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MAPING AND MONITORING FOR THE VALUATION OF SOIL FUNGI AND CHILI DAMPING OFF

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ABSTRACT

Capsicum annum is one of the most important spice crop of Pakistan which is threatened by damping off which is soil borne nursery disease and responsible for more than 70% losses in the yield. A systematic survey in chili field of Lahore District was conducted to estimate the incidence, prevalence and % mortality of the seedlings due to damping off disease. Another important objective of the research was to design a map for the distribution of this disease in Lahore. 15 villages around the main city of Lahore were visited for sampling and data recording. Maximum disease incidence (47%) was observed in the village Shamkibhattian while the minimum disease incidence (09%) was recorded in the village Chappa. For the disease prevalence and percentage mortality the maximum values were 100% and 39% while the minimum values were 67% and 4% respectively. A detailed map of the district was also designed highlighting the distribution of chili damping off in the Lahore. Population dynamics as well as percentage frequency of soil borne oomycetes and other fungi were calculated. Species of *Pythium, Aspergillus* and *Mucor* were among the frequently isolated species. The main objective of the following research is to map the damping off disease in Lahore district as well as to estimate the level of pathogenic population and disease incidence.

Key Words:

INTRODUCTION

Chili (*Capsicum annum* L.) is a vital vegetable commodity of Pakistan. This crop is used as spice, salad and also as dried condiments. Due to variety of applications and high cost this crop is getting significanceamong the vegetable crops (Govt. of Pakistan, 2006-07).The hot brands are cause of the digestive stimulating Capsicin (Mehboob*et al*., 1998).It contributes about 1.5% to the country's agricultural GDP in Pakistan, chilies are grown over an area of 62.7 thousand hectares with the total production of 150.3 thousand tons having an average yield of 2.7 tons/ha (GOP, 2013).According to an estimate the average yield of this crop is 2.5 tons per hectare. Khyber Pakhtunkhwa (KPK) contributed 0.6 thousand hectares with total production of 0.7 thousand tones and an average yield 1.2 tons per hectare (Govt. of Pakistan, 2008-09).

Chili is alsogrown for ornamental purposes because of its glossy fruits and a wide range of colours (Cronin, 2002; Reilly *et al.,* 2001). The enormous gardening, agricultural as well as natural variation of this crop has made it universally significant as a newly introduced vegetable crop and also a basis of the different food constituents (Bosland and Votava, 2000).Furthermore, the spices have their medicinal values and deliver the constituent for a non-lethal preventive or repellent to human and animal behaviour (Krishna, 2003).This crop is attacked by different a-biotic as well as biotic agents and causes great losses to the farmer ultimately. Among those pathogenic diseases damping off is one of the most significant disease that causes about 70 % of plant death as pre or post emergence damping off.Damping-off is one of the most common agricultural disease in the glasshouse or nursery which affects sprouting seeds and young seedlings (Hulvey*et al*., 2010).In pre-emergence damping-off, the seeds wilt or deteriorate which result in a thin and sporadic germination. In post-emergence damping-off, the pathogen infects the root collar tissue and within short time the seedlings become black, decay nearly to the soil line at lower portion of the stem, then wither and die. These diseases can blow-out rapidly in a few days depending on the environmental conditions of the greenhouse or nursery (Postma*et al*., 2005). Recent research work was conducted to map and locate the disease incidence, prevalence and mortality rate among the chili plants infected with damping off.

MATERIALS AND METHODS

The present study was accomplished at Plant Nematology Laboratory in the Institute of Agricultural Sciences (IAGS), University of the Punjab, Lahore, Pakistan. Field transportation and survey was conducted by the courtesy of Agriculture extension wing of the Agriculture department, Government of the Punjab, Lahore.

