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CORRELATION BETWEEN PHYSICOCHEMICAL CHARACTERS AND RHIZOSPHERIC MYCOFLORA FROM SOIL OF PIGEON PEA FIELD IN JALAUN DISTRICT

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Abstract- **Soil is an excellent culture media for the growth and development of various microorganisms. It is a mixture of five component i.e., organic matter, mineral matter, soil air, soil water and soil microorganism. Soil is not an inert static material but a medium pulsating with life. The amount proposition of different components in soil varies with localities in climate. The study was undertaken to investigate the relationship among physicochemical characteristic of soil and their impact on microfloral diversity and wilt disease incidence of pigeon pea, from ten different villages belonging to Jalaun district. Soil sample were analyzed for various characteristic pH, EC, soil moisture, N, P, K, organic carbon. Soil microflora was isolated. Wilt incidence was estimated. A total 20 fungal species belonging to 12 genera comprising of Aspergillus, Alternaria, Botrytis, Curvularia, Cladosporium, Fusarium, Pythium pathogenic and Aspergillus, Emericella, Mucor, Penicillium, Rhizopus, Trichoderma saprophytic fungi was isolated. There was no uniform trend visualized among the correlation of soil microflora with physical and chemical property of soil. Soil pH, Moisture, EC and P showed significant impact and other component like C, N and K also affect the growth of soil microbiota.**

Key Words: **Pigeon pea, Soil characteristic, Wilt disease incidence and Soil microflora.**

I. INTRODUCTION

Pigeon pea (Cajanus cajan (L.) Millspaugh) is an important legume crop predominantly cultivated in tropical and subtropical countries. It plays an important role in food and nutritional security of population because it is rich in protein, minerals and vitamins (1). It is cultivated in about 24 countries of the world. India is the biggest consumer and producer of the pigeon pea approximately 5.39 million hectares area is under pigeon pea cultivation with 4.87 million tones production (2). The production is decrease year to year because of several phytopathological disease caused by bacteria, virus and fungi and climatic issues. The disease affecting yield of pigeon pea is usually soil borne. The pigeon pea wilt caused by Fusarium udum Butler is a major threat to the growers of pigeon pea. Soil-borne fungi are an important biotic component of the soil. They not only cause a number of plant and animal diseases but also have some beneficial aspects like phosphate solubilization, organic matter decomposition etc. The growth of the crop plants is also influenced by the fungal population present in the soil. It is now a proven fact that the fungal genera which reside in soil are sensitive to one or the other soil physico-chemical characteristics (3, 4, 5). The members and kinds of micro-organisms present in soil depend on many environmental factors (6). Physical and chemical component of soil affects the density and diversity of microbial population (7). The growth of the soil fungi is favoured by amount of carbon, nitrogen, phosphorus, sulphur and water present in the soil (8, 9) and soil pH also regulates the availability of nutrients in soil. Soil microorganism is an important factor in controlling availability of nitrogen from organic matter of soil (10).

There have been positive correlations between the fungal population with soil pH, moisture content, organic carbon, total nitrogen concentration and available potassium (11).

II. MATERIAL METHOD

Study area and location:

Jalaun is a part of Jhansi division. The district lies entirely within the level plain of Bundelkhand, and is almost surrounded by the Yamuna, Batwa and Pahuj river from northern, southern and western boundaries. According to Koppen and Geiger system (12), climate of this district is warm and temperate and classified in Cfa category. There is a great shortfall of rainfall in Jalaun, even in the driest month. The average rainfall and temperature are 912 mm and 25.8°C respectively. The district has been under severe drought for last four year.

Method for collection of soil samples and diseased plant material:

The soil samples were collected from different villages of Jalaun district during November 2017 to January 2018. During the study at regular intervals the soil samples were collected thrice. Five samples were collected from five different 5 fields of each ten village. Overall 250 samples were collected. After taking a fresh soil sample for isolation of fungal flora, rest of the soil of each sample was air dried, crushed, sieved and stored in clean plastic bottles for chemical analysis and isolation of soil mycoflora. The disease incidence was assessed by counting the number of plants showing symptoms in 100 plants were randomly selected in each field.

