Isolation and identification of Soil Fungi from Selected Regions of Peshawar and Charsadda, Pakistan

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Abstract

The isolation and identification of fungi from soil play a crucial role in understanding the diverse microbial communities contributing to ecosystem dynamics. Fungi, as essential components of the soil microbiome, significantly influence nutrient cycling, plant health, and overall soil ecology. This study aimed to demonstrate the fungal diversity in a specific soil environment through a systematic isolation and identification process. The soil samples were collected from diverse locations in Peshawar and Charsadda, considering variations in vegetation, climate, and land use. Different dilution and plating techniques were employed to isolate fungi from the soil matrix. These isolated fungal colonies were then subjected to microscopic examination, employing staining methods to discern morphological characteristics such as hyphal structure, spore morphology, and reproductive structures. The morphological traits served as primary indicators for preliminary identification. The results revealed maximum number of species amongst fungal isolates belongs to Aspergillus and Rhizophus genera. Among all the identified species Aspergillus flavus was found in maximum numbers in collection sites, with highest species richness in soil sample of Jindi River (8), followed by Haryana and Warsak region (2 each). Additionally, the study provided insights into the fungi's adaptability to specific soil conditions. The isolation and identification of fungi from soil is pivotal for ecosystem management, agricultural practices, and environmental conservation efforts, underscoring the significance of fungi in maintaining soil health and ecosystem functionality.

Keywords: Agricultural Practice, Isolation, Identification, Microbial Communities, Soil Fungi

Introduction

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Microbial communities of plants and herbs preserve their resources in the soil that can produce nitrogen and CO_2 cycles. Microorganisms greatly influence the soil ecology. Through the breakdown of organic matter, nutrient recycling, and biological control, microbial composition and function change the quality of the soil (Gadde & Kalli, 2020b; Stefani*s et al.,* 2013). Fungi are one type of organism that has been extensively studied for its potential in the bioremediation of heavy metals. Fungi have a variety of biosorption properties and the capacity to flourish in adverse conditions (Gul *et al*., 2021; Akhtar *et al*., 2013). The soil used for agriculture is a dynamic environment where various microbes and fungi that are both harmful and harmless exist in close contact (Pauzi *et al*., 2019; Lowenfels and Lewis, 2006). Recycling carbon and nitrogen is made possible by soil microbes (Gadde & Kalli, 2021b). The beneficial substances produced by microorganisms contribute to the health of the soil, plant growth, and the biological balance of life on our planet. They also play a significant role in the nutritional chains that support these processes (Jan *et al*., 2023; Dar, 2009).

Typically, the physical traits of the colony and microscopic investigations are used to identify the fungal species (Gadde & Kalli, 2020a; Ciriminna *et al*., 2013). Microscopy and culture remain widely used and important instruments for identifying fungal species like Penicillium and Aspergillus, although molecular approaches continue to advance and become more quickly available (Gul *et al*., 2020; Klich, 2002). The cycle of the airborne fungi was observed, with spring and autumn having the highest frequency and summer having the lowest (Rahim *et al*., 2019. The most abundant species of Aspergillus in soil were *Aspergillus niger* and *Aspergillus flavus*. Cladosporium, Curvularia, Drechslera, Alternaria, and Penicillium were among the recovered common genera (Gadde & Kalli, 2021a; Alsharjabi & Al-Qaddafi, 2015). More than 90% of terrestrial plant species have mycorhizal fungi growing on their root systems in a mutually beneficial relationship (Zareef *et al*., 2023; Currah *et al*., 1990). The host plant's photosynthesis produces sugars that the fungus exchange for the necessary ion's phosphate and nitrate (Gul *et al*., 2019; Wipf *et al*., 2019). Hence the current studies focus on the isolation and identification of fungal strains form natural sources.

Materials and Methods

Collection of Soil and Water Sample Sites

Seven zones (Warsak, Warsak (Garhi Sherdad), Tarnab Farm, Sufaid sang, Zandai Tarnab, Haryana of Peshawar region, and, Jindi River of Charsadda region) were chosen for collection, and soil samples were

taken from each area close to the roots, where the majority of the activity of microbes is found. Using a variety of sterile, clean, dry polythene bags and a sterile spatula, soil samples (about 5g) were collected.

PDA Media Preparation

Potato tubers, peeled off and weighed 200g, then using a knife to cut the tubers into little pieces, added dextrose (20g) and agar (15g) to the extract, gently heated it, and then gave it a shake to help the ingredients dissolve. Then transferred medium into a litre cylinder. By adding drops of HCL or NaOH, the medium's pH was adjusted to 5.6 Put the medium into two Erlenmeyer flasks, top them with cotton plugs, cover them with foil or paper, and autoclave them for 20 minutes at 120°C. The flasks were taken out and stored at room temperature when the temperature decreased **(**Kour *et al*., 2019).

Preparation of soil sample and microbial culture

Samples of soil were diluted using 1g of soil in 10 ml of sterile distilled water. In sterile Petri plates that contained Potato Dextrose Agar, 1 ml of suspension was applied. The plates were incubated for 5-7 days at 28 °C. The majority of the fungi's isolated species are heavily sporulate. The pure culture was used to fill test tubes filled with brand-new agar slants of PDA medium. The spread plate technique was used for pure cultures because separating pure cultures from mixed colonies was difficult (Stefani*s et al.,* 2013).