Disease Survey: Preliminary surveys were conducted during the chili growing season (September-October) in 2012 and 2013 to collect the infected samples of chili plants with Damping off. Soil from the rhizospherealong with infected plant samples was also collected. During survey total 15 different locations comprising of 40 fields were explored during the months of September, October, November and December of 2013. Fields of rural and urban areas were surveyed for the evaluation of incidence, severity and collection of the diseased plants. Infected plants were keenly observed on the basis of symptomatology, infection level was recordedand morphological changes due to infestation was also observed and written on notebook. Samples of infected chili plants were collected gently with the help of a sharp knife along with some adjacent rhizospheric soil. Samples were photographed and kept in polythene sampling bags and labelled with the date and site of sampling(Iram*et al.,* 2011).

Zones for sample collection: To make the work more comprehensive the total surveyed areas were divided into two major zones on the basis of their mode of cultivation, intensity and severity of disease, soil features, disease history in the field and the attention of farmer towards the target crop.

Zone I: It includes the areas of Manawan, Marl Mari, GanjiSindhuwan, Dhoop Sari, Dhokywali, KhwajaFaiq, MoslleyWal, Hadiara and Chappa. In zone I areas were included with the cultivation of chili on more than 10 acres.

Zone II: It includes the areas of ShamkiBhattian, Sunder, Baath, Manga Mandi, Echo Gill and Jandiala. In zone II areas were included with the cultivation of chili on less than 6 acres.

Infected Plant Sample Collection: Diseased plant samples were collected randomly from the fields on the basis of symptoms on above and lower parts of the plants. Sampling was done randomly using one meter square quadrate. At each site the field was divided into "X" shaped and samples were collected along the two crossing lines. Percentage prevalence of the disease was calculated on the basis of the number of locations showing disease symptoms in an area. (Wszelaki and Miller, 2005;Madakbaş*et al.*, 2013). Percentage disease incidence, Prevalence and % Mortality were calculated by following formulas:

$$\% D \quad e \quad i\imath = \frac{N\ ,o \quad i\imath \qquad p}{T \qquad N\ .o \quad P} X\ 100$$

$$\% P\imath = \frac{N\ ,o \quad l\iota \qquad sho \qquad s\imath}{T \qquad N\ .o \quad l\iota} X\ 100$$

$$\% M = \frac{N\ ,o \quad d \qquad p}{T \qquad N\ .o \quad p} X\ 100$$

Each diseased sample was placed in different polythene bag, labelled and brought to laboratory for the isolation and other studies.

Pathological constraints and Collection of Soil Sample: During the surveys, field scouting for disease was made in diagonal fashion according to the field geometry. Starting point was 10 feet into the field and rest of the points were having space of 8 feet. A bird eye view was given to field for the occurrence or absence of Damping off disease. For each sampling soil was dug up to the depth of 30 cm to include surface (5 cm), sowing depth (10-15 cm) and plough depth (20-30 cm). Soil as well as plant samples were collected in polythene bags and labelled with site and date. The collected specimen were processed for identification and single spore culture.

Population dynamics of soil fungi in chilli field: Soil sample (10g) was taken in a 250ml containing 100ml distilled water. The flask was shaken on a electric shaker to get a homogenous suspension and serial dilution of the soil sample such as 10^{-1}, 10^{-2} and 10^{-3} were prepared. One ml of 10^{-3} dilution was plated in petri dishes containing PDA medium. The pH of the medium was adjusted to 5.6. Streptomycin sulphate ($100mg^{-1}$) was added to the medium to prevent bacterial growth. The plates were incubated at 25±2ºC for five days and the fungi appearing on the medium were recorded.

$$P \qquad o \quad f \qquad g - 10\, d \quad w\ .o \quad the\ s \quad . \frac{= M \qquad m\imath \qquad o \quad p\imath \qquad i\imath \quad d \qquad p}{W \qquad ht\ o \quad the\ d \quad s} x\ D$$

$$P \qquad F = \frac{N\ .o \quad s \quad s\iota \qquad f \qquad whi\iota h\ f \qquad w \quad r}{N\ .o \quad t\iota \quad s \quad s\iota} X\ 100$$

Disease rating scale: The disease rating for the evaluation of disease symptoms on plants was recorded by the visual symptoms and level of infection by following the modified protocol of (Saha, 2001). Following scales were made for disease evaluation.1-6: 1 = 0%, 2 = 10-25%, 3 = 25-50%, 4 = 50-70%, 5 = 70-85% and 6 = 85-100%.

