RESEARCH PAPER

Fungicide Resistance of Soil Borne Fungi from Agricultural Field of Pravara Area (Loni)

Kalpana Palghadmal
Post-Graduate Department of Botany and Research Centre, Padmashri Vikhe Patil College of Arts Science and Commerce, Pravaranagar 413 713, M.S., India
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ABSTRACT
Soil samples were collected from the rhizosphere region of agricultural fields from the Pravara area. Collected soil samples were used for isolation of soil borne fungi by serial dilution method on PDA plates. Rhizopus, Aspergillus, Fusarium and Mucor these developed fungal colonies were observed based on colony morphology, colour and growth pattern. These colonies were sub-cultured on fresh PDA plates for purification and identification. Different fungicides were selected based on their common use against soil-borne fungal pathogens in agriculture i.e. Bavistin, Indofil-M-48, Indofil-Z-78, Anthracol. The Poisoned Food Technique showed that the antifungal activity varied depending on the type of fungicide and fungal species. Fusarium oxysporum showed minimum growth of fungal colony to the 100 ppm conc. towards the Anthracol (0.8 cm), Indofil-Z-78 (0.4 cm), Indofil-M-48 (0.3 cm). However, Aspergillus niger showed minimum growth of fungal colony to the 100 ppm conc towards Bavistin (1.4 cm).
Keywords: Fungicide, ppm Concentrations, Soil sample, fungal pathogens etc.
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Introduction:

Soil provides home for the growth of all living organisms I,e plants, animals and microorganisms. Soil has moisture holding capacity and it maintains adequate aerations. Among all microorganisms grown in soil fungi grows dominantly (Ali-Shtayeh and Jamous, 2000; Rane and Gandhe, 2006) Soil harbors fungi and other macro and microorganisms viz bacteria, worms, beetles and plants etc. Soil, the mantle of weathered rock contains nutrients and minerals in addition to organic matter (Sylvia et al., 2005) Soil habitats are full of life and the soils which are rich in keratinous material are the most conductive for the occurrence and growth of keratino philic fungi (Ingham, 2007). Soil habitats can be different and the characteristics of soil affect what lives in it. Fungi belong to kingdom Myceteae and are cosmopolite in distribution. They can grow in any habitat where life is possible. The microscopic cells of fungi grow usually as long strands or threads called hyphae. The hyphae push their way between soil particles, roots and rocks. The significant characteristics of fungi are that, they are eukaryotic, heterotrophic or non-photosynthetic, lack tissue differentiation and their cell wall is composed of chitin or other polysaccharides and they propagate by spores sexuallyand/orasexually(Benson,2002) Fungi play important services in the fields of water dynamics, nutrient cycling, and disease suppression and they also play important role as decomposers along with bacteria in the soil food web. Decomposers, mutualists and pathogens are the three general categories of soil fungi created on the bases of how they get energy. Decomposers help in breaking down of dead organic matter, mutualistic fungi colonize the plant roots and provide nutrients to plant in exchange for shelter and carbon (an energy source). Pathogens are responsible for diseases or death of living organism on which they colonize and feed (Tugel etal., 2000; Brady and Weil, 2002) (Rafia Akhtar 2022).

Fungi are a diverse group of organisms that influence agricultural systems in both beneficial and harmful ways. While some species are recognized as plant pathogens with the potential to threaten crop production and food security, others function as biological control agents with the capacity to manage pests and diseases in an environmentally sound manner (Davies et al., 2021, Ayaz et al., 2023, Madlhophe et al., 2025). This functional duality has drawn significant attention to the role of fungi in integrated pest and disease management (IPDM), a strategy designed to utilize multiple compatible control measures to achieve pest suppression with reduced ecological disruption (Kumhar et al., 2020).The development of fungal biocontrol agents (BCAs) has expanded in response to concerns about the adverse effects of synthetic pesticides. Chemical pesticides, while effective in the short term, are associated with negative outcomes such as environmental contamination, development of pest resistance, and human health risks (Lahlali et al., 2022). The search for safer and more sustainable alternatives has led to an increased focus on biological control strategies. Fungal BCAs, including Trichoderma, Beauveria, Metarhizium, and Paecilomyces, have received attention due to their ability to suppress pathogens and insect pests through mechanisms such as parasitism, competition, production of secondary metabolites, and induction of plant defense responses (Lahlali et al., 2022).Eliakira Kisetu Nassary (2025).

Soil-borne plant pathogens are diverse, including filamentous fungi, bacteria, protozoans, nematodes, and viruses. All of them are thought to have evolved to survive into the next generation through the soil, and once they have occurred, they tend to become difficult-to-control diseases. Therefore, understanding the ecology of each pathogen is extremely important in planning and establishing control methods against soil-borne pathogens. On the other hand, scientific methods are indispensable for understanding complex natural phenomena. Based on scientific methodology, I have been conducting research on the ecology of soil-borne plant pathogenic fungi and nematodes.Masahiro Shishido (2025).

