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Isolation of fungal endophytes at diferent pH from two wild plants of Mirzapur district (U.P.) India

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ABSTRACT

The impact of pH on the microbial colonisation of endophytes on two plants (*Buchanania* and *Celastrus* sp.) was studied. The leaf and stem of two plant species were subjected to two PDA (Potato Dextrose Agar) medium at pH levels (4.5 and 9) for 5 weeks for isolation of fungal endophytes in aseptic condition. Over the testing period, pH 4.5 produced the highest fungal colony. At different pH values, the fungal populations were noticeably different, with consistently higher counts at pH 4.5 and 9, respectively. At pH 4.5 isolated fungi were *Aspergillus niger, Alternaria, Curvularia, Fusarium, Rhizopus, Mucor, and Penicillium,* and at pH 9.0 the isolated fungi were *Aspergillus fumigatus, Penicillium, Alternaria, Curvularia, Fusarium,* and *Rhizopus* respectively. It demonstrates that varied pH levels led to changes in the fungal populations in plant species, leaves and stems (resistance/ susceptibility to environmental influences).

Figures : 05 References : 39 Tables : 02
KEY WORDS : *Buchanania*, *Celastrus*, Colonization, Endophytes, Fungi, Leaf, Stem

Introduction

India has many regions of unique ecological niches harboring variety of medicinal plants. One such region is Vindhya region (U. P.), India. The Vindhya region of Uttar Pradesh lies between 82 degrees E to 83 degrees E longitudes and 22 degrees 45'N to 34'N latitudes, which includes districts Mirzapur and Sonbhadra. The forest of the Vindhya region is tropical dry deciduous type. The districts of the Vindhya region are inhabited by a large number of tribes such as Baiga, Bhil, Gond, Kharwar, Musahar, Parja, *etc.* A significant portion of the plant micro-biota is made up of a hyper-diversified population known as plant endophytes. When colonizing their hosts, endophytes develop a cooperative connection with the plant that is housing them.[31,36] Endophytic microorganisms can colonise the roots, leaves, stems, fruits, and seeds of plants. The role of endophytic bacteria for health has been extensively investigated.[10] They produce antibacterial chemicals and are known to stimulate plant growth and infection resistance.[2,3,12,32] and are sources of bioactive compounds [30].Endophytes enter plant primarily through the root system, as well as through the stems, cotyledons, radicles, stomata, and flowering parts. The pH of the soil plays a significant role because it influences the amount of minerals (Fe, Mg, Cu, Zn, Bo, HPO_4, Ca, and Mg) available for plant usage. At ideal pH ranges between 3 and 5, and at a temperature of 26°C, the growth of fungal infections and their transfer from one host to another are promoted. [3.] The objective of the study was to observe of occurrence and isolation of fungal communities at different pH.

Materials and Methods

Collection of Samples and Preparation

The leaf and stem of the chosen plants (*Buchanania* and *Celastrus* sp.) were surface sterilized with 0.5% NaOCl for 2 min and 0.1% mercuric chloride for 3 min and 7 min, respectively, followed by a 30 second dip in 70% ethanol and two rinses in distilled water before the experiment was set up.[15,39]

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TABLE-1 : Prevalence of endophytic fungal genus isolated from *Buchanania* species leaf and stem

Genera.	Number of isolates
Aspergillus niger	48
Alternaria spp.	27
Curvularia spp.	24
Fusarium spp.	18
Rhizopus spp.	16
Mucor sp.	11
Penicillium	04
Unidentified	89

TABLE-2 : Prevalence of endophytic fungal genus isolated from *Celastrus* species leaf and stem

Genera	Number of Isolates
Aspergillus fumigatus	39
Penicillum sp	27
Alternaria sp.	21
Curvularia sp.	18
Fusarium sp.	16
Rhizopus sp.	11
Unidentified	119

Preparation of pH levels

To achieve the appropriate pH levels of 4.5 and 9, respectively, dilute HCl (0.1%) and dilute NaOH were applied to potato dextrose agar (PDA) media.