Survey: A detailed survey map of all the sampling sites and the areas under survey was made. The sampling sites on map were spotted on the map with the shades to

represent the value of % disease incidence in all areas. Same colour means that two or more areas surveyed, were having the same value of disease incidence (Fig: 1). Moreover, % disease incidence, % mortality and % disease prevalence were calculated. The variation among the level of incidence and percentage mortality was observed at all the locations data was recorded (Figure 2,3,4). Maximum prevalence of Damping off disease in chilli was found in ShamkiBhattian (65%) and Dhoop Sari (62%) followed by Echo Gill (59%). Minimum disease occurrence was observed in Chappa (03%) and KhwajaFaiq (09%) (Figure: 2). Maximum disease incidence of damping off disease was calculated at ShamkiBhattian (47%), Dhoop Sari (45%) followed by Echo Gill (43%). High level of disease incidence was observed. Minimum disease incidence was calculated at DhokayWali(09%) (Figure: 3). Maximum mortality percentage due to Damping Off disease in chilli nurseries was observed at Dhoop Sari (39%), ShamkiBhattian (37%) followed by Echo Gill (36%) and GanjiSindhuwan (34%) respectively. Lack of proper space among the seedlings was the main factor observed, responsible for infection. Minimum percentage mortality was observed at Hadiara (06%), Chappa (07%) and MosleyWali(09%) (Figure: 4).

Population dynamics of soil borne fungi: A total of 18 soil borne fungal isolates were isolated from the infected soil which was collected from chilli fields. The total number of colonies isolated varied from 15.4 to 33 (per 10 gm of dry soil). The maximum number of colonies were found in the month of October. There was a decline in the number of colonies in the month of March and April. Qualitatively, the species spectrum of fungi was more from the month of October to December (Table:1)

Percentage Frequency of Soil borne fungal isolates: Among the different species of fungi, the species of *Pythium* and *Mucor*were frequently found isolated. The percentage frequency was as follows; *Pythium* (100%), *Mucor*(73.4%), *Fusarium* (66.7%). The species of *Cheatomium, Cladosporium, Arthobotrytis*and *Botrytis* were among the less frequently isolated (Figure: 5).

Disease rating scale: The disease rating for the evaluation of disease symptoms on plants was recorded by the level of infection according to following scale. Scale 0= plant healthy, scale 1 = 1-10% discoloration, scale 2 = 11-25% root discoloration, scale 3 = 26-50% root infection, scale 4 = 51-75 % root discoloration and infection and scale 5 = 100 % infection with the death of seedling (Table 2).

