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RESEARCH ARTICLE

Screening of Chilli Germplasm for the Sources of Resistance against *Pythium* and *Phytophthora Sp*.

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Abstract

Damping off caused by Pythium sp. is responsible for 90 percent of plant mortality, as well as pre or post emergence on farms and fields. *Phytophthora* also damages chilli crop by causing damping off. Screening of fifteen chilli cultivars was done against *Pythium* and *Phytophthor*a. Pathogens were isolated from collected soil during survey. All the fifteen varieties showed symptoms against *Pythium* and *Phytophthora* to some extent. No variety was found resistant against the pathogens. Out of fifteen varieties used, six varieties were found moderately resistant both against *Pythium* and *Phytophthora*. Four varieties were found moderately susceptible and five varieties were found susceptible to the *Pythium* and *Phytophthora*. The pathogens also effect the plant biomass like root length, shoot length, root weight and shoot weight.

Keywords: *Pythium*, *Phytophthora*, *Capsicum annum*, Damping off, Chilli.

Introduction

Chilli (*Capsicum sp.*) belongs to the Solanaceae family. There are five domesticated species of the genus *Capsicum* (*C. annum*, *C. frutescens*, *C. chinense*, *C. pubescens* and *C. baccatum*) of which the most *Capsicum annum* is widely cultivated specie worldwide (Andrews, 1984). It is believed that Christopher Columbus and other early European explorers in the sixteenth century introduced pepper in the Europe and pepper scattered throughout the world (Greenleaf, 1986). It resembles a small perennial shrub that has a white or greenish corolla, one or more peduncles at a knot with different sizes of fruits and shapes (Norman, 1992). Pepper Genotype are distinguished and divided into groups according to phenotypic characteristics such as fruit weight, flower color, fruit shape, height of plants, etc. The use of phenotypic characters in the description and classification of genetic material is the basic step in any characterization program (Smith and Smith, 1989).

Chilli fruit is used fresh, cooked, pickled and canned in sauces and as powder for hot spices. Green chillies are rich source of vitamins especially vitamin A, C, B1, B2 (Saimbhi et al, 1977; Sayed and Bagvandas, 1980) and is also rich in vitamin P (rutin), which is of immense pharmaceutical importance (Purseglove, 1977). Chilli contains more vitamins C than any other vegetable crop (Dexiang, 1994).

There was a decline in the yield from 86.5 (1994-95) to 55.8 thousand tons (2003-04) despite the importance of crop (Hussain et al, 2011). According to a report of Pakistan Agriculture Research Council (PARC), there has been a progressive decline in area and production of chilli

in Pakistan. The area under chilli crop has decreased from 86.8 to 48.7 thousand hectares and production from 115.5 to 90.5 thousand tons since 1999-00 to 2004-05. The yield of chilli obtained in Pakistan is for less than the potential exists.

Owing to the potentiality, intensive cultivation of chilli is attacked by several diseases leading to loss of yield in terms of quality and quantity. Among these diseases, damping off incited by *Pythium* spp. is responsible for 90 per cent of plant death either as pre or post-emergence in nurseries and fields (Sowmini, 1961). *Pythium* damping off is very common problem in fields and greenhouse, where the newly emerged seedlings are killed by organism (Jarvis, 1992).

Pythium species are soil borne plant pathogenic fungi, which cause pre and post emergence damping off (Shah-Smith and Burns, 1996). The thready oomycete pathogen genus *Phytophthora* capsici insite root, crown, foliar and fruit rot on variety of vital vegetables (Erwin and Ribeiro, 1996). *Phytophthora capsici* may be an extremely dynamic and harmful pathogen of vegetables, which raid all cucurbits, pepper, tomato and eggplant.

Genus *Phytophthora capsici* initially was represented in 1922 when it was absolutely redeemed from *capsicum* at the New Mexico Agricultural Experiment Station field plots in 1918 (Leonian, 1922).

Damping off in chilli is caused by two species of *Pythium* namely, *Pythium ultimum* and *Pythium aphanidermatum* (Edson). Among them, *P. aphanidermatum* infects chillies grown under hydroponic culture while, *P. ultimum* occurs relatively in higher frequency in dry areas (Zagade et al, 2012).

More than 60% mortality of seedlings is caused by damping-off incited by *Pythium aphanidermatum* (Edson) both in nurseries and field grown crops (Jadhav and Ambadkar, 2007). Management of *Pythium* damping off becomes as a key issue, because of the prolonged survival of propagules, rapid germination of sporangia (Osburn et al, 1989) and quick infection (Whipps and Lumsden, 1991).

