



Rhizosphere Mycoflora of Some Common Weeds

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Abstract:

The present study aimed to isolate and identify the rhizosphere mycoflora associated with three common weeds—*Parthenium hysterophorus*, *Amaranthus viridis*, and *Tridax procumbens*. Rhizosphere soil samples were collected from agricultural fields of Bavdhan village, Wai Tehsil, Satara district, Maharashtra. Entire plants with intact root systems were carefully uprooted, and soil adhering to the roots was collected in sterile bags. Serial dilutions of the samples were prepared, and aliquots were inoculated on Potato Dextrose Agar (PDA) and Czapek Dox Agar (CzDA). Fungal isolates were characterized based on macro- and micromorphological features, including colony appearance, pigmentation, aerial mycelium formation, and conidial structures. A total of 17 fungal species belonging to twelve genera were recorded. *Aspergillus niger* was the most dominant species, followed by *A. flavus*. Other fungi observed included *Rhizopus oryzae*, *R. stolonifer*, *Trichoderma viride*, *Aspergillus fumigatus*, *A. nidulans*, *Penicillium chrysogenum*, *P. expansum*, *Curvularia lunata*, and *Fusarium oxysporum*. The study highlights the diversity of fungal communities associated with the rhizosphere of common weeds.

Keywords: Fungal Diversity; Rhizosphere Fungi; Rhizosphere Soil; Soil Mycoflora; Weed Species

Introduction:

Weeds are plants that grow unintentionally along with cultivated crops and interfere with agricultural practices. They are generally regarded as undesirable because they compete with crop plants for essential resources such as nutrients, water, light, and space, thereby reducing crop yield and agricultural productivity. Weeds also cause economic losses by increasing the cost of cultivation, harboring pests and pathogens, and interfering with harvesting operations. Due to their aggressive growth and high adaptability, weeds often dominate cropping systems and negatively influence the national economy and agricultural sustainability.

Despite their harmful effects in agroecosystems, weeds also play important ecological roles when viewed from a broader environmental perspective. Weeds provide

vegetative cover that protects the soil surface from erosion caused by wind and rain. Their root systems penetrate deeper soil layers and mobilize nutrients, which are later returned to the upper soil layers through leaf litter and plant decomposition. In this way, weeds contribute to nutrient recycling and organic matter accumulation in soil. Additionally, weeds serve as a source of food and shelter for various organisms, including insects, birds, and small animals, thereby supporting biodiversity. Wild plant species related to crops also serve as valuable genetic reservoirs for crop improvement, particularly for traits such as resistance to pests, diseases, and environmental stress.

Soil itself is a complex and heterogeneous habitat that supports a wide range of organisms, including bacteria, fungi, protozoa, nematodes, and earthworms. These organisms interact with

one another and with their physical environment, contributing significantly to soil structure, fertility, organic matter decomposition, nutrient cycling, suppression of soil-borne pathogens, and detoxification of harmful substances (Prescott et al., 2005; Kirk et al., 2004; Kozdroj and Van Elsas, 2000).

The rhizosphere is a dynamic zone of soil that surrounds plant roots and is strongly influenced by root activity. It is governed by complex interactions between plant roots and associated microorganisms. Root exudates, which include sugars, amino acids, organic acids, and secondary metabolites, play a crucial role in shaping the composition, activity, and population density of rhizospheric microorganisms. The rhizosphere harbors a diverse community of culturable and non-culturable microorganisms that can be exploited for agricultural and environmental benefits. Many rhizosphere-associated microbes enhance crop productivity by improving nutrient availability, thereby reducing dependence on chemical fertilizers and supporting sustainable agricultural practices. Some microorganisms also protect plants against pathogenic organisms through mechanisms such as competition, antibiosis, and induced systemic resistance.

Among soil microorganisms, fungi play a particularly important role in maintaining soil health and plant growth. Rhizospheric fungi contribute to nutrient cycling, organic matter decomposition, and plant growth promotion, and they are also involved in biological control of plant pathogens (Thorn, 1997). Soil nutrient dynamics, particularly carbon, nitrogen, and phosphorus, vary with crop growth stages, with higher concentrations generally observed during early growth stages and a decline toward crop maturity (Asghar et al., 2013). In addition to essential nutrients, soils may also contain trace amounts of heavy metals such as lead (Pb),

cadmium (Cd), and nickel (Ni), which can influence microbial activity and soil health (Khan et al., 2012).