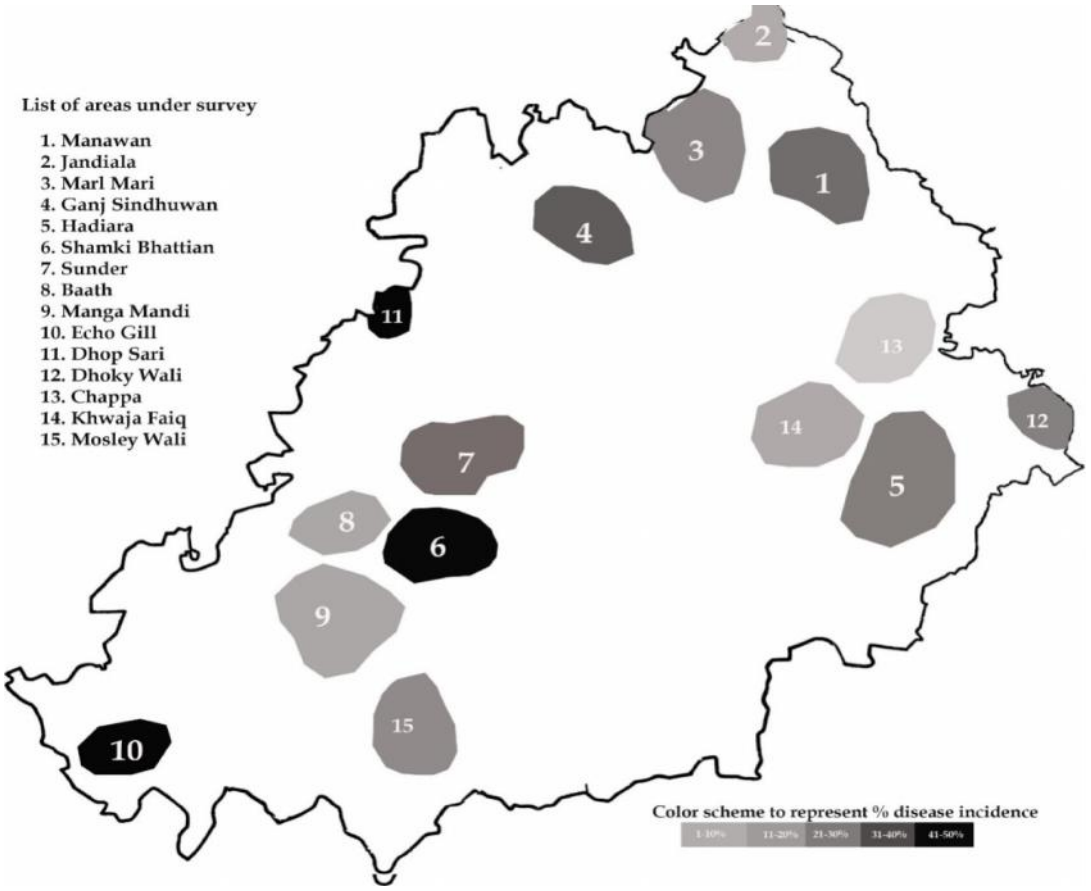

Figure 1. A detailed survey map showing sampling sites and level of disease incidence.

