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## INCIDENCE OF MAJOR FUNGAL ROOT DISEASES OF CHICKPEA IN LAYYAH AND BHAKKAR DISTRICTS OF PUNJAB, PAKISTAN

<sup>a</sup>Muhammad Shahjahan, <sup>a</sup>Muhammad Inam-ul-Haq, <sup>a</sup>Tariq Mukhtar, <sup>b</sup>Azeem Khalid

<sup>a</sup>Department of Plant Pathology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan.

<sup>b</sup>Department of Environmental Science, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan.

### ABSTRACT

Root diseases either soil or seed borne poses serious implications on seed germination and subsequently on the plant developmental stages. The attack of root diseases in earlier stages of the crop completely destroys the crop resulting in unrecoverable losses. This study was designed to evaluate the prevalence, incidence and severity of major fungal root pathogens infecting chickpea crop in major chickpea growing areas of Punjab such as districts Layyah and Bhakkar. Surveys were conducted during year 2013-14 and 2014-15 in the months of November to March. From each district twenty areas and three fields from each area were visited making total 120 fields. Results revealed 100% prevalence of root diseases in every single field and average highest incidence recorded in Layyah was 60.05% and in Bhakkar was 57.5%. The incidence in Layyah and Bhakkar ranged from 52%-72%, 48%-76% and 48%-76%, 50%-70% during the year 2013-14 and 2014-15 respectively. Disease severity ranged from 1-4 on the scale. Collected samples retrieved five fungal pathogens and the most frequent was *F. oxysporum* f. sp. *ciceri*, with average highest frequency of 88% followed by *M. phaseolina*, *Verticillium* spp. with 75.5% and 74.5% respectively. Results elucidate that fungal pathogens associated to roots of chickpea pose serious implications in growth and productivity of chickpea.

**Keywords:** Chickpea, fungal root pathogens, disease incidence, Layyah, Bhakkar.

### INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a highly nutritious pulse crop with good value of protein contents and therefore known as poor man's meat. It is usually grown under arid and semi-arid environmental conditions of the world (Millan *et al.*, 2006). The major producers of chickpea are India, Turkey, Pakistan, Iran, Mexico (FAOSTAT, 2010). In the chickpea cultivation Pakistan stands second in area and third in production around the world. From total cultivation area of chickpea, 82% is grown in Punjab, 9% in Khyber Pakhtunkhwa, 8% in Sindh and 1% in Baluchistan province. In Punjab, 90% chickpea is grown under rain fed conditions increasing the soil nutritional level. The production per hectare of chick pea is very poor in Pakistan in comparison to other countries of the world. Regardless of increasing

demand and high yield potential, chickpea yield is low and productivity is less with a world average production of 0.8 t/ha (Millan *et al.*, 2006).

About ninety percent losses in the yield occur due to the occurrence of root based diseases and pathogenic fungi (Zamani *et al.*, 2004; Haware, 1990; Sharma and Muehlbauer, 2007). Chickpea production is greatly influenced by root based pathogens in many countries of the world like India, Pakistan, Tunisia, Spain, Iran, Nepal and Burma (Nene, 1984). Root infecting pathogenic fungi involves mainly *Fusarium oxysporum* f.sp. *ciceris*, *Macrophomina phaseolina*, *Fusarium solani*, *Rhizoctonia solani* and *Phythium ultimum*. The pathogens can be in active phase even in the absence of its host for more than six years even they are seed or soil borne (Haware *et al.*, 1996; Ayyub *et al.*, 2003). It was published that *Fusarium wilt* (*Fusarium oxysporum*) damaged 10-15% yield losses in chickpea while black root rot (*M. phaseolina*) damaged 60-70% yield. Dry root rot caused by *Macrophomina phaseolina* damage over 500 host

\* Corresponding Author:

Email: mahar.phd@gmail.com

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plant species of the tropical and temperate regions of the world (Inam-ul-Haq *et al.*, 2015).

Although many strategies have been developed to control these diseases but the soil borne nature, occurrence in soil and availability of multiple hosts make control very difficult. The use of resistant to tolerant varieties is offers good control (Inam-ul-Haq *et al.*, 2015). This study was embarked to assess the prevalence, incidence and severity of root diseases on chickpea crop in major cultivation areas of Punjab, Pakistan that include Layyah and Bhakkar. Determining the losses and prevalence of fungal root pathogens is pre-requisite to allow implementation of management strategies accordingly.