Isolation and identification of fungi from the soil samples:

Isolation of rhizospheric soil mycoflora was done by soil dilution technique according to Johnson et al (13). 1 ml of suspension was taken out and inoculated onto PDA plates. Plates were incubated at 28 ± 2^0C for 7 days. After the incubation period isolated fungi were identified. The fungal morphology was studied macroscopically by observing colony features like colour and texture. In our study 20 species of fungi belonging to 12 genera were isolated. The fungal flora was analyzed from different samples of selected sites. The identification and classification of fungi was done by Gilman (14) and Alexopolus et al (15). It is simple and widely accepted. Data regarding the number of saprophytic and pathogenic fungi per gram soil was recorded.

Physico-chemical analysis of soil:

Air dried soil was ground to pass through 2 mm sieve. **Electrical conductivity** of saturated soil pastes extract (EC) and **pH** of saturated soil paste (pHs) were determined by digital conductivity and pH meters, respectively. For **nitrogen** determination, a known weight of soil was acid-digested followed by distillation on Kjeldahl apparatus. Olsen method was used to determine NaHCO3-extractable **phosphorus**. Ammonium acetate-extractable potassium was determined on flame photometer by comparing with a standard curve of known **potassium** concentrations (16).

Statistical analysis:

Data regarding number of total saprophytic and pathogenic fungi colonies were calculated in 1g of soil per plate. The percent contribution of each isolate was calculated by using the following formula:

Percent contribution = $\frac{\text{Total no.of CFU of an individual specie}}{\text{Total no.of CFU of all species}} \times 100$

***CFU= colony forming unit**

The percent disease incidence was calculated using the formula,

Percent disease Incidence= $\frac{\text{No of infected plants}}{\text{Total no of plant observed}} x100$

The data were analyzed by the standard statistical methods. The graphs and other statistical analysis were done using the software Microsoft Excel office 2019 (Window 10 Home). Correlation of disease incidence with different soil characteristics was calculated by Bivariate Pearson's correlation (One tailed test of significance) using IBM SPSS Statistics 20.

III. RESULT

Soil chemical characteristics: Analysis revealed that soil of the entire experimental field was normal to slightly alkaline within a pH range of 7.0 to 7.8. However, other chemical properties of soil of different pigeon pea field are different from each other. EC of soil is varying from 0.11-0.73milimhos/cm^2 and the organic carbon present in different soil samples is 0.34-0.86 %. The amount of phosphorus is insufficient in maximum selected field of pigeon pea varies from 2.9-25.0 Kg/h in the selected fields. In present study, nitrogen and potassium varies from 82-209 Kg/h and 161-304 Kg/h respectively. Maximum fields of pigeon pea have insufficient amount of nitrogen while potassium is present in high amount in fifty percent of selected villages. Study revealed that fields of villages Kadaura, Lamsar and Ookurua have more disease incidence in comparison to other villages.

Soil Mycoflora: As per our result total 601 colony forming units (CFUs) were isolated from selected villages in which 360 and 224 CFUs belong to saprophytic and pathogenic fungal category respectively and rest 17 were unidentified. A total of 20 fungal species belonging to 12 genera were isolated from various soil samples. Among the isolated fungi, the genus Trichoderma (4 species) was dominant with 154 fungal colonies (CFUs) followed by 3 species of Aspergillus with 116 fungal colonies (CFUs), Penicillium (3 species) with 71 fungal colonies (CFUs), Fusarium (2 species) with 84 fungal colonies (CFUs). All other genera were represented with one species each. The total no of CFU was high in the soil of village Chhonk and low in the soil of village Aata. A. niger, A. flavus, A. oryzae, Emericella nidulens, F. udum, F. oxysporum, Mucor sp. P. crysogenum, Penicillium citrinum, Rhizopus sp., T. harzianum, T. viride, T. koningii, T. psudokoningii were most common fungal species found in the rhizospheric soil of pigeon pea field. Out of these, A. niger, F. udum, F. oxysporum, P. crysogenum and Rhizopus sp. were frequently found in all the fields of selected 10 villages and

Botrytis sp. and Cladosporium sp. was found minimum in the soil of selected villages.

