RESEARCH ARTICLE

Relationship between Mycoflora and Soil Functionality in Pigeon Pea (*Cajanus cajan* L.) in some Districts of Uttar Pradesh, India

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Abstract

India is the largest producer of pulses in the world, with 25% share in the global production. The leading pulse producing states are Madhya Pradesh, Maharashtra, Rajasthan, Uttar Pradesh, Karnataka and Andhra Pradesh. The important pulse crops are chickpea (48%), pigeon pea (15%), mung bean (7%), urdbean (7%) and lentil (5%). In the present communication, a survey was conducted from 2015 to 2016 of pigeon pea fields in eighteen locations at Agra, Prayagraj, Aligarh, Basti and Hardoi, districts. The pathogens isolated from pigeon pea plants were *Fusarium udum., Sclerotinia rolfsii* and *Rhizoctonia solani* mainly. Physically the textures of soil samples were found clay to sandy at several places among them sandy soil was dominating. Moisture content varied from 6.58 to 11.25%. During the course of study it became evident that pigeon pea (leguminous) plants were found to be wilting. It is also evident that the percent occurrence of wilted pigeon pea plants in different villages of the five districts was in the range of 6.03%-16.01%, whereas, the average wilting occurrence among the district varied from 9.03%-14.5%. Basti district showed maximum percentage of wilt occurrence (14.5%) while Agra, Prayagraj, Aligarh, and Hardoi showed 9.05%, 12.5%, 9.3% and 9.35%, respectively. It was also found that pH of the soil also affected diseases development. As in the fields having pH range of 6.5-8.0, the plants were found to be infected with wilt disease. However, at pH 5.0-6.5 the diseases could not be detected. In Basti, Prayagraj and Aligarh, due to alkaline pH (7.45-7.68) wilting disease on pigeon pea plants occurred frequently. Sandy loam favoured pigeon pea wilt. Sandy loams provide sufficient aeration in the rhizosphere of pigeon pea, which may be required for population build up of inoculum concentration of *Fusarium* leading to high incidence of wilt.

Keywords: Biodiversity, *Cajanus cajan* L., *Fusarium udum, Rhizoctonia solani, Sclerotinia rolfsii,* Soil Mycoflora

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Introduction

India is the largest producer of pulses in the world with 25% share in the global production. While chickpea is the topper among pulses occupying 39% of pulse area, pigeon pea follows with 21% area share. The major pulse producing states are Maharashtra(20%), Madhya Pradesh (17%), Rajasthan (11%), Uttar Pradesh (11%) and Andhra Pradesh (11%) together accounting for 70% of the total production of 14.76 million tonnes from an area of 23.63 m, ha (2007-08). In general, the pulse production is not keeping place with the domestic requirements, hence has to import 1.5-2.8 million tonnes per year, and is a matter of concern. In Uttar Pradesh, decrease in effective cultivable area for pulses due to the expanding townships, depletion of available nutrients in the soil and also the contamination by in judicious use of agrochemicals has necessitated investigations on the role of amendments in the soil in increasing the qualitative seed reserves and the quantitative yield of pulses.