Materials and Methods

Fifteen different genotypes of chilli were collected from three different places as shown in table 1. A survey was done in the chilli growing areas of Rawalpindi. The areas which were surveyed include Taxila, Adiyala road, Gujar khan, Kalar sadian, Rawat, kotli sattian and chak shahzad.

Table 1: Chilli Germplasm Collection for Screening against *Pythium* and *Phytophthora*

S. No	Genotype	Source
1	Sanam	National Agricultural Research Center
2	Ghotki	National Agricultural Research Center
3	Green blunt	National Agricultural Research Center
4	Loungi	Chilli Research Station Kunri
5	NARC 16/7	National Agricultural Research Center
6	NARC 16/4	National Agricultural Research Center
7	Nagina	Open Market
8	NARC 16/9	National Agricultural Research Center
9	Black Master	Open Market
10	Hot queen	Open Market
11	Long Green	Open Market
12	Neelum	Chilli Research Station Kunri
13	Sangri	Chilli Research Station Kunri
14	32311	National Agricultural Research Center
15	32315	National Agricultural Research Center

$$\text{Disease incidance \%} = \frac{\text{Number of diseased plants}}{\text{Total number of plants observed}} \times 100$$

$$\text{Disease prevalence \%} = \frac{\text{Number of diseased plants}}{\text{Total number of plants observed}} \times 100$$

$$\text{Disease severity \%} = \frac{\text{sum of all disease rating}}{\text{Total number of rating} \times \text{maximum disease grade}} \times 100$$

Disease incidence and prevalence was observed and soil samples were collected from the fields for isolation of *Pythium sp* and *Phytophthora sp.*

Isolation of pathogen was done according to Saha et al, (2002). Bottle gourd was utilized as bait which invigorate development of the pathogen. The fruit was dipped in a solution containing carbendazim (500 ppm) and streptomycin (100 ppm) for 12 hrs to make the fruit succulent and prevent contamination from bacteria and other fungi.

The treated fruit was transversely cut into 2-4 pieces and buried into infested soil at a depth of 5-6 cm below the soil surface. The soil was kept moist. After 24 to 48 hours, the bait fruits was removed from the soil in such a way that minimum disturbance was to the pathogen invaded French bean/bottle gourd fruits.

The fruits was placed inside air filled plastic bag individually under aseptic condition and kept at room temperature for 24 hours and by that time white fluffy mycelia growth was noticed on fruits, was aseptically transferred. The mycelial growth was observed under microscope. Purification of the pathogen was done according to (Papias et al, 2016).

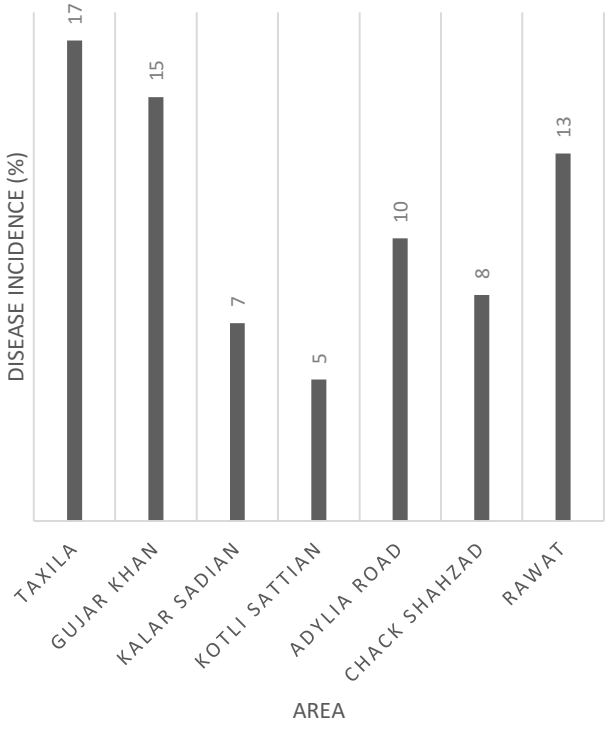
Refinement of *Pythium* culture was completed by cutting a little bit of the media with mycelia from the edge of a state and afterward sub refined onto corn meal agar (CMA) growth media. Unadulterated disconnects was exchanged to potato dextrose agar (PDA) inclines and following 14 days was put away at -20°C.

In the screening 10^5 spores/mL concentration per pot was used which was measured by hemocytometer. Disease severity was determined for each pot on the basis of standard procedures recommended by the international plant genetic resource institute, Rome, Italy. The rating was 1-5 where 1 is highly resistance and 5 is highly susceptible (IPGRI, 2001).