Correlation Studies: Correlation between total number of fungal colonies, number of saprophytic and pathogenic fungal species with C, N and K was insignificant. There was positive and significant (P≤0.01) correlation observed between total numbers of colonies, saprophytic fungi with soil moisture. Similarly, Correlation of saprophytic with EC is positive and significant (P≤0.05). pH of soil expressed negative and significant (P≤0.05) correlation with number of pathogenic fungi. Likewise, the correlation of saprophytic fungi with phosphorus is negative and significant (P≤0.05). The relationship between disease incidence and soil pH, EC, moisture, carbon and phosphorus was insignificant. Correlation of disease incidence with nitrogen was positive and significant (P≤0.05) while with potassium it was negative and significant (P≤0.01). The relationship between disease incidence with pathogenic fungal flora was positive and significant (P≤0.05) and negatively correlated with saprophytic fungi.

Table 1: Physicochemical Analysis of Rhizospheric Soil and Disease Incidence of Pigeon Pea Field in Different Villages of Jalaun District

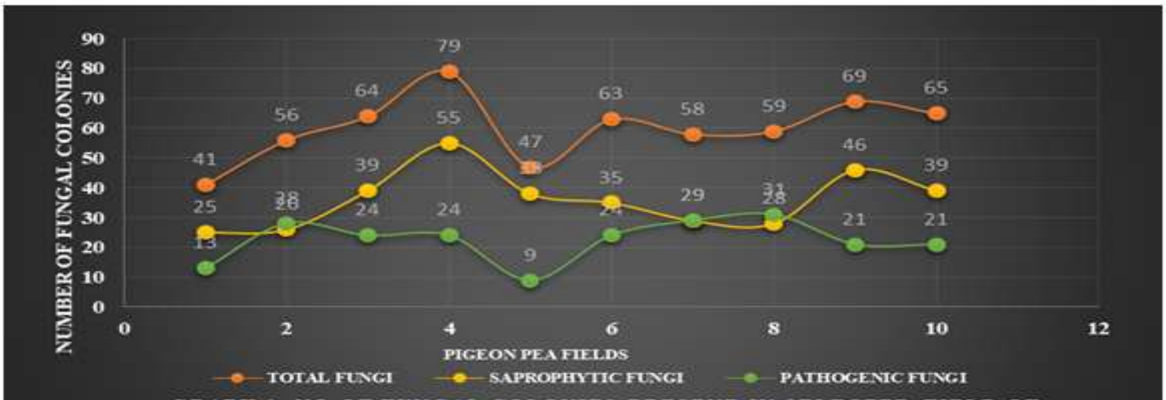
S. No.	Villages	Soil P^H	Soil EC	Soil Moisture (%)	C (%)	N (Kg/h)	P (Kg/h)	K (Kg/h)	Disease Incidence* (%)
1	**Aata**	7.5	0.15	5.86	0.43	132	23.5	248	8
2	**Babina**	7.0	0.35	17.94	0.65	167	15.8	304	6
3	**Bagi**	7.5	0.73	15.98	0.28	209	17.3	288	7
4	**Chhonk**	7.3	0.59	24.62	0.55	87	2.9	187	7
5	**Jolhopur**	7.8	0.38	9.32	0.63	108	8.6	343	5
6	**Kadaura**	7.0	0.52	9.63	0.86	201	18.4	158	11
7	**Lamsar**	7.3	0.11	12.0	0.92	178	13.7	161	14
8	**Ookurua**	7.2	0.28	7.98	0.37	203	25.0	201	12
9	**Saidnagar**	7.0	0.27	22.32	0.46	82	15.8	300	6
10	**Tagarepur**	7.0	0.43	19.87	0.34	198	21.4	276	8