Pigeon pea is grown in an area of 4.3 m ha and production of 3.3 million tonnes in Asia. India has the largest cultivated area (3.6 m ha) of pigeon pea. Maturity duration varies from about 120 days extra-early varieties to more than 260 days for long-duration varieties. Pigeon pea is an important constituent in the category of pulses among Indians. Availability of 20-21% protein in pigeonpea supplements the energy rich cereal diet. From natural resource management perspective cultivation of pigeon pea improves the soil quality status (upto 200 kg N ha^{-1}) ensuring better growth to succeeding crop by contributing about 40 kg ha^{-1}. Its stalks are sources of fuel and used for other socio-economic purposes in rural areas. Production levels of 1.7 million tonnes in 1950-51 increased to over 2.8 million tonnes in 2005-06 owing to the increase in acreage than the productivity per year. Consequently, per capita availability of pigeon pea has not been able to support the growing population. Pigeon pea varieties currently available has a potential to yield around 2.0 (short duration) to 3.5 (long duration) tonnes ha^{-1}. They are adapted to different agro-climatic intercropping niches, including low input conditions. The cultivated area has been increased from 3,33,000 ha in 1992-93 to 4,53,000 ha in 2006-08 in Andhra Pradesh, 1.01 m.ha to 1.14 ha in Maharashtra and 4,72,000 to 6,24 000 ha in Karnataka mainly due to the increase in productivity in these states to the tune of 109%, 64% and 103%, respectively. Gujarat registered 27% increased yield of pigeon pea. The average experimental yield in national trials is around 1300 kg ha^{-1}, but the national average yield is only 753 kg ha^{-1}, indicating slow pace of transfer of technology and that 75-90% increase in productivity can be achieved through improved adoption of existing technology and varieties.

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Commonly used soil amendments include municipal bio-solids, animal manures and litters, wood ash, coal combustion products such as fly ash, neutralizing lime products, composted bio-solids, and a variety of agricultural byproducts, as well as traditional agricultural fertilizers. Soil amendment reduces exposure by limiting many of the exposure pathways and immobilizing contaminants to limit their bio availability. The addition of amendments restores soil quality by balancing pH, adding organic matter, increasing water holding capacity, re-establishing microbial communities, and alleviating compaction. It enables site remediation, revegetation and revitalization.

Also for many years, microbiologists have tried to culture beneficial microorganisms for use as soil inoculants to overcome the harmful effects of phyto-pathogenic organisms, including bacteria, fungi and nematodes. It is necessary to thoroughly understand the individual growth and survival characteristics of beneficial microorganism, including their nutritional and environmental requirements. Their ecological relationships and interactions with other microorganisms, including their ability to coexist in mixed cultures and after application to soils needs to be investigated for optimum benefits.

Soil is a dynamic and species rich habitat containing all major groups of microorganisms (Hagvar, 1998). The soil micro-community plays a vital role as the global element cycles and thus for life on earth because 60-90% of the whole terrestrial primary production is decomposed in the soil and furthermore, many waste products of human society are detoxified there (Giller, 1996). Microorganisms in the soil form a part of the biomass and contribute to reserve the soil nutrients and are generally referred to as the microbial biomass. Microbial biomass regulates the transformation and storage of nutrients; these processes affect many nutrient cycling functions including soil fertility and soil organic matter turnover (Horwath and Paul, 1994). There is a relationship between microbial diversity and soil functionality, by considering that 80-90% of the processes in soil are reactions mediated by microbes (Coleman and Crossley, 1996; Nannipeiri and Badalucco, 2003).

The fungi (pathogenic and non pathogenic) associated with the soil are known as soilborne fungi and play a fundamental role for the functioning of the soil ecosystem (Doron and Parkin, 1994, 1996; Hawksworth *et al.,* 1996). Due to their ability to decompose complex macro molecules like lignin or chitin they are essential for making the locked up nutrients like C, N, P, S easily available. Moreover, the fungal mycelia play an important role in the stabilization of the soil because it binds soil aggregates and thus reduces erosion and helps to increase the water holding capacity (Kennedy and Gewin, 1997). Fungi are dominant in acid soils because an acidic environment is not suitable for the existence of either bacteria or actinomycetes, resulting in the monopoly of fungi for the utilization of organic substrates (Bolton *et al.,* 1993). In the frame of agriculture, the micro flora is of great significance because it has both beneficial and detrimental role in overall productions (Whitelaw, 2000). Farm practices including crop rotations and fertilizer or pesticide applications influence the nature and dominance of fungal species (Pelczar and Reid, 1972). Similar to Plant Growth Promoting Rhizobacteria (PGPR), some rhizosphere fungi able to promote plant growth upon root colonization are functionally designated as Plant-Growth-Promoting-Fungi (PGPF) (Hyakumachi, 1994; Srivastava and Sharma, 2014).