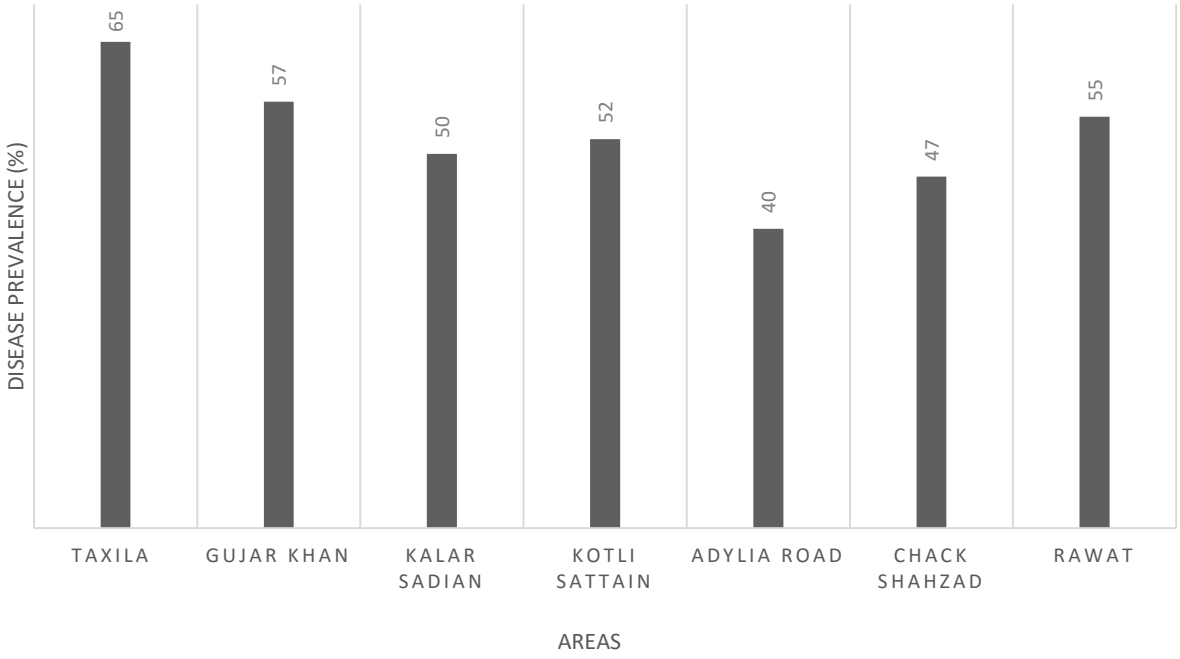
Results

The overall incidence of damping off in the areas of Rawalpindi was found to be 20 % and the prev-alence was recorded as 65 %.

Out of the seven areas of Rawalpindi, maximum disease incidence of 17 % was recorded in Taxila followed by Gujar khan showing 15 % disease incidence, followed by Rawat which showed disease incidence of 13%. On the other hand, minimum disease incidence was observed in area of kotli sattian as 5% as shown in the graph 1. The prevalence of the damping off chilli was also observed. The prevalence of the damping off disease was maximum in the Taxila (60%) followed by the Gujar khan showing 57% disease prevalence. On the contrary, the minimum disease prevalence was noticed in the area of kotli sattian as shown in graph 2.



Graph 1: Incidence of Damping off by *Pythium* and *Phytophthora* in Seven Areas of Rawalpindi



Graph 2: Prevalence of Damping Off By *Pythium* and *Phytophthora* in Seven Areas of Rawalpindi

Table 2: Screening of Chilli Germplasm against *Pythium* (Art-11)

S. No	Variety	Plants /pot	Germination %	Disease incidence%	Disease severity %
1	Sanam	10	80	37.5	40
2	Ghotki	10	80	12.5	20
3	Green blunt	10	70	28.5	35
4	Lounge	10	60	33.3	50
5	NARC 16/7	10	70	28.5	35
6	NARC 16/4	10	80	25	30
7	Nagina	10	60	33.3	35
8	NARC 16/9	10	80	37.5	40
9	Black master	10	70	14.2	20
10	Hot queen	10	60	16.6	30
11	Long green	10	80	12.5	35
12	Neelum	10	80	37.5	50
13	Sangria	10	90	11.11	20
14	32311	10	80	12.5	20
15	32315	10	70	28.5	45

All the fifteen varieties were screened against highly virulent isolate of *Pythium* and *Phytophthora*. The results of screening are shown in tables 2 and 3. The response of chilli cultivars against *Pythium* and *Phytophthora* are shown in tables 4 and 5.