Isolation of endophytic fungi

Streptomycin 1.0 g/L was added to Potato Dextrose Agar (PDA), medium to prevent the growth of bacteria. After surface sterilization, leaf and stem of the plants were put on PDA medium in petri dishes. The petri dishes were cultured at 28°C for 4 weeks before being evaluated[27, 39].

Characterization of fungal isolates Fungal isolate colonies were examined after 48 to 96 hours after inoculation. Each fungal colony was isolated and grown in another petri plate as a pure culture. After the full growth of the fungal colony, it was observed under the microscope and identified with the help of relevant literature[13].

Statistical analysis

The sampling time for *Buchanania,* and *Celastrus* leaf and stem were 2, 4, 6, 8 10 14, 16, 22, 24, 26, days at pH 4.5 and pH 9.0 respectively.

Results

Fungal isolates were observed in all the plant samples although *Aspergillus species* were the most often found. At different pH values, the fungal populations were noticeably different, with consistently higher counts at pH 4.5 and 9, respectively. *Aspergillus niger, Alternaria, Curvularia, Fusarium, Rhizopus, Mucor*, and *Penicillium*

Fig. 1 : Mixed culture of fungal endophytes

Aspergillus niger *Aspergillus flavous* *Fusarium sp.* *Rhizopus sp.*

Fig.2 : Pure culture of fungal endophytes

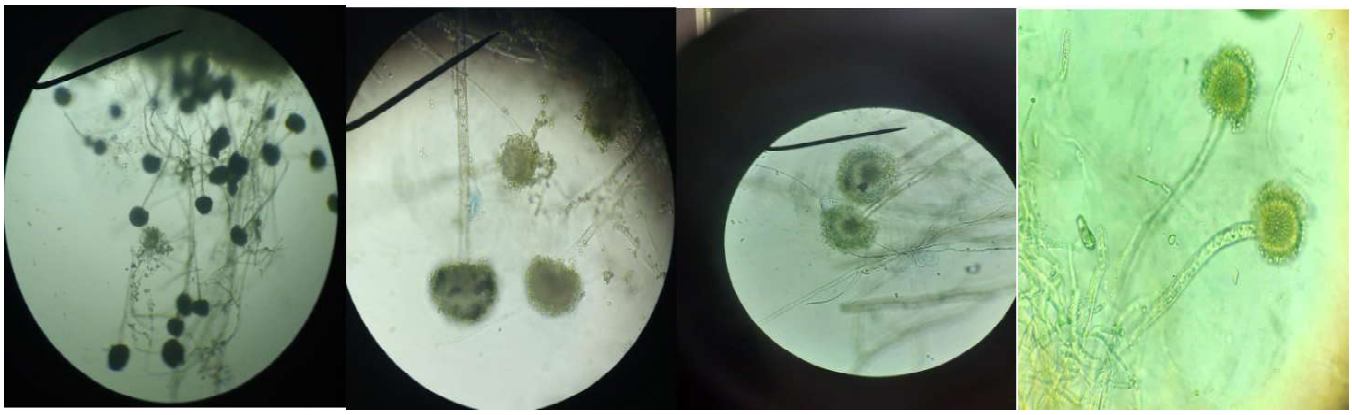

Fig. 3 : Microscopic *Aspergillus sp.*

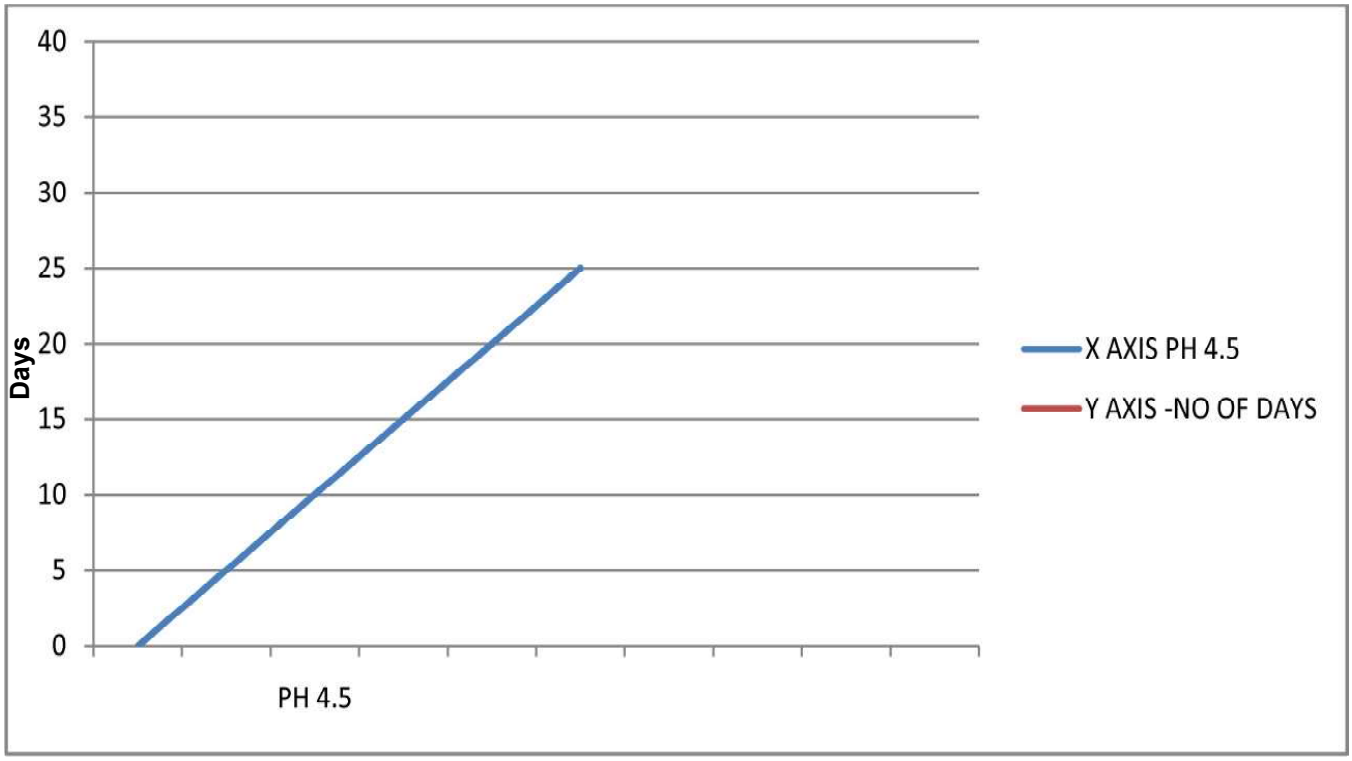

Fig. 4 : Growth rate (Days) of endophytic fungal genus isolated from *Buchanania* sp. at pH 4.5