Therefore, it is an attempt to explore the soil mycoflora in some selected pigeon pea growing agricultural lands of UP.

Materials and methods

Sampling sites

The study was conducted in pigeon pea fields of eighteen locations at Agra, Prayagraj, Aligarh, Basti and Hardoi districts.

Collection of soil samples

Soil sample was collected from different agricultural areas [healthy and diseased (problem) soil] in three replicates. In the collection of soil samples, first a soil profile was extracted and the surface of the profile was cleaned (Brown, 1958). Vertical samples were taken from 0-5 cm, 5-10 cm, 10-15 cm and 15-20 cm depths with a disinfected spatula. The spatula was applied perpendicular to the vertical surface of the profile. Samples were kept in sterile polythene in refrigerator.

Soil analysis

The soil texture was determined by wet sieving technique (Barbour *et al,* 1980). Soil reaction (pH) was determined by using digital pH meter. Moisture content of soil samples was calculated by oven drying the soil and determining the weight loss (Garrett, 1963).

Isolation of soil mycoflora

The soil micro fungi were isolated by Soil Dilution (Waksman, 1927) and Soil Plate Methods (Warcup, 1950) using Potato Dextrose Agar media (PDA). In the Soil Plate Method, soil samples (0.01 g) were dispersed in 1 ml of sterile distilled water in a sterilized petri dish and then approximately 10 ml of molten, cooled sterile agar was added and mixed. The soil particles were distributed through out the medium by rotating the petridish. The petridishes were incubated for 5-7 days at 25±2°C.

For Serial Dilution, soil samples (1.0 g) were suspended in 10 ml of sterilized water, giving a dilution of 1:10. Serial dilutions of 1:100, 1:1000, and 1:10,000 were prepared, and then a 1 ml aliquot from the 1:1000 dilutions were added to a petridish containing streptomycin (200 g L^{-1}). Thereafter, approximately 10 ml of molten, cooled sterile agar medium was added to each dish. Each dilution was replicated three times and the dishes were incubated for 5-7 days at 25± 2°C.

Identification was done by microscopic analysis using taxonomic guides, standard procedures and other relevant literature available on fungal systematic (Raper and Fennell, 1965; Ellis, 1971; Moubasher, 1993; Barnett, 1967; Gilman, 2001) in Mycology and Plant Pathology Laboratory, Lucknow University, Lucknow.

Survey of some pigeon pea field in Uttar Pradesh for the analysis of wilted plants

A survey of pigeon pea field was carried out in Pigeon pea wilting areas of Uttar Pradesh covering eighteen locations of Agra, Prayagraj, Aligarh, Basti and Hardoi districts. Regular trips were made during the years of 2015-2016. So as to collect wilted plants were selected randomly stage of plant growth. 3-4 villages were selected randomly in each district and three plots per village were taken for the collection of wilted specimens of pigeon pea plants. Three plots of one square meter of the fields were taken at random in each plots and wilted as well a healthy plants were counted. The wilted plants were kept separately in polythene bags after labeling the name of the district, village, plot, and spot number and brought to the laboratory for the isolation of wilt causing fungi (Singh, 2002).

$$\text{Percent occurrence wilted plants in spot} = \frac{\text{Total no. of wilted plants}}{\text{Total no. of plants in the spot}} \times 100$$

Results and Discussion

Survey was conducted from 2015 to 2016 of pigeon pea fields in eighteen locations of Agra, Prayagraj, Aligarh, Basti and Hardoi, districts. The data on villages study and severity of pigeon pea wilt is presented in Table 1 and Map 1.