#### MATERIALS AND METHODS

For determination of incidence and severity of root infecting fungal pathogens, a systematic survey of major chickpea cultivating areas such as Layyah and Bhakkar was conducted in the months of November to March during the year 2013-14 and 2014-15. From each district, 20 areas were selected and from each area 3 fields were visited totaling 120 fields. Samples were collected by taking ten plants from five different places along a diagonal and the hierarchical sampling strategy was adopted (McDonald and Martinez, 1990).

Disease prevalence (DP) was given by:

$$DP (\%) = \frac{\text{number of infected fields}}{\text{total number of fields visited}} \times 100$$

Disease Incidence (DI) percentage was measured according to the formula:

$$DI(\%) = \frac{\text{Number of plants infected}}{\text{total number of plants observed}} \times 100$$

Disease severity scale 0-4 (Casas and Díaz, 1985) was used to assess the percentage yellowing or necrosis in an acropetal progression (0= 0%, 1= 1-33%, 2= 34-66%, 3= 67-100%, 4= dead plant).

**Isolation of root fungal pathogens:** The collected samples were processed for isolation of root associated fungal pathogens. Roots of the plants were cut into small pieces (0.5 cm) and surface disinfected with 1% sodium hypochlorite and subsequently washed thrice with sterile distilled water (SDW) followed by drying on sterile filter paper. The root pieces were placed on water agar (WA) and potato dextrose agar (PDA) media. The plates were incubated at 26 °C for 2-3 days. After 2-3 days, composite cultures were observed under stereoscope and individual spores type were picked and placed on separate media plates to get purified cultures

of each spore type. Wet sieving and dilution technique (Sheikh and Ghaffar, 1975) was used for isolation of *Macrophomina phaseolina*.

**Identification of fungal pathogens:** Each type of spore was observed under microscope for identification on the basis of morphology. *Fusarium* spp. was identified according to the described keys (Booth, 1977; Nene and Haware, 1980; Haware *et al.*, 1992), *Macrophomina* was identified by keys of Barnett and Hunter (1972). *Verticillium* spp. was identified according to Smith (1965), *Rhizoctonia* was identified as described keys (Sneh *et al.*, 1991). *Sclerotium* spp. identification was done according to the described keys (Aycock, 1966) and (Punja, 1985).

#### RESULTS AND DISCUSSION

Survey of the areas elucidated that root based diseases are very important limiting factors in chickpea productivity in these areas. During both years of survey, root diseases were prevalent in each field in both districts and 100% prevalence was recorded and not a single field was found free of infection. From each field, wilted, dead and plant exhibiting chlorosis were observed. General symptoms observed were yellowing of leaves, chlorosis, root rot, collar rot, completely wilted and dead plants. When the infected plants were uprooted, vascular tissue discoloration was observed in most of them. In general, average root diseases incidence was slightly higher in Layyah (60.05%) as compared to Bhakkar (57.5%). In Layyah, lowest disease incidence was observed in Mochi moor (52%) during 2013-14 while during 2014-15, lowest DI was recorded in Sahoowala with 48% incidence. Highest DI in Layyah district was recorded in Asarwala with 72% and 76% during 2013-14 and 2014-15 respectively. Overall DI ranged from 52-72% and 48-76% during 2013-14 and 2014-15 respectively in Layyah. In Bhakkar, DI ranged from 48%-76% and 50%-70% during 2013-14 and 2014-15 respectively. Area-wise in Bhakkar, highest DI was recorded in Dagarshada during both years of survey with figures of 76% and 70% respectively while lowest DI was observed in Panjgirain and Kiri wala during each year of survey (Table 1). Disease severity was variable and ranged from minimum to maximum (1-4) in both districts. Even in a single field, there was variable severity and fields exhibited 1-4 severity.

From the infected samples, the isolation retrieved five fungal pathogens viz. *F. oxysporum* f.sp. *ciceri* (Foc), *M.*

*phaseolina*, *Verticillium* spp., *Sclerotium rolfsii* and *R. solani*. The highest frequency among the isolated fungi was of Foc which was recovered from 85% samples of Layyah and 91% samples of Bhakkar, followed by *Verticillium* spp. and *M. phaseolina*. *S. rolfsii* and *R. solani*

were least frequent and were retrieved from few samples. *F. solani* was not recovered from any sample (Figure 1). More than one fungus was retrieved from most of the collected samples making it difficult to elaborate the loss from individual pathogen.

Table 1. Fungal root disease incidence and prevalence in Layyah and Bhakkar district.