Considering the influence of weeds on soil properties and microbial diversity, the present study is designed to investigate the rhizospheric soil fungi associated with selected weed species. Understanding the diversity and functional role of rhizospheric fungi associated with weeds may provide valuable insights into their potential application in sustainable cropping systems and soil health management.

Materials And Methods:

Collection of Rhizosphere Soil Samples:

Rhizosphere soil samples of selected weed species were collected from the field by carefully uprooting the plants with minimal disturbance to the root system. The loosely attached bulk soil was gently removed by shaking, and the soil particles firmly adhering to the root surface were considered as rhizosphere soil. This rhizospheric soil was carefully scraped from the root surface using a sterile spatula and collected in sterile polythene bags.

The collected samples were transported to the laboratory within 12 hours of collection. A portion of the fresh rhizosphere soil was used immediately for fungal isolation studies to maintain microbial viability. The remaining soil samples were air-dried at room temperature, passed through a 2 mm mesh sieve, and preserved in properly labeled containers for the analysis of physicochemical properties. For experimental work, ten grams of soil were used, while the rest of the samples were stored for further analysis.

Isolation of Rhizospheric Soil Fungi:

The occurrence and diversity of fungi in the rhizosphere soil samples were studied using the **soil dilution plate technique** as described by Warcup (1950). The air-dried and sieved soil

samples were thoroughly mixed, and one gram of soil was accurately weighed. The soil was suspended in a 250 mL conical flask containing 100 mL of sterile distilled water to prepare the stock suspension. The flask was agitated vigorously on a mechanical shaker for 15 minutes to ensure uniform dispersion of soil particles and microbial propagules and then allowed to stand briefly for sedimentation of coarse particles.

From the stock suspension, 1 mL was aseptically transferred using a sterile pipette into a test tube containing 9 mL of sterile distilled water to obtain a 10^{-1} (1:10) dilution. Further **serial decimal dilutions** were prepared up to 10^{-5} (1:100,000) by transferring 1 mL aliquots into successive tubes containing 9 mL sterile distilled water. The final dilution (10^{-5}) was mixed thoroughly, and 1 mL of the suspension was poured onto sterile culture plates under aseptic conditions. The plates were gently rotated to ensure uniform distribution of the inoculum over the surface of the medium.

Selection of Culture Media:

The isolation and enumeration of soil fungi require appropriate culture media due to the diverse nutritional requirements of different fungal groups. Since no single medium is suitable for the growth of all soil fungi, several commonly used fungal media were evaluated to obtain maximum fungal diversity and population. The media tested included Potato Dextrose Agar (PDA), Martin's Rose Bengal Peptone Dextrose Agar (Martin, 1950), Waksman's Agar (Waksman, 1916), and Czapek's Dox Agar medium (Raper and Thom, 1948).

Among the tested media, **Dextrose–Peptone Agar (DPA)** and **Czapek's Dextrose Agar (CzDA)** were found to be most suitable for fungal isolation and colony enumeration. DPA supported the growth of a wide range of saprophytic fungi and yielded higher colony

counts, whereas CzDA favored the growth of fungi belonging mainly to *Aspergillus* and *Penicillium* groups. Based on overall performance, Dextrose–Peptone Agar was selected as the most suitable medium for fungal isolation and quantitative analysis.

Composition of Media:

Dextrose–Peptone Agar (g/L):

Dextrose – 10.0 g
Peptone – 5.0 g
Potassium phosphate – 1.0 g
Magnesium sulphate – 0.5 g
Agar – 25.0 g
Distilled water – 1000 mL

Czapek's Dextrose Agar (g/L):

Sucrose – 30.0 g
Sodium nitrate – 2.0 g
Potassium phosphate – 1.0 g
Potassium chloride – 5.0 g
Magnesium sulphate – 0.1 g
Ferrous sulphate – 0.1 g
Agar – 15.0 g
Distilled water – 1000 mL

The pH of the media was adjusted to 4.0–4.5 using 1 N H_2SO_4 or 1 N NaOH prior to sterilization. The media were sterilized at 15 lb pressure for 25 minutes. After cooling to about 45–50 °C, streptomycin sulphate (30 mg/L) was added aseptically to suppress bacterial growth before pouring the media into sterile Petri plates.