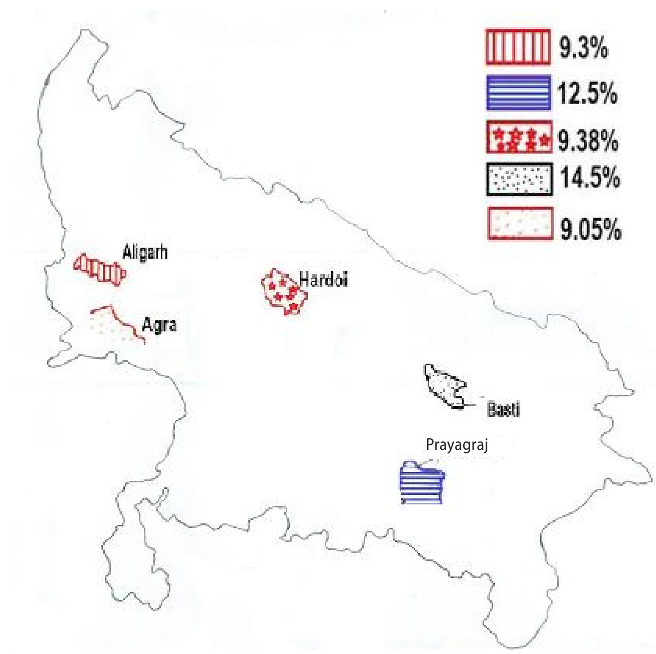
Physico-chemical properties of soil

Physically the textures of soil samples were clay to sandy dominating. Moisture content varied from 6.58 to 11.25%. The pH values ranged from 6.00 to 7.68 (diseased and healthy soil) and differed significant statistically (P>0.05) (Table 1).

Isolation of soil mycoflora

A total No. of 13 genera of micro fungi were isolated from all the pigeon pea growing agricultural lands of Uttar Pradesh. Highest number of species were isolated from upper soil (0-10 cm depth) while the lowest number of species were isolated from deeper profile (10-20 cm). The isolated fungi include *Alternaria* spp., *Aspergillus niger, Botrytis cineria, Chaetomium* spp., *Curvularia* spp., *Fusarium udam, Mucor racenosus., Penicillium* spp., *Rhizoctonia solani, Rhizopus arrhizus, Sclerotinia rolfsii, Trichoderma* spp. and *Verticillium* spp. (Table 2, Fig. 1-2). The dominated fungi in diseased soil *Fusarium udum, Sclerotinia rolfsii* and *Rhizoctonia solani* were observed whereas in healthy soil other fungi as *Aspergillus* spp., *Curvularia* spp., *Penicillium* spp., *Trichoderma* spp., *Chaetomium* spp. were observed.

During the course of study it became evident that pigeon pea (leguminous) plants were found to be wilting (Fig. 3-7). It is evident



Map 1: Severity of pigeon pea wilt in Uttar Pradesh.

Table 1: Soil pH, texture and moisture content at different sampling sites.

		Parameters		
S.N.	*Sampling sites*	*pH range*	*MC %*	*Texture*
1	Agra	6-6.8	7.65	clay
2	Aligarh	6.8-7.48	9.54	loam
3	Prayagraj	6.7-7.68	11.01	clay sand
4	Basti	6.90-7.45	11.25	clay sand
5	Hardoi	6-7.45	6.58	clay

Table 2: Status of soil fungi in different regions of Uttar Pradesh

	Different location of Utter Pradesh				
Fungi (isolates)	*Agra*	*Prayagraj*	*Aligarh*	*Basti*	*Hardoi*
Alternaria spp	++	+	+	++	+
Aspergillus niger	++	+	++	+	+
Botrytis cineria	+	+	-	++	-
Chaetomium spp	+	+	+	++	+
Curvularia spp	+	+	+	+	+
Fusarium udam	+	++	+	+++	+
Mucor racenosus	+	+	+	+	+
Penicillium spp	+	+	+	+	+
Rhizoctonia solani	++	++	+	+++	+
Rhizopus arrhizus	-	+	+	+	++
Sclerotinia rolfsii	++	++	+	+++	+
Trichoderma spp	++	+	++	+	+
Verticillium spp	+	+	++	+	+
Unidentified sterile mycelia	++	++	++	++	++

-: Not observed, +: Observed, ++: Moderate observed, +++: Highly observed

Fig. 1: Collection of soil from different locations of Uttar Pradesh.