For some 35 years now the agricultural industry has faced problems arising from the development of resistance in fungal pathogens of crops, against the fungicides used to control them. Since the first cases of widespread resistance arose, agrochemical manufacturers, academic and government scientists, and crop advisers, have put a great deal of effort into analyzing the phenomenon and establishing countermeasures. In 1994 the Fungicide Resistance Action Committee (FRAC), now affiliated to Crop Life International, commissioned a broad review of progress world-wide in dealing with fungicide resistance, and of the outstanding difficulties that need to be overcome **(**St Raphael 2007).

Soil is arguably the most important resource for food production. It is a very complex system whose functions not only depend on its physical properties, but also on its biological components. In particular, soil microorganisms are essential players in the cycling of several elements essential to life, including C, N, and P [1]. Understanding the effect of fungicides on the beneficial activities of microorganisms is important to assess the hazards associated with fungicide used in agriculture. Crop productivity and economic returns will be maximized with the use of products controlling well fungal pathogens, but preserving beneficial organisms. Different organisms may possess identical or similar mechanisms and constituents, and fungicides targeting nonspecific binding sites can directly affect non target organisms. For example, the toxicity of carboxylic acid fungicides is derived from the ability of these chemicals to bind on DNA to isomerase II, as common enzyme that unwind, and wind, DNA to allow protein synthesis and DNA replication. This enzyme found in fungi but also in prokaryotic cells. Some glucopyranosyl antibiotic fungicides are toxic to bacteria, in which they may inhibit the synthesis of amino acids. These fungicides are also toxic to certain non fungal higher eukaryotic organisms. Indirect non target effects are also possible. Microorganisms are either functionally or nutritionally connected with each others, and changes in a component of a microbial community may influence the structure of the whole community. This is particularly true for plant-associated microorganisms which is influence on and are influenced by the plant metabolic status. In order to establish a proper regulation for the use often many fungicidal substances promoted by industry in sustainable agriculture, fungicide action modes and possible side effects on nonfungal microorganisms must urgently be clarified. Fungicide action modes have never been well classified, and the side effects of these important chemicals are not fully understood. (Chao Yang 2011).

Plant pathogenic fungi cause severe crop diseases with global repercussions, and their management involves a careful balance of chemical and non-chemical approaches. Chemical management of plant diseases plays a significant role in modern agriculture and horticulture by effectively controlling and preventing the spread of plant diseases. Fungicides have been employed by farmers for more than 200 years to safeguard their crops against fungi, despite the toxic residues they leave behind and have a negative impact on other organisms like beneficial and non-target organisms, birds, fish etc. Over time there's a failure in control of plant diseases in field even after lump sum application of recommended fungicides. This is the first sign of fungicide resistance development in the field. The baseline serves as the reference point for the acknowledged fungal responsiveness to a particular fungicide. To observe any potential shift towards resistance in the reference, it is imperative to be aware of the sensitivity baseline for the fungus-fungicide combination. This knowledge enables the monitoring of fungicide effects on the fungus, allowing for the detection of any changes in its susceptibility over time. Fungicide resistance poses significant challenges to effective disease control and ecosystem health, emphasizing the need for sustainable and responsible agricultural practices. Addressing fungicide resistance requires a comprehensive approach that considers economic implications, optimizes fungicide use, and preserves beneficial fungi. Educating the farmers about fungicide resistance, proper fungicide use, and the necessity of fungicide rotation strategies is essential. The ultimate goal is not just to manage resistance but to prevent or delay its development by understanding baseline sensitivity and tracking fungicide's impact on fungi.Around 1970, benzimidazoles began to exhibit resistance to many pathogens. (Chandana Y. Kundargi 2023)

Materials and Methods:

For the present study soil sample were collected from the rhizosphere region of agricultural fields in the pravara area(Loni village) Collected soil sample were used for isolation of soil borne fungi by serial dilution method .1 g soil sample was transferred aseptically into 9 mL sterile distilled water to prepare 10^{-1} dilution and mixed thoroughly by shaking. From this, 1 mL suspension was transferred into another test tube containing 9 mL sterile distilled water to obtain 10^{-2} dilution. Similarly, serial dilutions were prepared up to desired dilution level ($10^{-5}/10^{-6}$). (Renu Jangid *et.al* 2025). For isolation of fungi, Potato Dextrose Agar (PDA) medium was prepared. (Kumari Palak *et.al* 2025).Prepared medium was transferred into conical flasks and sterilized in an autoclave at 121°C, 15 psi for 15–20 minutes. After sterilization, molten medium was cooled to about 45–50°C and aseptically poured into sterile Petri plates. Plates were allowed to solidify. From appropriate serial dilutions, 0.1 mL or 1 mL suspension was transferred onto PDA plates and spread uniformly using sterile spreader (spread plate method).Inoculated plates were incubated in inverted position at 25–28°C for 5–7 days for fungal growth. (Sylvester Boakye Owusu *et.al* 2024).Developed fungal colonies were observed based on colony morphology, colour and growth pattern. Distinct colonies were sub-cultured on fresh PDA plates for purification and identification.(Magdalena Frąc *et.al* 2016) .Different fungicides ie, Bavistin, Indofil-M-48, Indofil-Z-78 and Anthracol. Were selected based on their common use against soil-borne fungal pathogens in agriculture. The selected fungicides were procured from local agricultural stores/laboratory and used for evaluating their effect on fungal growth under laboratory conditions.