Counting of Fungal Colonies:

After gentle swirling, the inoculated plates were incubated at room temperature. Visible fungal colonies appeared within three to four days of incubation. The colonies were counted on the fifth day to avoid overcrowding and overlapping growth. The relative occurrence of individual fungal isolates was expressed as a **percentage of the total colony count**, following the method described by Aneja (2003).

Pure Culture of Fungal Isolates:

During primary isolation, fungal colonies exhibited rapid growth and competition for space and nutrients, which often restricted proper development of individual isolates. To obtain healthy and well-defined cultures, selected colonies were subcultured onto **M2 Agar (glucose–yeast extract medium)**, which supports better growth and sporulation.

Composition of M2 Agar (g/L):

- Glucose – 10.0 g
- Yeast extract – 5.0 g
- Glycerol – 10.0 g
- Potassium dihydrogen phosphate – 0.1 g
- Magnesium sulphate – 0.05 g
- Agar – 25.0 g
- Distilled water – 1000 mL

Distinct fungal colonies were picked aseptically from PDA or CzDA plates using a sterile inoculating needle or loop and transferred onto M2 agar slants for further growth and purification.

Preservation and Maintenance of Cultures:

Pure cultures of fungi isolated from rhizosphere soil were maintained by periodic subculturing on M2 agar slants under aseptic conditions. For long-term preservation, the cultures were stored under sterile mineral oil. The slants were sealed with cotton plugs and wrapped with aluminum foil to prevent contamination and desiccation.

Identification of Fungal Isolates:

Microscopic preparations of fungal cultures were made using **lactophenol cotton blue stain**. Lactophenol was prepared using lactic acid (20 mL), phenol crystals (20 g), glycerol (40 mL), and distilled water (20 mL). Polyvinyl alcohol (PVA) mounting medium was also used, prepared from polyvinyl alcohol (11 g), glycerol

(10 mL), phenol (25 drops), lactic acid (25 drops), and distilled water (100 mL).

Microscopic observations and photomicrographs were obtained using a Leitz microscope (Model 307-107.015) equipped with a Janvel photomicrography unit. Identification of fungal isolates was carried out based on cultural and microscopic characteristics using standard manuals and monographs, including Gilman (1957), Subramanian (1971), Ellis (1971, 1976), and Barnett and Hunter (1972).

Result And Discussion:

During the present investigation, the rhizosphere mycoflora associated with some common weed species from the study area was examined at selected localities of Wai (Bavdhan). Observations on weed species at each locality were recorded separately. The rhizosphere soil samples collected from the selected weed species were analyzed for their physicochemical properties, and the results are presented in Table 1. The rhizosphere soil of the weed species showed a slightly alkaline pH ranging from 7.2 to 7.5, which is considered favorable for the growth of a wide range of soil fungi. The electrical conductivity (EC) values ranged from 0.43 to 0.66 mS cm⁻¹, indicating non-saline soil conditions. Organic carbon content varied between 0.47 and 0.98%, suggesting moderate to high organic matter availability, which supports microbial proliferation in the rhizosphere.

The nitrogen content of the soils ranged from 125.44 to 150.53 kg ha⁻¹, while available phosphorus (P) ranged from 198.51 to 313.76 kg ha⁻¹ and potassium (K) from 467.60 to 991.31 kg ha⁻¹. The comparatively higher levels of macronutrients indicate nutrient-rich rhizosphere conditions that favor fungal colonization and activity. Micronutrients such as copper (Cu), iron (Fe), zinc (Zn), and manganese (Mn) were present in variable concentrations in some samples, which

may further influence fungal diversity and metabolic activity.

Overall, the physicochemical characteristics of the rhizosphere soils associated with the selected

weed species provide a conducive environment for the development and diversity of soil fungi, thereby influencing rhizospheric microbial communities.