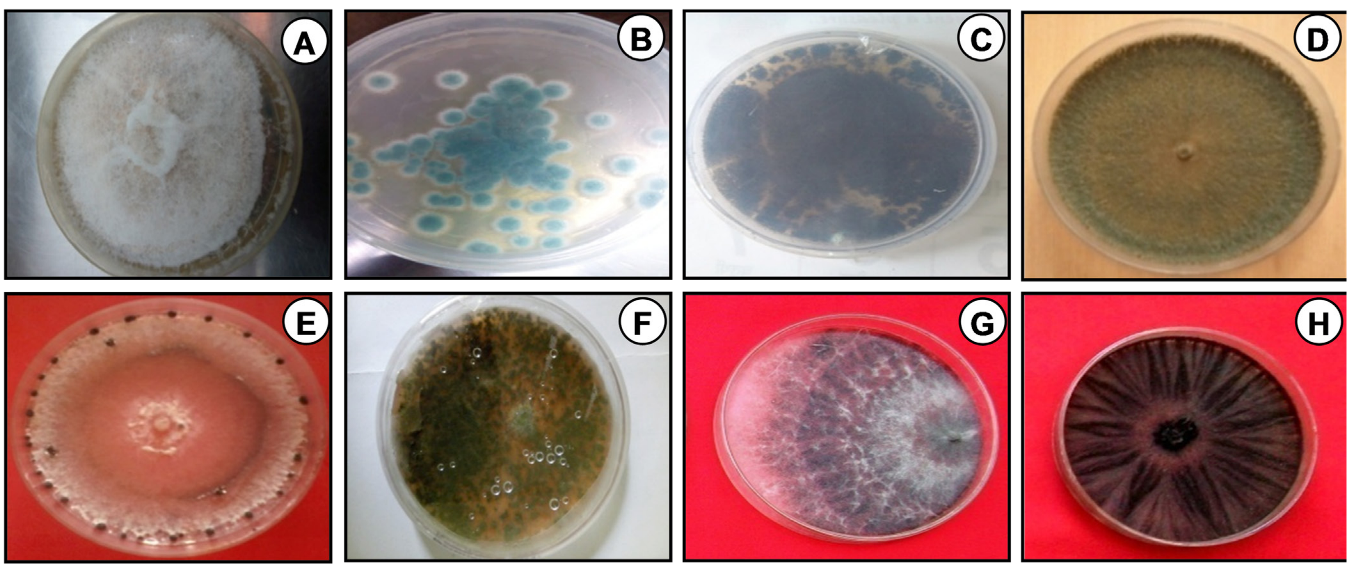


Fig. 2: Different types o f microfungi isolates from healthy and diseased (problem) soil. A. *Fusarium udum*, B. *Penicillium* spp, C. *Aspergillus* spp, D. *Trichoderma* spp, E. *Sclerotinia rolfsii,* F. *Alternaria* spp, G. *Curvularia lunata,* H. *Rhizoctonia* spp

that the percent occurrence of wilted pigeon pea plants in different villages of the five districts was in the range of 6.03-16.01%, whereas, the average wilting occurrence among the district varied from 9.03-14.5%. The average per cent of wilted plants of pigeon pea in Aligarh, Prayagraj, Basti and Hardoi district are 9.03, 12.5, 14.5 and 9.38%, respectively (Fig. 8). The minimum per cent wilting is reported in village Gulabgadi, district Aligarh (9.03%) and maximum in Gaur (16.01%) in Prayagraj district.