*** Value are the mean of five fields per village**

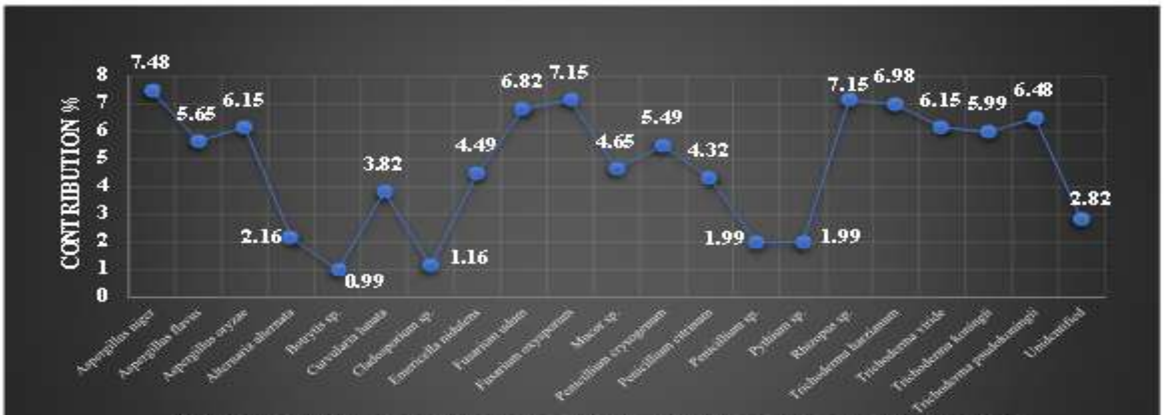
Table 2: Occurrence of Rhizospheric Soil Mycoflora of Pigeon Pea Field in Different Villages of Jalaun District

S. No.	Rhizospheric soil Mycoflora	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	Total (individual sp.)	Contribution %
PATHOGENIC FUNGI													
1	**Aspergillus niger**	3	5	6	4	2	4	8	3	5	5	**45**	**7.48**
2	**Aspergillus flavus**	2	6	4	5	-	1	3	2	7	4	**34**	**5.65**
3	**Alternaria alternate**	-	1	4	3	-	2	2	1	-	-	**13**	**2.16**
4	**Botrytis sp.**	1	-	-	2	1	2	-	-	-	-	**6**	**0.99**
5	**Curvularia lunata**	2	4	1	2	-	-	3	5	2	4	**23**	**3.82**
6	**Cladosporium sp.**	-	3	1	-	-	2	-	-	1	-	**7**	**1.16**
7	**Fusarium udum**	3	4	2	4	3	7	5	8	3	2	**41**	**6.82**
8	**Fusarium oxysporum**	2	4	3	2	3	6	6	8	3	6	**43**	**7.15**
9	**Pythium**	-	1	3	2	-	-	2	4	-	-	**12**	**1.99**

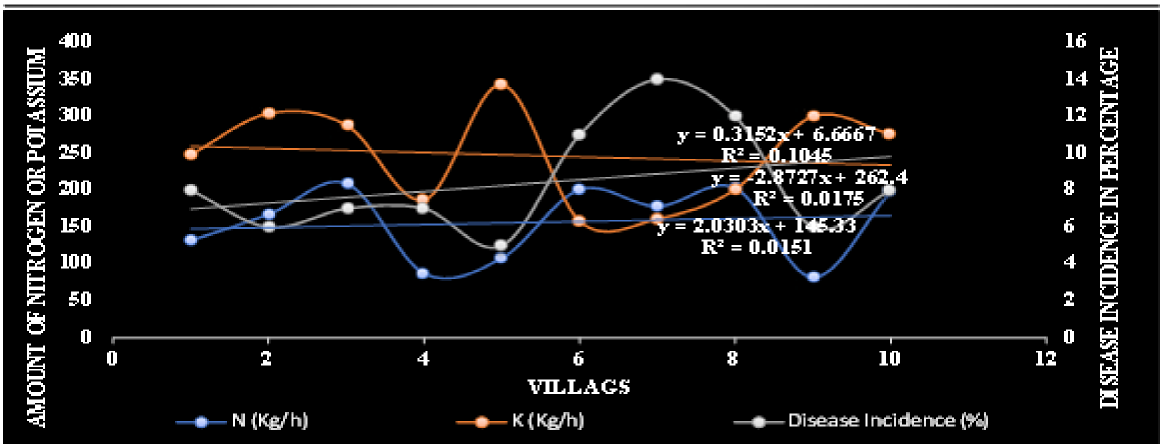
	sp.												
SAPROPHYTIC FUNGI													
10	**Aspergillus oryzae**	3	4	6	5	5	1	-	1	8	4	**37**	**6.15**
11	**Emericella nidulens**	2	-	1	4	6	4	5	3	1	1	**27**	**4.49**
12	**Mucor sp.**	-	5	2	6	4	4	2	1	1	3	**28**	**4.65**
13	**Penicillium crysogenum**	3	2	3	3	2	1	4	6	4	5	**33**	**5.49**
14	**Penicillium citrinum**	-	2	4	4	1	1	3	3	2	6	**26**	**4.32**
15	**Penicillium sp.**	4	-	-	3	2	-	-	-	2	1	**12**	**1.99**
16	**Rhizopus sp.**	2	4	5	6	2	7	3	5	4	5	**43**	**7.15**
17	**Trichoderma harzianum**	4	-	6	8	4	3	6	2	4	5	**42**	**6.98**
18	**Trichoderma viride**	4	2	4	7	5	5	2	3	5	-	**37**	**6.15**
19	**Trichoderma koningii**	3	4	3	5	4	5	2	-	7	3	**36**	**5.99**
20	**Trichoderma psudokoningii**	-	3	5	4	3	4	2	4	8	6	**39**	**6.48**
21	**Unidentified**	3	2	1	-	-	4	-	-	2	5	**17**	**2.82**
22	**Total Mycoflora (CFUs)**	**41**	**56**	**64**	**79**	**47**	**63**	**58**	**59**	**69**	**65**	**601**	