Different concentrations of fungicide were prepared using sterile distilled water under aseptic conditions. Required quantity of fungicide was measured and mixed properly to obtain desired concentrations. (10 ppm, 25 ppm, 50 ppm, 100 ppm). (Zafar Iqbal et.al 2010).Sterilized Potato Dextrose Agar (PDA) medium was prepared and cooled to about 45–50°C. Different fungicide concentrations were added separately into molten PDA medium and mixed thoroughly. The medium was then poured into sterile Petri plates and allowed to solidify.(Geethanjali R et.al 2023).A small fungal disc (5 mm diameter) taken from actively growing fungal culture was placed at the center of each Petri plate containing fungicide-amended medium. Control plates without fungicide were also maintained. The inoculated plates were incubated at 25–28°C for 5–7 days.

Results and Discussion:

Table No.1.Effect of different conc of Bavistin against growth of isolated fungi

Results summarized in table no.1 supported that 100 ppm conc. Bavistin showed highest resistance to colony growth of *Aspergillus niger* (1.4 cm) as compare to other fungal isolates and control

Fungal Isolates	Colony diameter (cm)				
	10 ppm	25 ppm	50 ppm	100 ppm	Control
Mucor globosus	7.5	6.2	3.4	2.3	9.1
Fusarium oxysporum	6.0	5.0	4.3	3.0	7.0
Aspergillus niger	7.2	4.0	3.1	1.4	9.5
Rhizopus stolonifer	8.3	6.4	3.8	1.9	9.9

Table No.2.Effect of different conc. of Indofil-M-48, against growth of isolated fungi

Fungal	Colony diameter (cm)

Isolates	10 ppm	25 ppm	50 ppm	100 ppm	Control
Mucor globosus	3.2	2.1	1.0	0.4	5.6
Fusarium oxysporum	2.7	1.3	0.9	0.3	3.9
Aspergillus niger	5.8	3.9	2.4	1.3	6.4
Rhizopus stolonifer	2.5	1.9	1.5	0.8	4.5

Results summarized in table no.2 supported that 100 ppm conc. Indofil-M-48 showed highest resistance to colony growth of *Fusarium oxysporum* (0.3 cm) as compare to other fungal isolates and control

Table No.3.Effect of different conc. of Indofil-Z-78, against growth of isolated fungi

Fungal Isolates	Colony diameter (cm)				
	10 ppm	25 ppm	50 ppm	100 ppm	Control
Mucor globosus	5.2	4.0	2.1	0.6	7.2
Fusarium oxysporum	4.7	2.8	1.6	0.4	6.7
Aspergillus niger	5.3	3.6	2.4	1.2	8.1
Rhizopus stolonifer	4.5	2.0	1.3	0.8	9.0

Results summarized in table no.3 supported that 100 ppm conc. Indofil-Z-78 showed highest resistance to colony growth of *Fusarium oxysporum* (0.4 cm) as compare to other fungal isolates and control

Table No.4.Effect of different conc. of Anthracol against growth of isolated fungi

Fungal Isolates	Colony diameter (cm)				
	10 ppm	25 ppm	50 ppm	100 ppm	Control
Mucor globosus	5.5	4.4	2.5	1.3	6.0
Fusarium oxysporum	4.7	3.1	1.9	0.8	8.1
Aspergillus niger	6.8	4.5	3.2	1.2	9.0
Rhizopus stolonifer	7.4	5.2	2.9	1.0	9.3

Results summarized in table no.4 supported that 100 ppm conc. Anthracol showed highest resistance to colony growth of *Fusarium oxysporum* (0.8 cm) as compare to other fungal isolates and control

Conclusion:

The results obtained from the study clearly indicated that increase in fungicide concentration resulted in gradual reduction of fungal growth. Among all tested concentrations, 100 ppm showed minimum colony growth in all fungal isolates, whereas maximum was observed at 10 ppm concentration and control. The antifungal activity varied depending on the type of fungicide and fungal species. *Fusarium oxysporum* showed minimum colony growth to the 100 ppm conc. towards the Anthracol (0.8 cm), Indofil-Z-78(0.4 cm), Indofil-M-48(0.3cm), however . *Aspergillus niger* showed minimum colony growth to the 100 ppm conc towards Bavistin(1.4 cm).

The study demonstrated that fungicides play an important role in controlling soil-borne fungi under laboratory conditions. However, variation in fungal sensitivity towards fungicides was observed, indicating that different fungi respond differently to chemical treatments. Continuous and excessive use of fungicides may lead to development of fungicide resistance in fungal pathogens. Therefore, proper selection and optimum use of fungicides is essential for effective disease management.

Overall, the present investigation concluded that fungicides are effective in suppressing the growth of soil-borne fungi and higher concentrations provide poor fungal growth. The study may be useful for understanding fungicide sensitivity of fungal pathogens and for developing suitable management strategies for controlling fungal diseases in agricultural fields.

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