As agriculture remains a soil based industry, major increase in productivity is unlikely to be attended without ensuring that plants have and adequate and balanced supply of nutrients. Declining soil fertility and mismanagement of plant nutrients have made this task different. An integrated nutrient based approach to maintain and enhance soil, where both natural and manmade source of plant nutrients are used, is required for our state (Srivastava and Sharma, 2014). Soil moisture, pH and organic matter content influence the activity of soil microorganisms (Rama Rao, 1970; Behera and Mukerji, 1985). Fungi generally grow well in acidic conditions (Dix and Webster, 1995), but Jensen (1931) and Yamanaka (2003) have proved that fungi are abundantly found in alkaline soils and play a dominant role in the microbiological activity of such soils (Waksman, 1927).

In the present study soil pH ranges from neutral to alkaline which favors micro fungal growth. It was also found that pH of the soil also affected disease development. As in the fields having

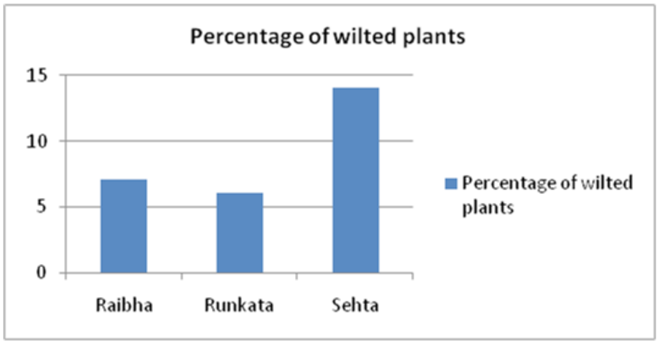


Fig. 3: Percent occurrence of wilted pigeon pea plants in different villages of Agra district. Average % of wilted plants in the district 9.05%; Range of % of wilted plants in the district 6.04-14.03%.

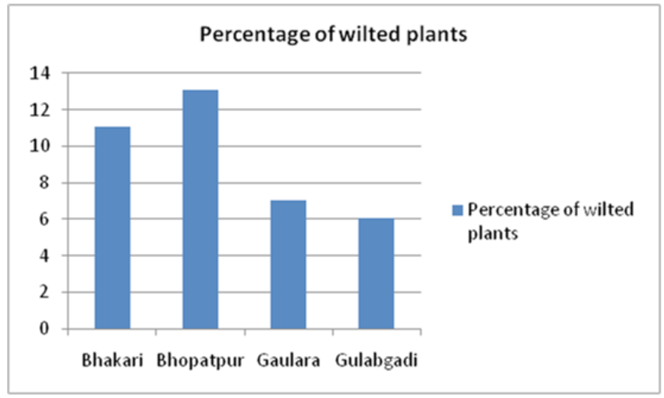


Fig. 4: Percent occurrence of wilted pigeon pea plants in different villages of Aligarh district. Average % of wilted plants in the district 9.3%; Range of % of wilted plants in the district 6.03-13.06%.

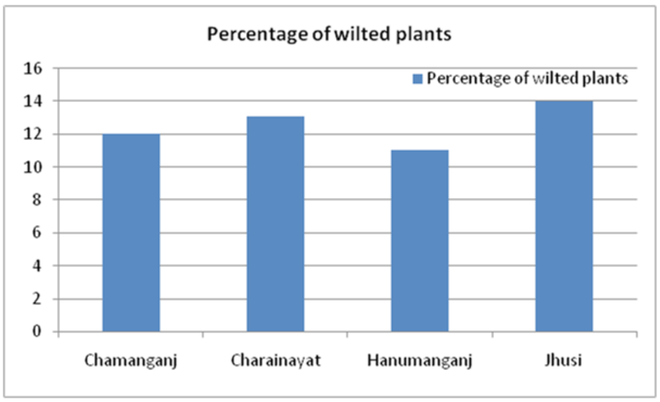


Fig. 5: Percent occurrence of wilted pigeon pea plants in different villages of Prayagraj district. Average % of wilted plants in the district 12.5%; Range of % of wilted plants in the district 11.04-14.04%.