GRAPH 1: NO OF FUNGAL COLONIES PRESENT IN SELECTED FIELD OF PIGEON PEA



GRAPH 2: CONTRIBUTION % OF RHIZOSPHERIC SOIL MYCOFLORA OF PIGEON PEA FIELD IN JALAUN DISTRICT



GRAPH 3: RELATIONSHIP BETWEEN DISEASE INCIDENCE AND AVAILABILITY OF NITROGEN AND POTASSIUM IN RHIZOSPHERIC SOIL OF PIGEON PEA FIELD

Table 3: Correlation between number of fungal colonies and disease incidence with soil characteristic of pigeon pea

	pH	**EC**	**Soil Moisture (%)**	**C**	**N**	**P**	**K**	**Disease incidence (%)**
Total No of colonies	-0.496	0.544	0.810**	-0.082	-0.098	-0.402	-0.298	-0.005
No of colonies of pathogenic fungi	-0.610*	0.046	0.222	0.148	0.506	0.162	-0.546	0.586*
No of colonies of saprophytic fungi	-0.017	0.570*	0.746**	-0.179	-0.532	-0.668*	0.048	-0.415
Disease incidence	-0.224	-0.366	-0.431	0.425	0.556*	0.355	-0.834**	

**Correlation is significant at the 0.01 level.
*Correlation is significant at the 0.05 level.

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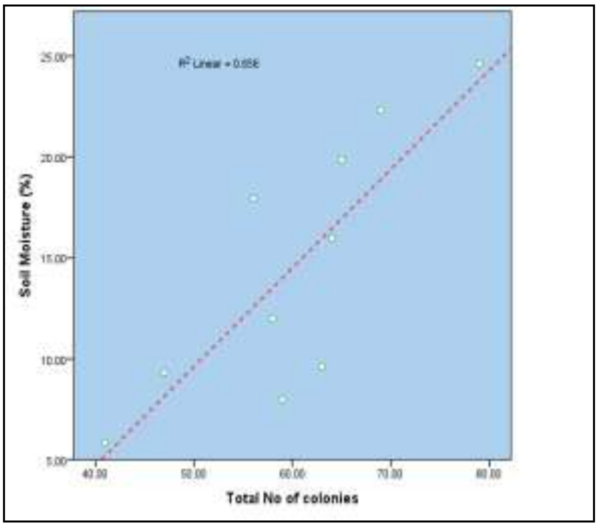
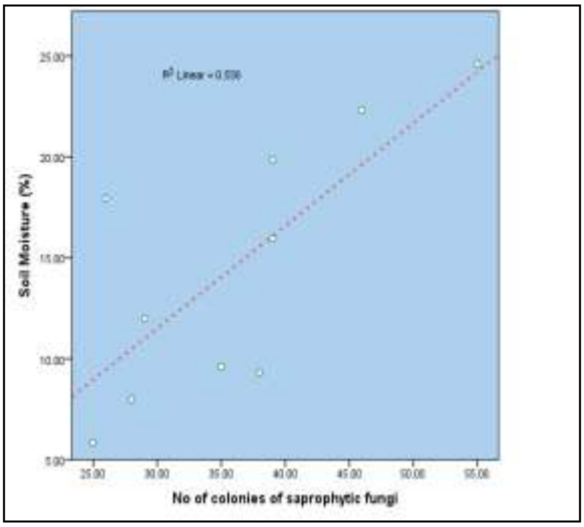
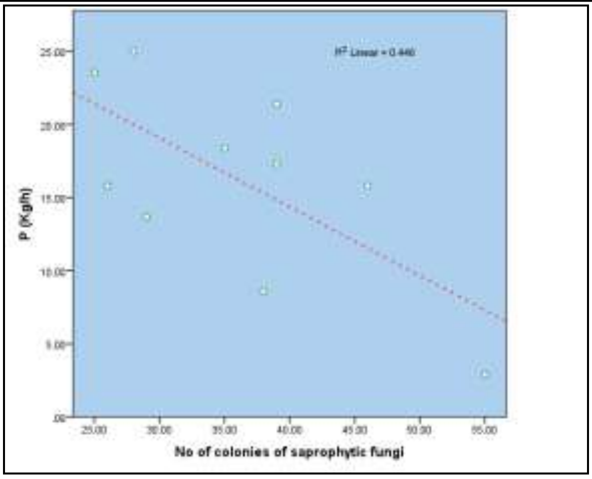


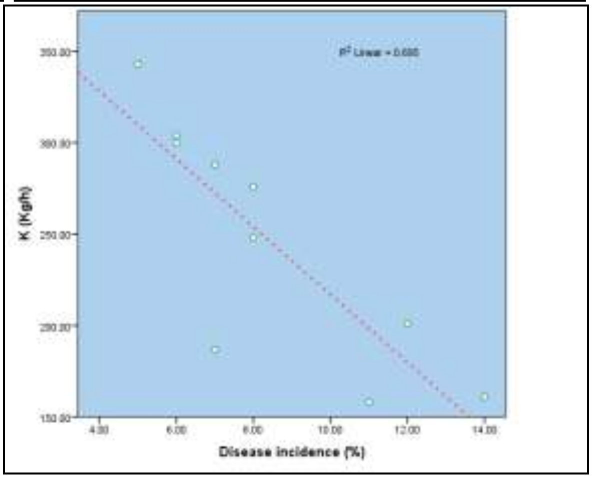
The graph represents strong positive correlation in between soil moisture and total number of colonies.



The graph represents strong positive correlation in between soil moisture and number of colonies of saprophytic fungi.



The graph represents moderate negative correlation in between phosphorus and number of colonies of saprophytic fungi.



The graph represents strong negative correlation in between potassium and disease incidence (%).

From experimental findings it can be concluded that there is some relationship between fungal flora, physicochemical characters of soil and the incidence of wilt disease. In the soil samples of some villages (Jolhopur and Saidnagar) the amount of the amount of potassium is higher than nitrogen and the number of saprophytic fungi was higher than pathogenic fungi. In these villages wilt incidence was 5 and 6 % respectively. In the same manner the disease incidence in village Bagi and Chhonk was 7% and the soil sample possessed lesser amount nitrogen to potassium. In Babina, the number of pathogenic fungi is high but the incidence of wilt disease is low. Here the amount of potassium was more than nitrogen this imparted negative impact on wilt disease. Similar condition was observed in village Aata and Tagarepur. Reverse condition was visualized in village Kadaura. In Lamsar highest disease incidence (14%) was recorded with nitrogen 178Kg/h and potassium 161Kg/h. Pathogenic and saprophytic fungi was similar but the virulence of pathogenic fungi is higher over saprophytic fungi. In village Ookurua, number of pathogenic fungi was higher than saprophytic fungi and the amount of nitrogen was higher than potassium.