Table 1. Physicochemical Properties of Rhizosphere Soil Associated with Selected Weed Species

Sr. No.	Soil Properties	<i>Parthenium hysterophorus</i>	<i>Amaranthus viridis</i>	<i>Tridax procumbens</i>
1	pH	7.2	7.2	7.5
2	EC (mS cm ⁻¹)	0.54	0.66	0.43
3	Organic Carbon (%)	0.98	0.85	0.47
4	Nitrogen (kg ha ⁻¹)	150.53	150.53	125.44
5	Phosphorus (kg ha ⁻¹)	220.15	313.76	198.51
6	Potassium (kg ha ⁻¹)	991.31	841.68	467.60
7	Copper (ppm)	Not analysed	3.96	1.48
8	Iron (ppm)	Not analysed	3.71	17.39
9	Zinc (ppm)	Not analysed	1.69	1.78
10	Manganese (ppm)	Not analysed	99.00	99.00

The present investigation revealed that the rhizosphere soil of selected weed species from the study area exhibited a diverse assemblage of fungi. Fungal isolates obtained from the rhizosphere soils showed distinct morphological and cultural characteristics such as colony growth pattern, texture, colour, presence or absence of aerial mycelium, surface wrinkles and furrows, and pigment production. These characteristics were used for preliminary identification of the fungal isolates, and the observations are summarized in **Table 2**.

Potato Dextrose Agar (PDA) supported the growth of a wide range of fungal isolates and was therefore used as the primary isolation medium. PDA is widely reported as an efficient medium for mycelial growth due to its simple composition and high carbohydrate content (Maheshwari et al., 1999; Saha et al., 2008). For purification and maintenance of cultures, Czapek's Dox Agar (CzDA) was preferred, as it reduced bacterial contamination and supported uniform fungal growth.

A total of **12 fungal species** belonging to different genera were isolated and identified from the rhizosphere soil samples of the selected weed species. Altogether, **17 occurrences** of fungal

isolates were recorded across the three weed species. The frequency of occurrence of individual fungal species is presented in **Tables 3–5** and illustrated in **Figure 1**.

Among the isolated fungi, *Aspergillus niger* showed the highest percentage frequency of occurrence (**17.64%**). This dominance may be attributed to its high sporulation capacity, ecological adaptability, and efficient utilization of organic substrates in soil environments. *Aspergillus flavus*, *Rhizopus oryzae*, and *Trichoderma viride* each showed moderate frequencies (**11.76%**), indicating their common association with weed rhizospheres. Other fungal species such as *Aspergillus fumigatus*, *Aspergillus nidulans*, *Rhizopus stolonifer*, *Penicillium expansum*, *Penicillium chrysogenum*, *Curvularia lunata*, *Curvularia clavata*, and *Fusarium oxysporum* exhibited lower frequencies (**5.88%**).

The dominance of *Aspergillus*, *Penicillium*, and *Rhizopus* species in the rhizosphere soil is consistent with earlier studies, which reported that these genera are common soil inhabitants due to their rapid growth, tolerance to variable soil conditions, and ability to decompose complex organic matter. The presence of *Trichoderma viride*, a well-known biocontrol

fungus, further suggests the ecological importance of weed rhizospheres as reservoirs of beneficial fungi. Overall, the results indicate that weed rhizospheres provide a favorable

microenvironment for diverse fungal communities, which may play a significant role in nutrient cycling and soil health.

Table 2. Morphological and Cultural Characteristics of Fungal Isolates

Fungal Isolates	Colony Characteristics
<i>Aspergillus niger</i>	Colonies compact, initially white to yellowish, later turning dark brown to black with dense sporulation
<i>Aspergillus fumigatus</i>	Colonies forming a dense felt of dark green conidiophores with aerial hyphae
<i>Aspergillus flavus</i>	Colonies powdery with yellowish-green spores on the upper surface and reddish-gold pigmentation on the reverse
<i>Aspergillus nidulans</i>	Woolly colonies with white mycelium and septate hyphae
<i>Rhizopus stolonifer</i>	Whitish colonies becoming greenish-brown due to dark sporangia
<i>Rhizopus oryzae</i>	Colonies whitish, turning grey with age; stolons smooth to slightly rough
<i>Aspergillus oryzae</i>	Colonies with aerial mycelium, pale greenish-yellow initially, later dull brown
<i>Penicillium chrysogenum</i>	Fast-growing, velvety colonies ranging from blue-green to grey-green
<i>Penicillium expansum</i>	Dense felt-like colonies, fast growing, usually green in colour
<i>Trichoderma viride</i>	Colonies with scattered light green to yellowish conidia
<i>Curvularia clavata</i>	Colonies with aerial mycelium, greyish-white surface and dark grey reverse
<i>Curvularia lunata</i>	Shiny, velvety black colonies with fluffy branching growth
<i>Fusarium oxysporum</i>	Cottony colonies with aerial mycelium, pink to light violet pigmentation