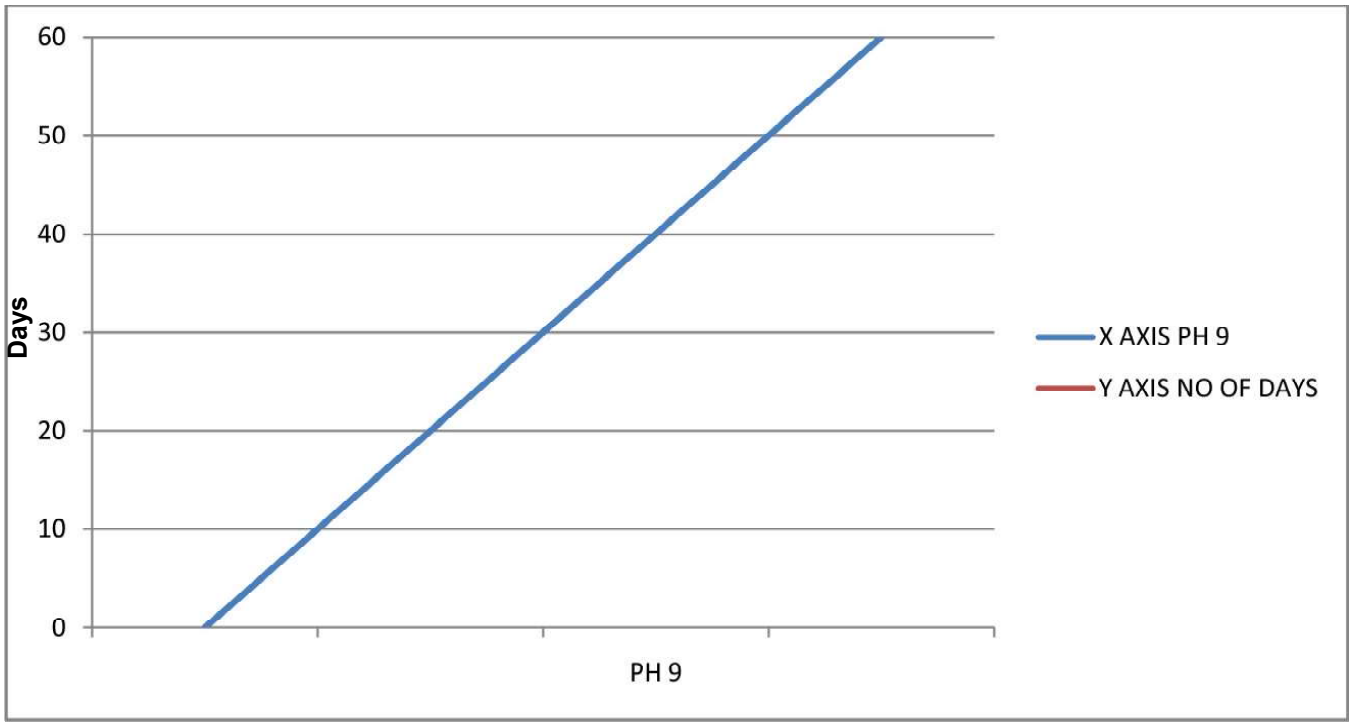

Fig. 5 : Growth rate (Days) of endophytic fungal genus isolated from *Celastrus* species at pH 9

were isolated at pH 4.5, while *Aspergillus fumigatus, Penicillium, Alternaria, Curvularia, Fusarium*, and *Rhizopus* fungi at pH 9 correspondingly. It demonstrates that varied pH levels led to changes in the fungal populations in plant species, leaves, and stems (resistance/susceptibility to environmental influences).

Discussion

The research emphasizes how variables including salinity, pH, temperature, altitude, and nutrition availability can have an impact on fungal diversity and colonization density. Fungi were found to grow at varied pH levels. This agrees with observation[16] that mycorrhizal fungus had a generally greater range of pH tolerance in symbiosis than in pure culture[16]. Difficulties were observed from investigations conducted in pure culture to symbiotic systems. Many of the saprotrophic fungi grew well at the pH 7 or 8[37]. At pH 5 or 6, the ectomycorrhizal species exhibited their best development. In particular, fungi that cause root rots (such as *Fusarium verticillioides, Fusarium avenaceum*, and *Acremonium strictum*, which cause black bundle disease and late wilt) are stressed and killed by high pH, which favours bacteria and actinomycetes. *Fusarium verticillioides, F. oxysporum, F. proliferatum,* and *F. solani* were considered to be "phyllosphere competent" among the *Fusarium sp.* known to colonise maize roots because they grow saprophytically, reproduce in the rhizosphere, and cause root rot when host plants are under stress[24,38].

Conclusion

Fungal growth rapidly decreased from approximately 25 p/mol/g acetate g^{-1} h^{-1} to 5 p/mol. Thus, it was found that fungi grew most effectively at a pH of around 4.5. Endophytes are capable of growth and development over wide pH ranges. The ability to control extracellular pH is an important aspect of fungal physiology that contributes to fitness within the host *via* the modulation of virulence factors to best fit the host[39].We noticed that pigment production, mycelium color, and other aspect were affected. It is concluded that pH impacted a wide range of fungal physiological processes.

References

1. Anthony D. pH and its effects on plants. Enterobactercloacae; Mol. *Plant-Microbe Interact.* 2003; **7:** 440-448.
2. Arnold A E, Herre E A. Canopy cover and leaf age affect colonization by tropical fungal endophytes: Ecological pattern and process in *Theobroma cacao* (Malvaceae). *Mycologia.* 2003;**95**: 388-398.
3. Atmosukarto I, Castillo U, Hess WM, Sears J, Strobel GA. Isolation and characterization of *Muscodor albus* I-41.3s, a volatile antibiotic-producing fungus. *Plant. Sci*. 2005;**169**: 854-861.