In this study we concluded that the incidence of diseases is affected by chemical properties of the soil and the presence of soil mycoflora. Presence of saprophytic fungi changes the effectiveness of the pathogen. The effectiveness of pathogenic fungi or saprophytic fungi is depending upon the virulence range, geographical distribution, soil type, and climatic condition. The changes in any factor are also affecting the impact of disease incidence in a crop life.

IV. DISCUSSION

Fungal diversity of soil in an area is affected by many environmental factors such as pH, organic content, temperature and moisture (17, 18) as well as physicochemical factors. The moisture content, organic carbon, nitrogen, phosphorus, potassium are the fertility parameters of a cultivated land. It has been widely established that plant growth and productivity are highly affected by amount and type of organic and inorganic nutrients in soil (19, 20, 3). Current analysis of soil samples for nitrogen and phosphorus was 82-209 Kg/h and 161- 304 Kg/h respectively. This study indicated that most of the selected fields have inadequate amount of nitrogen and phosphorus for healthy plants even less than recommended amount. Soil pH and electrical conductivity (EC) are the important characteristics that describe the suitability of the soil for growing plants and a good indicator of available nutrients to plants. EC affects the membrane permeability of plant for available nutrients in soil (21). It is an important factor for plant health. In the present study a wide range of EC 0.11-0.73 millimho/cm^2 was recorded from the different soil samples collected. It is recommended that for the normal plant growth EC should be less than 1 millimho/cm. Although crop plants vary in salt tolerance but as a general rule ECe up to 4 dS m-1 (4000 μS cm-1) is considered normal for plant growth (22, 4). Trolldenier (23) suggested that soil with pH 5.5 provides optimum environment for fungal growth, however at higher pH bacteria dominate over fungi. In our study the pH range varied from 7.0 to 7.8 in different soil sample. Sampling was done at the mid age of pigeon pea, in this duration the atmospheric condition become favourable for the growth of fungi. Moisture present in the different soil samples was 5.86 to 24.62%. It's a mere fact that microbial activity is always higher in monsoon season followed by winter while it lowers in summer. Organic matter and moisture content favours the growth of microbial population (24). According to Devi and Chhetry (25), some species of Gliocladium virens, Phoma sp., Rhizopus nigricans, Nigrospora sp., and Verticillium sp. were isolated from rhizospheric soil of pigeon pea plant. Shukla and Mishra (24) isolated A. dutrenticum, A. fumigatus, Chaetomium globosum, Fusarium sp., P. citrinum, P. crysogenum, Rhizoctonia solani, Rhizopus nigricans, Alternaria tenuis, A. dutrenticus, and Aspergillus sydowii from different depth of rhizospheric soil of pigeon pea. In our study A. niger, Rhizopus sp., F. oxysporum, T. harzianum, F. udum, T. psudokoningii, A. oryzae and T. viride was dominant. Jalander and Gachande (26, 27) obtained species of Aspergillus, Fusarium Penicillium Cladosporium and Curvularia were very commonly isolated from the rhizospheric soil of pigeon pea.

It has been established by many researchers that level of potassium in soil is negatively correlated with the prevailing pathogenic flora (28, 4). According to Karimi et al (29) higher soil pH reduces the disease incidence, due to decreased availability of the micronutrient that is essential for the growth, sporulation and virulence of the pathogen (30). Studies have also shown that high levels of nitrogen fertilization in agricultural soils generally increase wilt development (31, 32). High nitrogen and low potassium favoured the disease, while low nitrogen and high potassium retarded disease development (33).

V. CONCLUSION

In the present study, on the estimation of correlation of soil nutrient status with fungal population, it can be concluded that pathogenic fungal population show insignificant relation with soil nutrient while saprophytic population is adversely affected by phosphorus. The relationship between disease incidence with nitrogen is positive and significant (($P \leq 0.05$) while with potassium is negative and significant (($P \leq 0.01$) which indicate that the amount of nitrogen and potassium present in the soil significantly affects the wilt disease incidence.

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