Table 3. Occurrence of Fungal Isolates in Weed Rhizosphere Soil

Legend: * = Present _ = Absent

Sr. No.	Fungal Isolates	<i>Parthenium hysterophorus</i>	<i>Amaranthus viridis</i>	<i>Tridax procumbens</i>	No. of Occurrences
1	<i>Aspergillus niger</i>	*	*		3
2	<i>Aspergillus fumigatus</i>	*	_	_	1
3	<i>Aspergillus flavus</i>	*	*		2
4	<i>Aspergillus nidulans</i>	_	_	*	1
5	<i>Rhizopus oryzae</i>		*	*	2
6	<i>Rhizopus stolonifer</i>	*	_		1
7	<i>Penicillium expansum</i>	_	*	_	1
8	<i>Penicillium chrysogenum</i>	*	_	_	1
9	<i>Trichoderma viride</i>	*	*		2
10	<i>Curvularia clavata</i>			*	1
11	<i>Curvularia lunata</i>	*			1
12	<i>Fusarium oxysporum</i>	_	*	_	1
	Total				17

Table 4. Frequency of Occurrence of Fungal Isolates

Fungal Isolates	No. of Occurrences
<i>Aspergillus niger</i>	3
<i>Aspergillus fumigatus</i>	1
<i>Aspergillus flavus</i>	2
<i>Aspergillus nidulans</i>	1
<i>Rhizopus oryzae</i>	2
<i>Rhizopus stolonifer</i>	1
<i>Penicillium expansum</i>	1
<i>Penicillium chrysogenum</i>	1
<i>Trichoderma viride</i>	2
<i>Curvularia clavata</i>	1
<i>Curvularia lunata</i>	1
<i>Fusarium oxysporum</i>	1
Total	17

Table 5. Percentage Frequency of Occurrence of Fungal Isolates

Fungal Isolates	Frequency (%)
<i>Aspergillus niger</i>	17.64
<i>Aspergillus fumigatus</i>	5.88
<i>Aspergillus flavus</i>	11.76
<i>Aspergillus nidulans</i>	5.88
<i>Rhizopus oryzae</i>	11.76
<i>Rhizopus stolonifer</i>	5.88
<i>Penicillium expansum</i>	5.88
<i>Penicillium chrysogenum</i>	5.88
<i>Trichoderma viride</i>	11.76
<i>Curvularia clavata</i>	5.88
<i>Curvularia lunata</i>	5.88
<i>Fusarium oxysporum</i>	5.88

Values represent the average of three replicates.