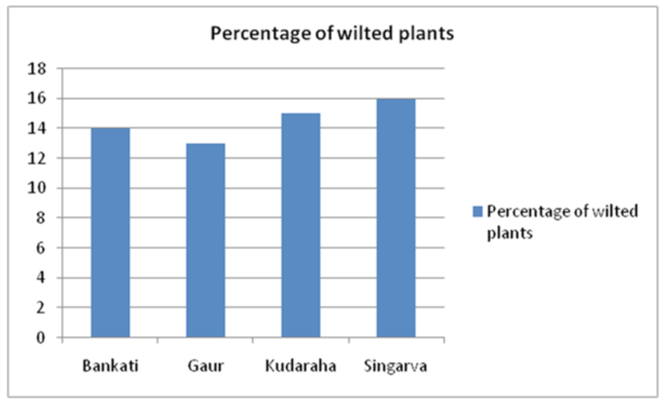


Fig. 6: Percent occurrence of wilted pigeon pea plants in different villages of Basti district. Average % of wilted plants in the district 14.5%; Range of % of wilted plants in the district 13.02-16.01.

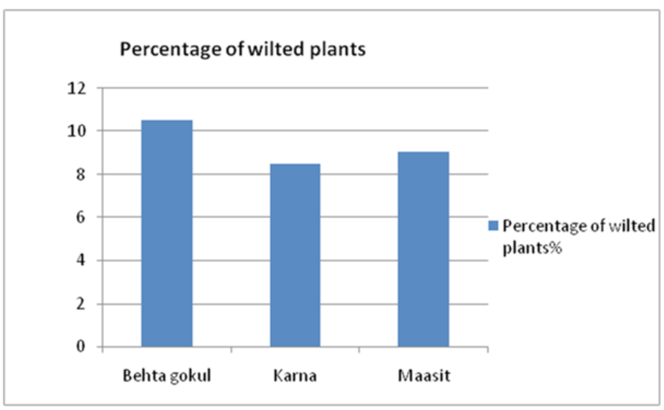


Fig. 7: Percent occurrence of wilted pigeon pea plants in different villages of Hardoi district. Average % of wilted plants in the district 9.38%; Range of % of wilted plants in the district 8.5-10.5%.

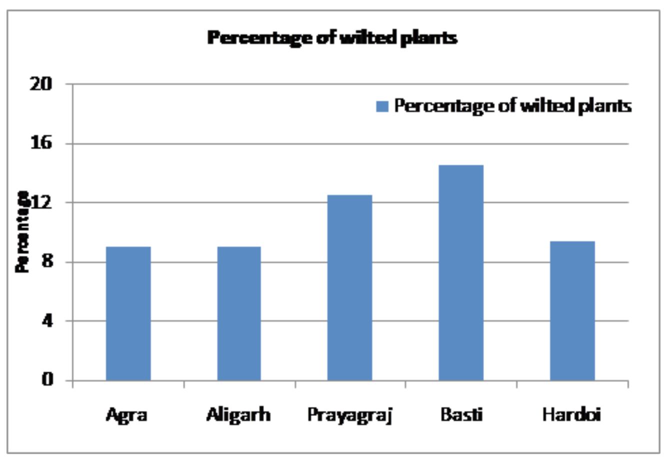


Fig. 8: Percent occurrence of wilted pigeon pea plants in different district of Uttar Pradesh.