4. Avidano L, Gamalero E, Paolo Cossa G, CarraroE. Characterization of soil health in an Italian polluted site by using microorganisms as bioindicators. *Appl. Soil Ecol.* 2005; **30:** 21-33.
5. Balows A, Trüper HG, Dworkin M, Harder W, Schleifer WKH. The Prokaryotes, a Handbook on the Biology of Bacteria. SpringerVerlag Berlin, Heidelberg, New York. 1992.
6. Bashan Y. Interactions of Azospirillum in soils: a review. *Biol. Fertil. Soils.* 1999; **29**: 246-256.
7. Black CA. Methods of Soil Analysis: Part I Physical and mineralogical properties. American Society of Agronomy, Madison, Wisconsin, USA.1965.
8. Brock TD ed. Thermophiles: General, Molecular and Applied Microbiology: Wiley, New York. 1986; p. 316.
9. Ceja-Navarro JA, Rivera FN, Patiño-Zúñiga L, Govaerts B, Marsch R, Vila-Sanjurjo A, Dendooven L. Molecular characterization of soil bacterial communities in contrasting zero tillage systems. *Plant Soil.* 2010; **329**: 127-137.
10. Chanway CP. Endophytes: They are not just fungi*! Canadian J. Bot.* 1996;**74**: 321-322
11. Ching-Hong Y, David E. Rhizosphere Microbial Community Structure in Relation to Root location and Plant Iron Nutritional status. *Appl. Environ. Microbiol.* 2000; **66**(1): 345-351.
12. Clay K, Schardl C. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *Am. Naturalist.* 2002;**160:** 99-127.
13. Dayan MP. Fungal Diseases of forest tree seeds and control measures: A Giidebook. *DENR Recommends.* 2004; **13**: 1-25.
14. Di Cello F, Bevivino A, Chiarini L, Fani R, Paffetti D, Tabacchioni S, Dalmastri C. Biodiversity of a *Burkholderia cepacia* population isolated from the maize rhizosphere at different plant growth stages. *Appl. Environ. Microbiol.* 1997;**63**: 4485-4493.
15. Dietmar B, Patricia G, Munoz-Rojas J, Estrella D, Moreno-Morilla S, Sanchez L, Ramos JL. Rhizormediation of lindane by root colonizingSphingomonas. *Microbial Biotechnol.* 2008**; 1**: 87-93.
16. Erland S, Söderström B, Andersson S. Effects of liming on ectomycorrhizal fungi infecting *Pinus sylvestris* L. II. Growth rates in pure culture at different pH values compared to growth rates in symbiosis with the host plant. *New Phytol.* 1990;**115**: 683-688.
17. Gaviria C. Normas para interpreter e reporter a contagem “standard” em placa. Sao Paulo Merck. 1v. Haney RL, Senseman SA, Hons FM, Zuberer DA. Effect of Glyphosate on soil microbial activity and biomass. *Weed Sci.* 1978;**48**: 89-93.
18. Krieg NR, Holt JG, Murray RGE, Brenner DJ, Bryanth MP, Moulder JW, Pfenning N, Sneath PHA, Staley JT (eds.). *Bergey’s Manual of Systematic Bacteriology.* 1984; Vol. **1**, The Williams and Wilkins Co., Baltimore.
19. Larkin RP. Characterization of soil microbial communities under different potato cropping systems by microbial population dynamics, substrate utilization, and fatty acid profiles. *Soil Biol. Biochem.* 2003;**35:** 1451-1466.
20. Le´ vesque CA, Rahe JE. Herbicide interactions with fungal root pathogens, with special reference to glyphosate. *Annu. Rev. Phytopathol.* 1992; **30**: 579-602.
21. Mejia LC, Rojas EI, Maynard Z, Arnold AE, Kyllo D, Robbins N, Herr EA. Inoculation of beneficial endophytic fungi into *Theobroma cacao* tissues. Smithsonian Tropical Research Institute. Apartado 2072. Balboa, Rep. of Panama. 2008.
22. Miller HJ, Henken G, van Veen JA. Variation and composition of bacterial popula: 656-660.
23. Narisawa K, Currah RS, Hashiba T. The root endophytic fungus *Phialocephala fortint* i suppresses Verticillium yellows in Chinese cabbage. *Proceedings* options in the rhizosphere of maize, wheat, and grass cultivars. *Can. J. Microbiol.* (1989); 35 *the 8th International Congress of Plant Pathology,* Christchurch, New Zealand, 2003; pp. 39.
24. Ocamb CM, Kommedahl T. Growth of rhizosphere competent and incompetent *Fusarium* species from corn on carbon substrates. *Phytopathology.* 1994; **84**: 508-514.
25. Oliveira ALM, Canuto EL, Silva EE, Reis VM, Baldini JI. Survival of endophytic diazotrophic bacteria in soil under different moisture levels. *Brazilian J. Microbiol.* 2004; **35**: 295-299.
26. Orole OO, Adejumo TO. The activity of fungal endophytes against four maize wilt pathogens. *Afri. J. Microbiol.*