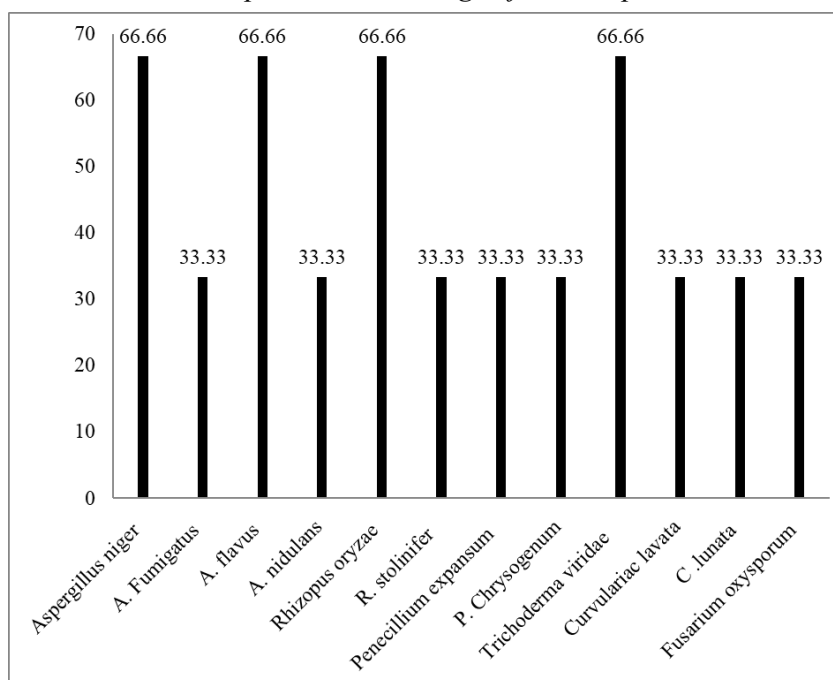
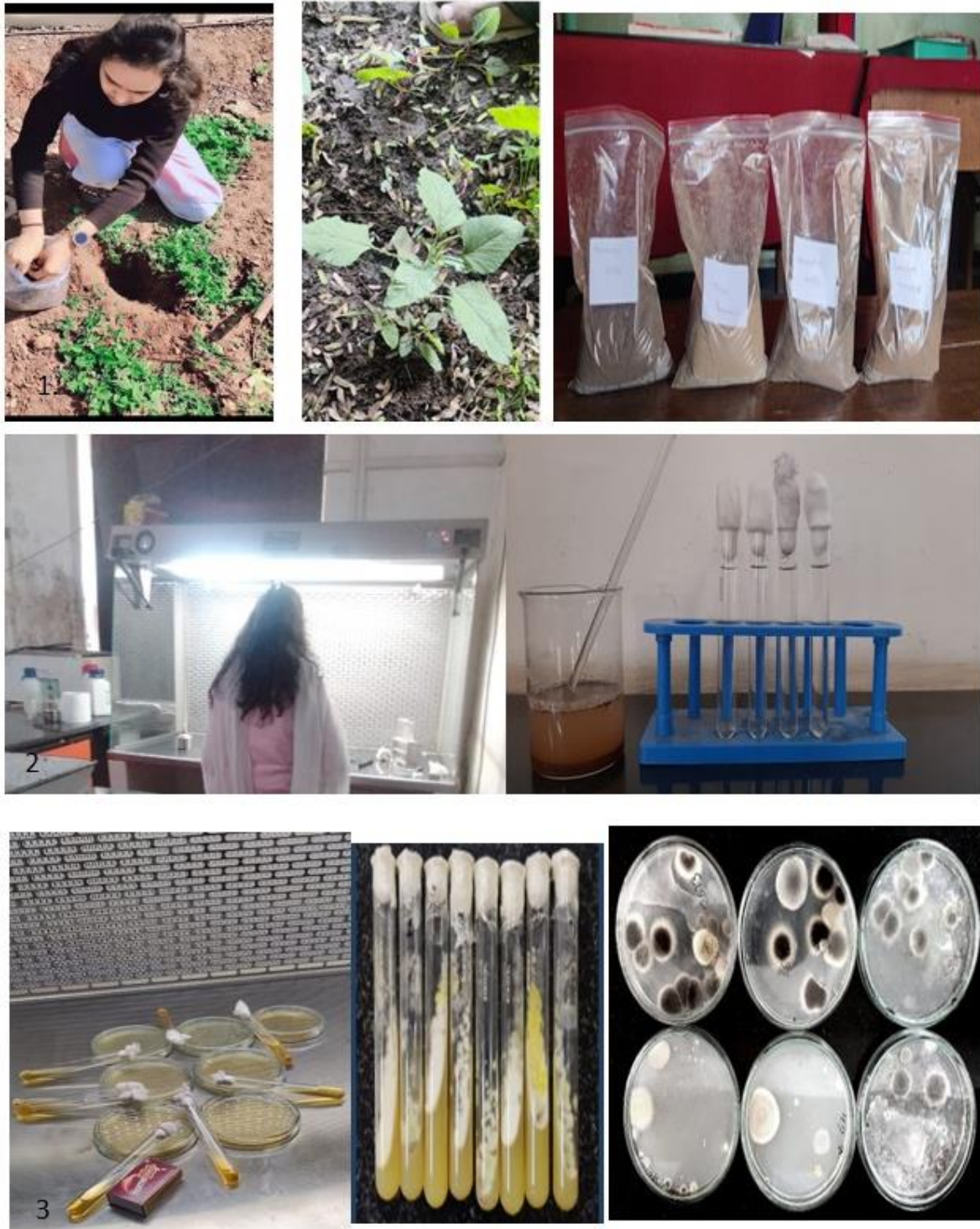