District	Areas	2013-14			2014-15			Mean DI (%)
		DI (%)	DP (%)	DS range	DI (%)	DP (%)	DS range	
Layyah	Sahoowala	56	100	1-4	48	100	1-4	60.05±6.74
	Shah wala	58	100	2-4	54	100	2-4	
	Turkey wala	70	100	2-4	62	100	2-4	
	Sobaywal	66	100	2-4	70	100	2-3	
	Asarwala	72	100	2-4	76	100	2-4	
	Bhularwal	58	100	1-3	52	100	1-4	
	Bhaliawala	56	100	2-4	58	100	2-4	
	Sheirowal	62	100	2-4	66	100	2-4	
	Yasinwal	70	100	2-4	68	100	2-4	
	Janay wal	54	100	1-4	62	100	1-4	
	Mochiwala	60	100	1-4	56	100	1-4	
	Shumarwala	62	100	2-4	54	100	2-4	
	Asargillaniwala	54	100	2-3	52	100	2-4	
	Okrnaywala	56	100	2-3	54	100	2-3	
	Mochi moor	52	100	2-4	56	100	2-4	
	Lungerwala	56	100	2-4	62	100	2-3	
	Deranawabsewag	70	100	1-4	60	100	1-3	
	Shumar moor	68	100	2-4	56	100	2-4	
	Darbarmaiwleet	68	100	2-4	52	100	2-4	
	Manjhiwala	60	100	1-4	56	100	1-4	
	Mean	61.4±6.43	100		58.7±7.06	100		
Bhakkar	Mankera	48	100	1-4	54	100	1-4	57.5±6.78
	Mani	56	100	1-4	52	100	1-4	
	Kuriya	62	100	1-4	56	100	1-4	
	Lunghawala	56	100	2-4	62	100	2-4	
	Kiri wala	56	100	2-3	50	100	2-3	
	Patiblinda	68	100	1-4	64	100	1-4	
	Dagarshada	76	100	2-4	70	100	2-4	
	Wig sadar	50	100	2-4	48	100	2-4	
	Khanser	52	100	2-3	50	100	2-4	
	Dullewala	56	100	1-3	62	100	1-3	
	Jundanwala	58	100	1-4	60	100	1-4	
	Magasiwala	66	100	2-4	62	100	2-4	
	ThalHayderabad	62	100	2-3	64	100	2-4	
	Tehsil deria khan	54	100	2-4	52	100	2-4	
	Maibalsharif	56	100	2-4	50	100	2-4	
	Kallurkot	64	100	2-4	56	100	2-4	
	Chandni chowk	66	100	1-3	54	100	1-3	
	Bhatti wala	56	100	2-4	50	100	2-3	
	PanjGirain	48	100	2-3	50	100	2-4	
	BastiMianPunja	60	100	1-4	64	100	1-4	
	Mean	58.5±7.10	100		56.5±6.45	100		

DI= disease incidence, DP=disease prevalence, DS= disease severity.

Root diseases either seed or soil borne is generally a serious growth and development implication for plants

and especially for agricultural crop as it has to feed growing population. The losses which are estimated to

be ranged from 10-100% (Bretag, 1982; Halila and Strange, 1996) in chickpea by these root diseases can pose a great yield loss causing economic and food security issues. In this study it was observed that chickpea is the major crop for Layyah and Bhakkar and

major domestic production of chickpea relies on these areas. We have noticed that root diseases are prevalent and are playing key role in reducing the chickpea production by reducing the number of plants thus reducing the number of pods significantly.