Res. 2009; **3** (12): 969-973.

27. Poling SM, Wicklow DT, Rogers KD, Gloer JB. *Acremonium zeae* a protective Endophyte of maize, produces Dihydroresorcylide and 7 – hydroxydihydroresorcylides. *J. Agri. Food Chem.* 2008; **56**(9): 3006-3009.
28. Posada F, Vega FE. Establishment of the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales) as an endophyte in cocoa seedlings *(Theobroma cacao). Mycolo. Soc. Am.* 2005; **97**(6): 1195-1200.
29. Prusky D., Yakoby N. Pathogenic fungi: leading or led by ambient pH? *Molecular Plant Pathology.* 2003; **4**: 509–516.
30. Rodrigues-Heerklotz KF, Drandarov K, Heerklotz J, Hesse M, Werner C. Guignardic acid, a novel type of secondary metabolite produced by endophytic fungus *Guignardia* sp.: isolation, structure elucidation and asymmetric synthesis. *Helv. Chim. Acta.* 2001;**84**: 3766-3771.
31. Saikkonen K, Wäli P, Helander M, Faeth SH. Evolution of endophyte - plant symbioses. *Trends Plant Sci.* 2004; **9**: 275-280.
32. Santos RMG, Rodrigues Fo E, Rocha WC, Teixeira MFS. Endophytic fungi from *Melia azedarach. World J. Microbiol. Biotechn.* 2003; **19**: 767-770.
33. Soonthornpoct P, Trevathan LE, Ingram D. The colonization of maize seedling roots and rhizosphère by *Fusarium* spp. in Mississippi in two soil types under conventional tillage and no-tillage Systems. *Phytoprot.* 2000; **81**: 97-106.
34. Talaro KP. Foundations in Microbiology. 5th Edn., McGraw-Hill Companies, Inc., New York, USA.2005; p. 407.
35. Ventura LA. The effect of Soil pH on plant growth. Science Experiments on File. Revised. *Facts on the File, Inc.* 2000; **4**(34): 1-5
36. Wilson D. Fungal endophytes: out of sight but should not be out of mind? *Oikos.* 1993; **68**: 379-384.
37. Yamanaka T. The effect of pH on the growth of saprotrophic and ectomycorrhizal ammonia fungi *in vitro. Mycologia.* 2003; **95**(4): 584–589.
38. Young TR, Kucharek TA. Succession of fungal communities in roots and stalks of hybrid field corn grown in Florida. *Plant Dis. Rep.* 1977; **61**: 76-80.
39. Zinniel DK, Lambrecht P, Harris NB, Zhengyu F, Kuczmarski D, Higley P, Ishimaru CA, Arunakumari A, Barletta RG, Vidaver AK. Isolation and Characterization of Endophytic Colonizing Bacteria from Agronomic Crops and Prairie Plants. *Appl. Environ. Microbiol.* 2002; **68**(5)**:** 2198-2208.