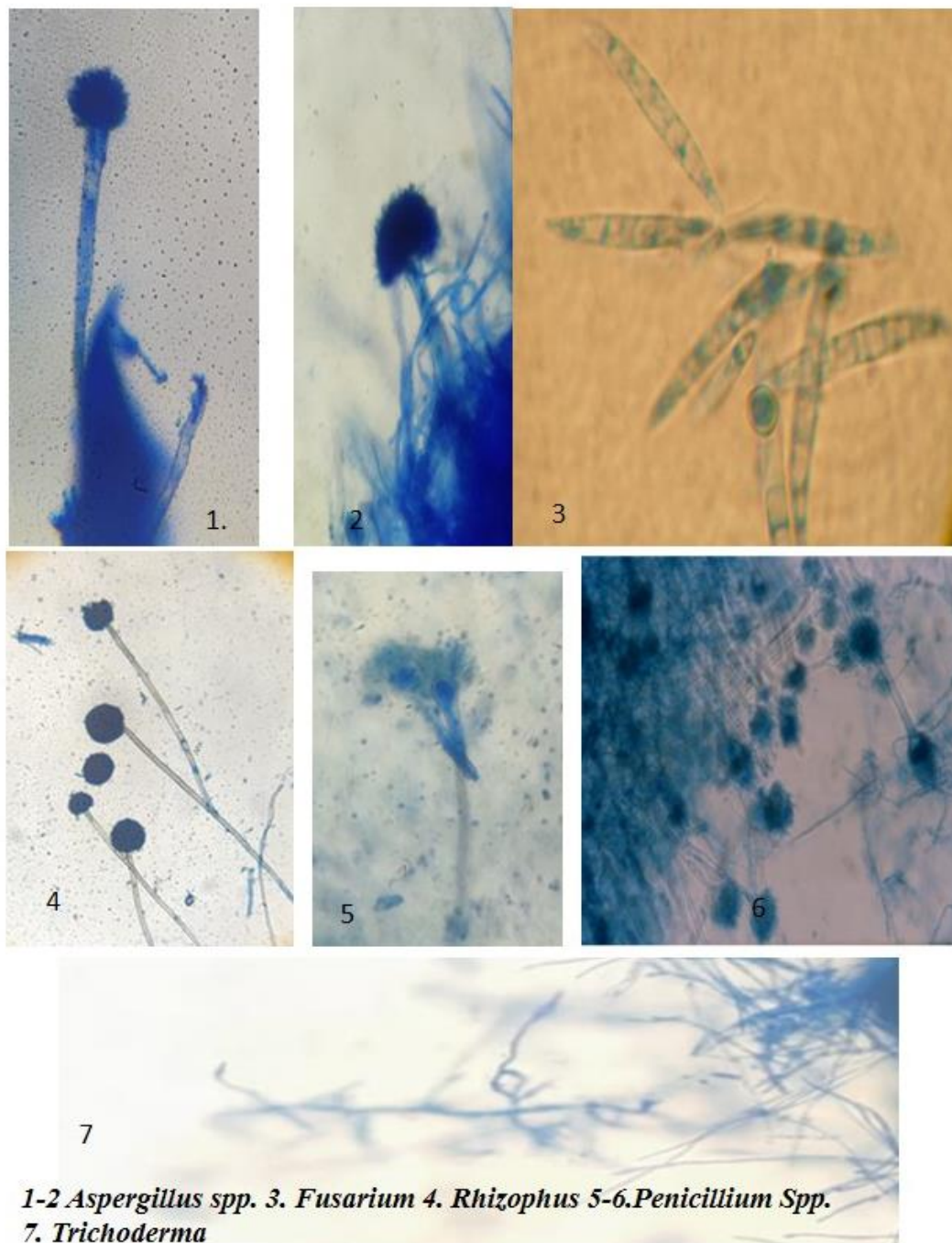