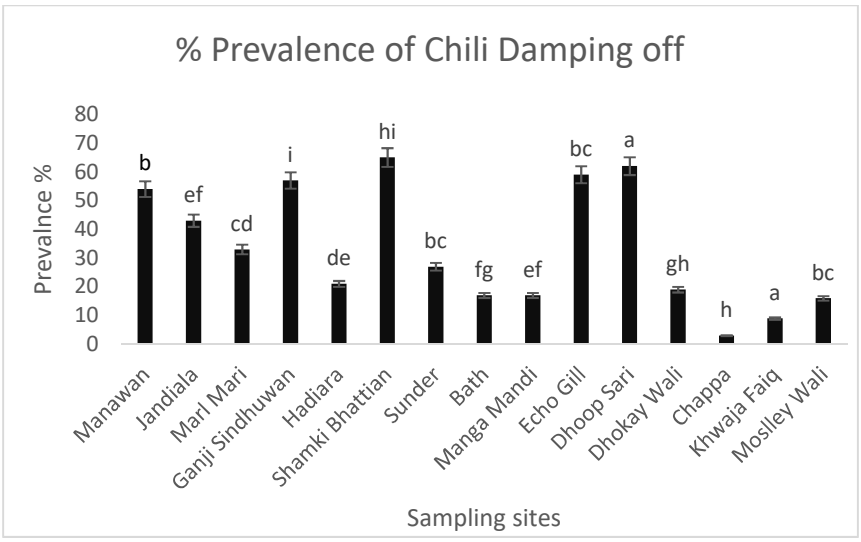

Figure 2. Percentage Prevalence of Damping Off in Chili growing areas

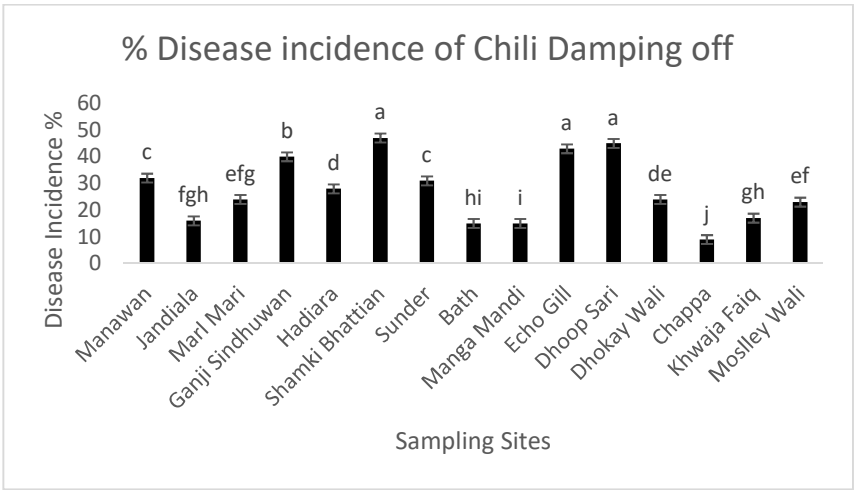

Figure 3.Percentage Incidence of Damping Off disease in chilli growing areas.

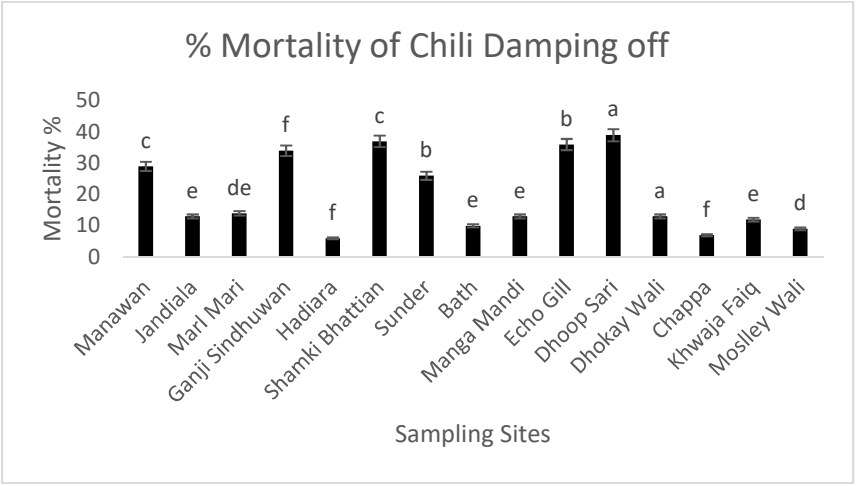

Figure 4.Percentage Mortality of Plants due to Damping Off disease

Table 1.Population dynamics of soil borne fungi in Chili growing areas of Lahore.

Locality	Weight of the soil sample	Population /10 gm of soil
Manawan	10	33
Jandiala	10	30.8
Marl Mari	10	36.3
GanjiSindhuwan	10	29.7
Hadiara	10	20.9
ShamkiBhattian	10	23.1
Sunder	10	18.7
Bath	10	25.3
Manga Mandi	10	25.3
Echo Gill	10	20.9
Dhoop Sari	10	15.4
DhokayWali	10	24.2
Chappa	10	27.5
KhwajaFaiq	10	26.4
MoslleyWali	10	31.9