pH range of 7.0-8.0, the plants were found to be infected by wilt disease, however, at pH 5-6.5 the diseases could not be detected. Species confined to upper layers were rarely found in deeper soils. This specific distribution is ruled by the availability of organic matter and oxygen to CO_2 ratio in the soil atmosphere of various depths (Giri *et al.,* 2005). These results are in agreement with the previous findings that biochemical activities tend to be greatly increased in the surface soil layer (0-10 cm) along with nutrient concentration (Aon and Colaneri, 2001). Further, surface soil of grazing lands and crop lands are comparatively rich in decaying organic matter with majority of annual herbal constituents which support a variety of microbes including microfungi (Guleri *et al.,* 2013). Highest number of species were isolated from upper soil (0-10 cm depth) while the lowest number of species were isolated from deeper profile (10-20 cm). The isolated fungi as *Alternaria* spp., *Aspergillus niger., Botrytis cineria., Chaetomium* spp., *Curvularia* spp., *Fusarium udam., Mucor racenosus,., Penicillium* spp., *Rhizoctonia solani., Rhizopus arrhizus., Sclerotinia rolfsii., Trichoderma* spp and *Verticillium* spp from the soil were observed. In diseased soil, the dominated fungi isolated from pigeon pea plants were *Fusarium udum, Sclerotinia rolfsii* and *Rhizoctonia solani* were observed. Whereas other fungus as *Aspergillus* spp., *Curvularia* spp., *Penicillium* spp., *Trichoderma* spp,, *Chaetomium* spp. is observed as in healthy soil.

The majority of the taxa showed qualitative variation with increasing soil depth. In agriculture system, wide changes in community structure take place at the surface layer due to activity of herbaceous undergrowth. At greater depth, differences of distribution level or patterns are usually reversed according to the prevailing conditions (moisture, potential oxygen and substrate availability) at various intervals after the respective operations (Domsch, 1986). The occurrences of fungal populations are also correlated with the availability of mineral nutrition and other factors including temperature, moisture, etc (Vanvuurde and Schippers, 1980). For a given community, it is generally observed that one or a few species are numerically predominant and may strongly affect environmental conditions for other species (Durall and Parkinson, 1991). In the present study, few species were regularly isolated at relatively high frequencies. These species also had the most widespread and least aggregated distribution. The low levels of aggregation observed for these fungi may reflect a relatively broad or diverse niche space that may be the result of successful adaptation to many dimensions in the system. The purpose in categorizing soil fungi based on their appearance in all or some sites and on their abundance is to indicate their chances of disappearing from the areas. The soil fungi with a species distribution that was categorized as common or frequent, but not rare, will not disappear easily from the wheat fields, while those that distribution was categorized as moderate or rare will disappear easily from the sites.

Wilt (also known as vascular wilt or true wilt) cause by *Fusarium udum,* Snyder and Husen is the most economically devastating disease of pigeon pea. In India wilt is a serious problem on all over the pigeon pea growing area. Butter (1906) reporting the wilt disease recorded 15-25% mortality of plant with an indication of its rise to > 60% in coming years. The average incidence has been found to be varying from 00.1% to 22.6% (Singh. 2002).

During the course of study it became evident that pigeon pea (leguminous) plants were found to be wilting **.**it is also evident that the percent occurrence of wilted pigeon pea plants in different villages of the five districts was the in the range of 6.03%-16.01, whereas, the average wilting occurrence among the district varied from 9.03%-14.5%. Basti district showed maximum percentage of wilt occurrence (14.5%) while Agra, Prayagraj, Aligarh, and Hardoi showed 9.05%, 12.5%, 9.3% and 9.35%, respectively.

Luitel and Koirala, (2009) reported that, there was no marked variation in soil texture of the different sites and there is no significant effect of texture on fungal populations. But in our study, sandy loam favoured pigeon pea wilt. Sandy loam provide sufficient aeration in the rhizosphere of pigeon pea, which may be required for population build up of inoculum concentration of *Fusarium* leading to high incidence of wilt disease.

The primary role of soil amendments is to provide nutrients for crop growth or to provide materials for soil improvement. Misuse of soil amendments can result not only in damage to crops but can also cause negative impacts on the receiving soil, water, air or habitat environment. Low agricultural production is closely related to a poor coordination of energy conversion which, in turn, is influenced by crop physiological factors, the environment, and other biological factors including plant and soil microorganisms. The soil and rhizosphere microflora can accelerate the growth of plants and enhance their resistance to disease and harmful insects by producing bioactive substances.

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