Fig.1 Frequency of occurrences of fungal isolates

PHOTOPLATE -I

- 1. Collection of soil samples.**
- 2. Serial dilution and inoculation**
- 3. Culture plates and slants**

PHOTOPLATE -II

**Discussions:**

Soil is a highly complex and dynamic system in which biological activity plays a crucial role in regulating nutrient cycling and ecosystem functioning (Chiang and Soudi, 1994). Among soil microorganisms, fungi constitute a major component due to their ability to decompose organic matter, adapt to diverse environmental conditions, and interact closely with plant roots

(Gnanasekaran et al., 2015). The present study revealed a diverse assemblage of rhizosphere fungi associated with selected weed species, with a total of **12 fungal species and 17 occurrences** recorded from the study area. The dominance of fungi belonging to the genera *Aspergillus*, *Penicillium*, *Rhizopus*, and *Trichoderma* indicates that weed rhizospheres provide a favorable microhabitat for fungal colonization.

Among the isolated fungi, *Aspergillus niger* exhibited the highest frequency of occurrence. The dominance of *Aspergillus* species, particularly *A. niger*, can be attributed to their rapid growth rate, high sporulation capacity, and ability to utilize a wide range of organic substrates. These fungi are well adapted to slightly alkaline soils with moderate to high organic carbon content, as observed in the present study. The soil pH range (7.2–7.5) and appreciable levels of organic carbon and macronutrients (N, P, and K) likely supported the proliferation of saprophytic fungi, especially *Aspergillus* and *Penicillium* species.

Moderate frequencies of *Trichoderma viride*, *Aspergillus flavus*, and *Rhizopus oryzae* suggest their active involvement in rhizosphere processes such as organic matter decomposition and nutrient transformation. *Trichoderma* species are well known for their antagonistic properties against plant pathogens and their role in promoting plant growth, indicating that weed rhizospheres may act as reservoirs of beneficial fungi. In contrast, fungi such as *Fusarium oxysporum* and *Curvularia* species occurred at lower frequencies, possibly due to competitive exclusion by fast-growing saprophytic fungi or less favorable microenvironmental conditions for their establishment.

The fungal diversity observed in the present investigation is comparable with earlier studies conducted in agricultural soils. Kumar et al. (2015) reported 18 fungal species belonging to six genera from paddy field soils of Tekkali Mandal, with *Aspergillus* and *Penicillium* species predominating. Similarly, Gaddeyya et al. (2012) documented the dominance of *Aspergillus*, *Penicillium*, *Trichoderma*, *Fusarium*, and *Curvularia* species in soils collected from various crop fields. The similarity in fungal composition supports the present findings and confirms that

these genera are common and ecologically successful soil inhabitants.

The predominance of **asexual fungal forms (mitosporic fungi)** in the present study may be explained by their efficient dispersal mechanisms, rapid asexual reproduction, and adaptability to fluctuating soil conditions. Weed rhizospheres, enriched with root exudates and organic residues, create nutrient-rich niches that enhance fungal richness and diversity. These fungi contribute significantly to soil fertility, organic matter turnover, and overall soil health.

In conclusion, the present study highlights the ecological importance of weed rhizospheres as habitats for diverse fungal communities. The dominance of saprophytic and beneficial fungi suggests that weeds play a crucial role in maintaining soil microbial diversity and functional stability. The findings are in close agreement with earlier reports and emphasize the potential of weed-associated rhizosphere fungi in sustainable soil and crop management strategies.

A similar pattern of fungal dominance has also been reported by Bhuyan et al. (2022), who investigated soil mycoflora from agricultural fields of Kaborugaon, Golaghat district. Their study recorded **15 fungal genera**, with *Aspergillus*, *Penicillium*, *Rhizopus*, and *Trichoderma* consistently dominating across all sampling sites. The prevalence of these genera was attributed to their high adaptive capacity, rapid growth, and efficient utilization of organic substrates in soil environments. Furthermore, Thinrat (2016) also reported the dominance of similar fungal groups in cultivated soils, emphasizing their widespread distribution and ecological significance.

The observations made by Bhuyan et al. (2022) and Thinrat (2016) closely corroborate the findings of the present investigation, where the rhizosphere soils of weed species were likewise dominated by *Aspergillus*, *Penicillium*, *Rhizopus*,

and *Trichoderma*. This strong agreement suggests that these fungal genera are universally dominant components of agricultural and weed-associated rhizosphere soils, irrespective of geographical location, due to their ecological versatility and competitive ability.

Kaborugaon, Golaghat district, was investigated by Bhuyan et al. in 2022. There were found to be 15 different genera of fungal forms. *Aspergillus*, *Penicillium*, *Rhizopus*, and *Trichoderma* were discovered to be dominant in all agricultural fields of all areas mentioned. The conclusions and observations made by Bhuyan et al. in 2022 and Thinrat in 2016 closely match the work done in the present.

Conclusion:

The fungal forms *Aspergillus*, *Penicillium*, and *Trichoderma* that are frequently found associated in rhizosphere soil are useful for farmers, agronomists, researchers, and microbiologists for future activities in the view of soil ecosystem conservation, conservation of soil microbial diversity, and sustainable agriculture, according to our research findings and literature review.

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