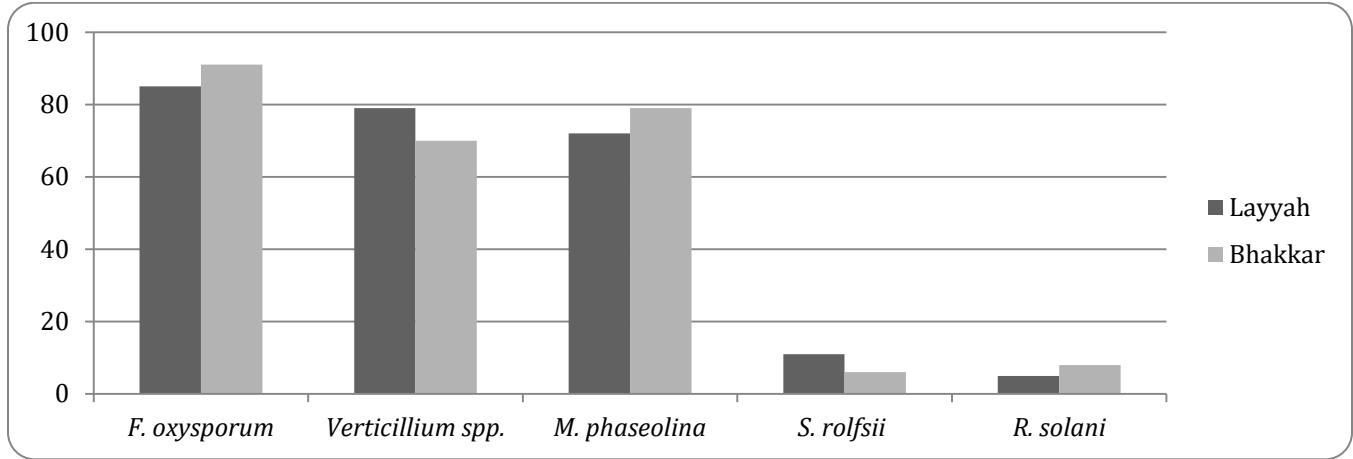


Figure 1. Frequency of chickpea root fungal pathogens from samples collected from Layyah and Bhakkar.

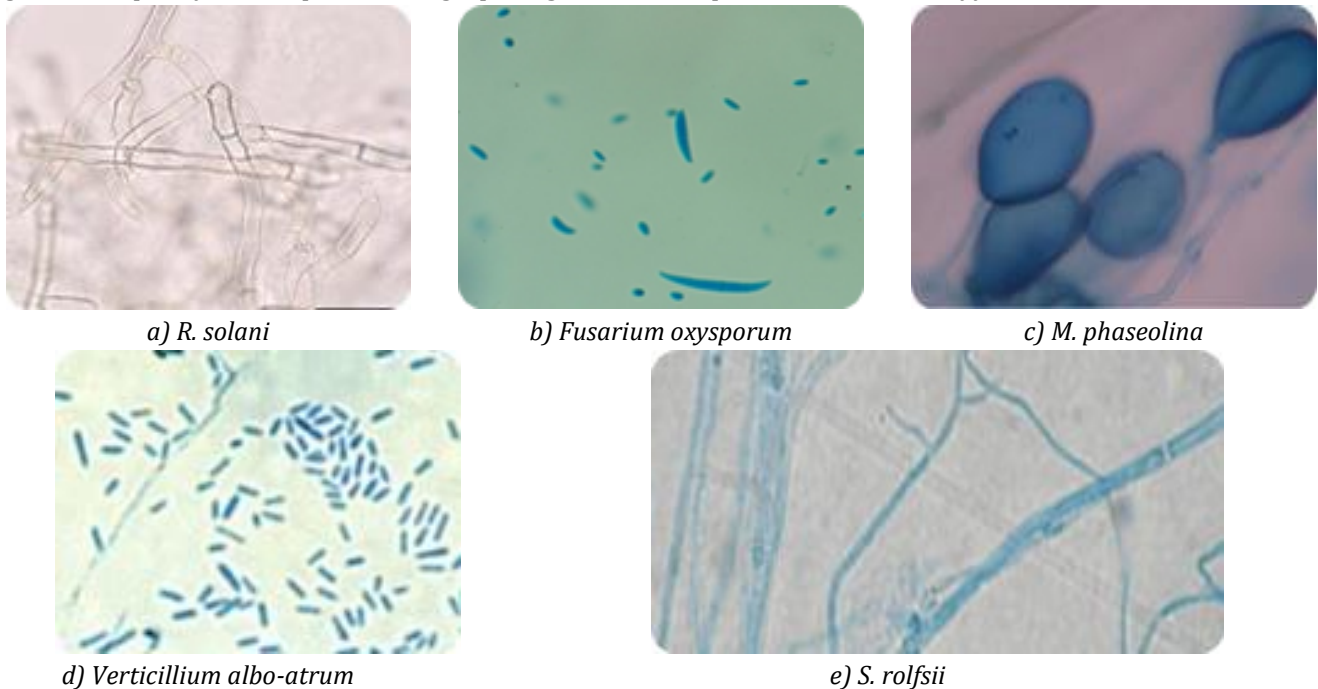


Figure 2. Morphological characteristics of fungal pathogens.

Major factor that play role in increased disease incidence is drought as less rainfall is received by these areas which favors the growth and development of several fungal pathogens (Ahmad, 2010). This disease cause 10-15% annual losses of chickpea grain yield ( Casas and Díaz, 1985), while (Haware *et al.*, 1990) reported that 10 to 15 % loss in the grain yield is the regular feature. In Pakistan the disease may cause 10-50 percent crop loss every year (Khan *et al.*, 2002).

Also it was observed in our study that among the fungal pathogens *F. oxysporum* is the most prevalent fungal pathogen responsible for wilting and highest recovery percentage was obtained with this pathogen. It has been observed that inoculum level in the soil determines the disease severity and ultimately losses. Thus it is imperative to work out on the ways to reduce the inoculum and lessen the incidence of root diseases of chickpea for better yield.

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