DICK MW. 1990. Keys to *Pythium*. Reading, UK: University of Reading, School of Plant Science, Department of Botany. 64 p.
- DIN UI, RAZIQ F, HUSSAIN S, SAIFULLAH. 2012. Occurrence of phytophthora root rot (*Phytophthora capsici* Leonian) of pepper in Peshawar and Malakand divisions. Sarhad J Agric 28: 443–450.
- ERWIN DC, RIBEIRO OK. 1996. Phytophthora diseases worldwide. St. Paul, Minnesota: APS Press. 592 p.
- GNANAMANICKAM SS, PRYIYADARASANI V, NARAYANAN NN, VASUDEVAN P, KAVITHA S. 1999. An overview of bacterial blight disease of rice and strategies for management. Curr Sci 77: 1435–1444.
- HARTMAN GL, HUANG YH. 1995. Characteristics of *Phytophthora infestans* isolates and development of late blight on tomato in Taiwan. Plant Dis 79: 849–852.
- HENDRIX FF JR, CAMPBELL WA. 1973. *Pythium* as plant pathogens. Ann Rev Phytopathol 11: 77–98.

- HUSSAIN A, AHMED MN, AKHTER AS. 1990. Protect chillies crop from Phytophthora, a new disease in Pakistan. Capsicum Newsletter 59: 8–9.
- HUSSAIN F, SHAUKAT SS, ABID M, USMAN F, AKBAR M. 2013. Filamentous fungi infecting fruits and leaves of *Capsicum annuum* L. in lower Sindh. Int J Biol Biotech 10: 101–108
- INAM-UL-HAQ M, MEHMOOD S, REHMAN HM, ALI Z, TAHIR MI. 2012. Incidence of root rot diseases of soybean in Multan Pakistan and its management by the use of plant growth promoting rhizobacteria. Pak J Bot 44: 2077–2080.
- IQBAL S, ASHFAQ M, SHAH H, INAM-UL-HAQ M, AZIZ-UD-DIN. 2012. Prevalence and distribution of *Cucumber mosaic* virus (CMV) in major chili growing areas of Pakistan. Pak J Bot 44: 1749–1754.
- JAVARIA S, KHAN MQ, HABIB UR RAHMAN, BAKHSH I. 2012. Response of tomato yield and post harvest life to potash levels. Sarhad J Agric 28: 227–235.
- KHAN SM, RAJ SK BANO T, GARG VK. 2006. Incidence and management of mosaic and leaf curl diseases in cultivars of chili (*Capsicum annuum* L). J Food Agric Environ 4: 171–174.
- KROBER H. 1985. Erfahrungen mit Phytophthora de Bary und Pythium Pringsheim. Mitteilungen der Biologischen Bundesanstalt fur Land-und Forstwirtschaft 225: 1–175.
- LEE YS, HOY JW. 1992. Interactions among *Pythium* species affecting root rot of sugarcane. Plant Dis 76: 735–739.
- LODHI AM, SHAHZAD S, GHAFFAR A. 2004. First Report of Pythium ostracodes Drechsler from Pakistan. Pak J Bot 37: 187– 191.
- MALIK SA, KHAN MA. 1944. Parasitic fungi of the North-West Frontier Province. Ind J Agric 13: 522–527.

- MIRA JH, QURESHI MSA. 1978. Fungi of Pakistan. [Dissertation], University of Agriculture Faisalabad, Pakistan.
- MUSHTAQ M, HASHMI MH. 1997. Fungi associated with wilt disease of *Capsicum* in Sindh, Pakistan. Pak J Bot 29: 217–222
- NASEEM S, MAHMOOD S, ALI Z. 2016. Occurrence of *Citrus tristeza virus* in Pakistan: A GIS based approach combining host distribution and disease reports. Pak J Agri Sci 53: 513–521.
- SALEEM A, BOKHARI MH, HAMAD K, ANSAR M. 1996. Mycoflora associated with root and collar rot disease of chillies in different districts of the Punjab (Pakistan). Pak J Bot 9: 80–84.
- SALEEM A, ANSAR M, HAMID K, JAMIL FF. 1998. Effect of physical parameters on the incidence of root and collar rot disease in chillies. Pak J Bot 30: 39–43.
- SHAHZAD S, GHAFFAR A. 1993. New record of *Pythium* species in Pakistan. Pak J Bot 25: 237–238.
- SOWMINI R. 1961. Studies on phycomycetes in agricultural soils with special reference to *Pythiaceae*. [M. Sc. Dissertation], University of Madras, Chennai, India.
- VAN DER PLAATS-NITERINK AJ. 1981. Monograph on the genus *Pythium*. Stud Mycol 21: 1–244.
- ZAGADE SN, DESHPANDE GD, GAWADE DB, ATNOORKAR AA, PAWAR SV. 2012. Biocontrol agents and fungicides for management of damping off in chili. World J Agric Sci 8: 590–597.