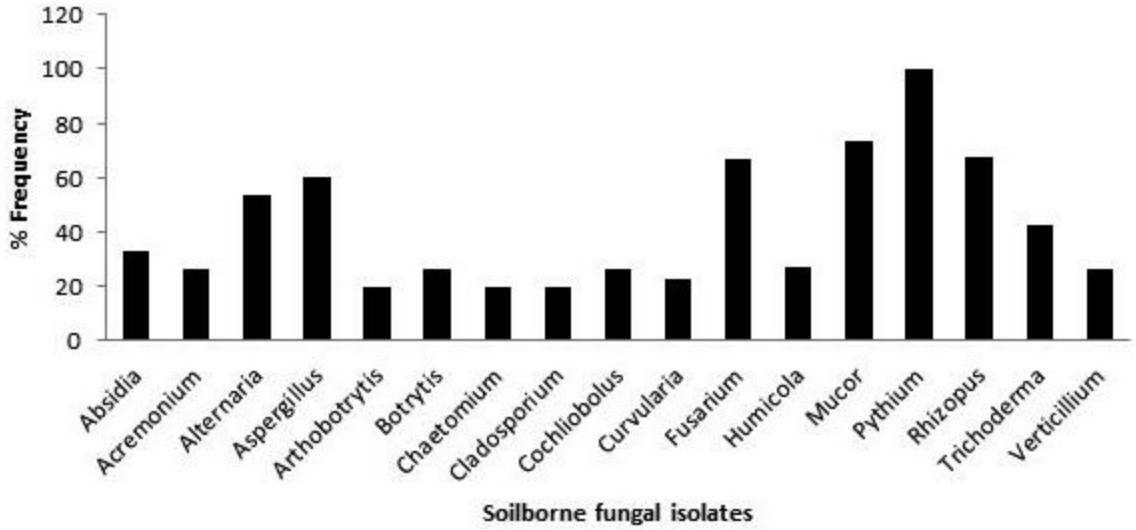

Figure 5. Percentage Frequency of soil borne fungal isolates

Table 2: Disease rating scale explaining the different levels of infection due to damping off.

Rating Scale	Level of Infection (%)	Description of symptoms	Visual symptoms
0	**0**	No discoloration of the root system	

1	1-10%	1-10% of the root system discoloured	
2	11-25 %	11-25 % of the root system discoloured	
3	26-50 %	26-50 of the root system discoloured	
4	50-75%	50-75% of the root system discoloured	
5	76-100 %	Seedling dead	

Fig 2. Infected samples of chili seedlings with Damping off symptoms

DISCUSSION

A detailed survey was conducted during the winter of 2012 and 2013 to evaluate and map the occurrence, incidence and prevalence of damping off in chilli crop. A total of 15 villages were surveyed (Fig: 1). Damping off is one of the most serious soil borne, seedling disease of this spice crop. During the survey the disease was observed more prominent at the places with excessive moisture level in soil. The incidence of disease was observed at all the sites but the values of percentage incidence were also different at all locations. Percentage disease incidence was calculated was 32%, 16%, 24% , 40% , 28% , 47% , 31%,15% , 43%, 45% , 24% , 9% , 17% , 23% at Manawan, Jandiala, Marl mari, GanjiSindhuwan, Hadiara, Shamkibhattian, Sunder, Baath and Manga Mandi, Echo Gill, DhoopSari, DhokayWali, Chappa, KhwajaFaiqand MoslleyWali, respectively (Table:1). The same trend in the disease was also observed by Shah (2007) and Shah and Khalid (2001) while working on the incidence and occurrence of viral diseases in different chilli growing areas of Pakistan. This variability in the disease incidence and disease severity can be due to different biotic and a-biotic factors like the poor management of field practices, location of the nursery field, type of crop variety and the level of fungal

inoculums in the soil. The same observations were also recorded in the research work of Shumaila*et al*., (2012). Infected plants were collected on the basis of symptoms. The plants with the dark and shrivelled portions near soil line and in severe conditions the fallen and dead seedlings were collected (Figure: 2). A disease rating scale was also designed to differentiate the different disease developmental stages on the hosts (Table: 3). According to the findings of Black *et al*., (2010) the fall of seedlings is due to the excess or very low level of soil moisture but it also can be due to the attack of different pathogenic organisms. It was also observed that the fields which were previously not cultivated by chilli crops were having low level of disease incidence and it can be due to the absence or low level of fungal inoculums in the soil. Thus, along with the infected plants the soil samples were also collected for further study (Table: 2).

Hence, this research work revealed that this nursery or seedling disease of chilli is causing a regular loss in the final crop yield as the level of inoculums overwinters and multiply in the soil environment. Producers are well aware of the a-biotic constraints such as level of moisture and location of the field but they are not trained to assess the role of biotic factors in low yield. This research has also adopted new strategies to record data and mapping to highlight the areas with the incidence of this disease.

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