Table 3: Screening of Chilli Germplasm against *Phytophthora* (ILS78-0)

S. No	Variety	Plants /pot	Germination %	Disease incidence%	Disease severity %
1	Sanam	10	60	33.3	35
2	Ghotki	10	70	14.3	20
3	Green blunt	10	70	28.5	35
4	Lounge	10	60	33.3	40
5	NARC 16/7	10	80	25	30
6	NARC 16/4	10	70	28.5	30
7	Nagina	10	60	33.3	35
8	NARC 16/9	10	80	37.5	35
9	Black master	10	60	16.6	20
10	Hot queen	10	70	14.2	25
11	Long green	10	80	12.5	20
12	Neelum	10	80	37.5	30
13	Sangria	10	80	12.5	20
14	32311	10	80	12.5	25
15	32315	10	70	28.5	30

Table 4: Reaction of Chilli Cultivars towards *Pythium*

S. No	Mean disease index	Disease incidence %	Variety	Reaction
1	1-2	0-10	N/A	Resistant
2	2-3	11-20	Ghotki , Black master, Hot queen, Long green, Sangri , 32311	Moderately resistant
3	3-4	21-30	NARC16/7, NARC16/4, 32315, Green blunt	Moderately susceptible
4	4-5	31-40	Loungi, NARC16/9, Neelum, Nagina, Sanam	Susceptible

Table 5: Reaction of Chilli Cultivars towards *Pytophthora*

S. No	Mean disease index	Disease incidence %	Variety	Reaction
1	1-2	0-10	N/A	Resistant
2	2-3	11-20	Ghotki , Black master, Hot queen, Long green, Sangri , 32311	Moderately resistant
3	3-4	21-30	NARC16/7, NARC16/4, 32315, Green blunt	Moderately susceptible
4	4-5	31-40	Loungi, NARC16/9, Neelum, Nagina, Sanam	Susceptible

Discussion

In the present study, an overall incidence of 20% and prevalence of 65% of the damping off of chillies caused by *Pythium* and *Phtophthora* was recorded in the areas of Rawalpindi. Incidence and prevalence varied among the chilli growing areas of Rawalpindi which were visited.

The maximum disease incidence of 17% was recorded from Taxila while the minimum disease incidence of 5% was recorded in kotli sattian. In these chilli growing areas of Rawalpindi

farmers mainly uses some local varieties over the years.

In the recent study, pathogens were isolated from the infected soil sample collected from the fields infected by damping off by *Pythium* and *Phytophthora* during the survey. Isolation of pathogen was done according to Saha et al, (2002). Purification of the pathogen was done by Papias et al, (2016).

Fifteen cultivars were screened against the *pythium* and *phytophthora* for the sources of resistance. The varieties were tested in the pots. The isolate which was found most pathogenic during the pathogenicity test was used in screening. The isolate was inoculated in the autoclaved soil at the time of transplantation of nursery. Data was taken after 14 days and symptoms of disease were observed. All the fifteen varieties showed symptoms against *Pytium* and *Phytophthora* to some extent. There was no variety found resistant. Out of fifteen varieties used six varieties were found moderately resistant both against *Pythium* and *Phytophthora*. The varieties were found moderately resistant were Ghotki, Black Master, Hot Queen, Long Green, Sangri and 32311. Four varieties were found moderately susceptible according to disease index, these were green blunt, NARC16/7, NARC16/4 and 32315. Five varieties out of fifteen were found susceptible to the damping off disease, the varieties found susceptible were Loungi, NARC16/9 and Neelum, Sanam and Nagina. The *Pythium* was more pathogenic and causes more disease incidence and severity then the *Phytophthora.*

The present study is related with the study of (Saleem et al, 1999) in which sixty six chilli varieties/lines evaluated in pots and 51 in the field, 16 varieties belonging to *capsicum annum* were found disease free both in pots and field against *Phytophthora capsici*, mainly emerging from the crosses of Italian varieties with Mexican wilt type *Capsicum annum*. 24 varieties belonging to the *Capsicum annum*, *Capsicum Chinese* and *Capsicum frutescent* were highly susceptible and gave 90 to 100% mortality in pots while in the field these varieties exhibited 58.33 to 100 % disease incidence (Saleem et al, 1999)

Conclusion

Damping off caused by *Pythium* and *Phytophthora* is widely prevalent in the country. *Pythium* and *Phytophthora* can be isolated from the infected soil. Moderately resistance reaction have been identified in some of the cultivars against highly virulent isolate of the fungal pathogens among the indigenous germplasm of chilli.

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