Fungi isolation from soil sample

Two methods were used to count the soil micro fungi: the use of the soil plate and soil dilution plate methods on potato dextrose agar containing medium. To create microbial suspensions (10-1 to 10-5), ten ml of double distilled water were used to suspend 1 gramme of soil sample. To isolate fungus, 10-3, 10-4, and 10-5 fold dilutions were utilised. Each concentration of the microbial solution was applied in triplicate to 15 ml of sterile Potato Dextrose Agar in sterile Petri dishes. Identification of the soil fungi involved studying their morphology under a compound microscope for conidia, conidiophores, and the arrangement of spores as well as macroscopically examining colony characteristics (colour and texture) (Stefani*s et al.,* 2013).

Determination of Colonies

It was determined how many colonies there were on each plate in 1g of soil. Using the following formula, the % contribution of each isolate was determined:

% contribution = Total no. of CFU of an individual specie/ Total no. of CFU of all species × 100 *CFU-Colony Forming Unit

Fungal Staining

The fungus either produces hyaline (colorless) or different colored fungal spores. Using lactophenol and cotton blue, the cytoplasm and hyaline mycelia, spores, and conidia were examined. To create a light blue the background, cytoplasm was stained with cotton blue. Lactophenol has cleaning properties. The specimens that were stained were examined using a light microscope (Magnus MLXi plus) to identify them at a magnification of 10X × 40X (Stefani*s et al.,* 2013).

The impact of Lacto Phenol Cotton Blue (LPCB)

LPCB is a stain used to create fungus microscopic preparations that are semi-permanent. The three elements listed below make up the LPCB stain. Phenol is an organism killer. Lactic acid: protects the fungal structures the cotton blue dye deeply colours the chitin and cellulose present in the fungal cell wall (Stefani*s et al.,* 2013).

Optimization of culture condition for fungi

Potato dextrose agar medium were utilised to determine the ideal conditions for isolated fungus. The pH 4 to 7 adjustments were made. To increase the incubation period, the culture plates were incubated for four to seven days at 28°C (Stefani*s et al.,* 2013).

Identification of Fungi

Examining the colonies for slow or rapid growth, topography (flat, piled, folded regularly or irregularly), texture (yeast like, powdery, granular, velvety or cottony) and other macroscopic and microscopic characteristics Micromorphological (Hyphae, macro conidia, micro conidia, chlamydospores) and morphological (surface pigmentation and reverse pigmentation) and using the most recent identification keys, appropriate medium, slide cultures, and other unique fungal structure) features (Stefani*s et al.,* 2013).

Results

A total of 13 different fungal isolates were obtained from the soil sample. These 13 fungal isolates from soil samples were identified with the help of pathologist; Dr. Sana Ishteyaq from Agricultural Research Institute (ARI) Tarnab Farm Peshawar. According to the results maximum number of species amongst fungal isolates belongs to *Aspergillus* and *Rhizophus* genera. Among all the identified species *Aspergillus flavus* was

found in maximum numbers in collection sites, with highest species richness in soil sample of Jindi River (8), followed by Haryana and Warsak region (2 each).

Soil samples Study

Any soil's fungal diversity is dependent on a number of variables, including pH, moisture, and organic matter. Varied fungal group were isolated from soil sample of Tarnab Farm presented in Table (4.1). According to the result dominant species were *Rhizopus* with a common CFU value 4, having white and yellowish white colony colour, followed by *Aspergillus flavus* (green) with CFU (3), *Aspergillus niger* (black) and *Penicillium* (yellowish) with common CFU value 2. Nature of hyphae was Aseptate in *Rhizophus* and septate in rest of the species. Total number of colonies observed in soil sample collected from Jindi river was 19, colour of colony was green and nature of its hyphae was septate shown in Table (4.2). Haryana site was dominated by *Aspergillus flavus* with CFU (14), having colony colour green, followed by *Aspergillus Niger* having CFU value (5), with colony colour black, *Rhizopus* having CFU (2), with colony colour white and *penicillium* with least CFU value (1) having yellowish colony colour (Table 4.3). Results of the data collected from Warsak area revealed existence of two fungal species *Aspergillus niger* with highest CFU value (10), followed by *Rhizopus* CFU (6). The colour of the colony was black and white respectively. Nature of the hyphae was septate and Aseptate (Table 4.4).

Species isolated from Sufaid Sang area include *Rhizopus* with maximum CFU (9), *penicillium* and *Aspergillus niger* with common CFU (2), *Aspergillus flavus* with CFU (1) (Table 4.5). The respective color of the colony was White, Yellowish, Black and green respectively. Nature of hyphae was Aseptate in case of *Rhizopus,* whereas rest of the species have Aseptate hyphae. Results displayed in (Table 4.6) revealed the occurrence of *penicillium* with maximum CFU (6), followed by *Aspergillus flavus* with CFU (5), *Rhizopus* with CFU (4) and *Aspergillus niger* with CFU (3). Colony color was yellowish, green, white and black respectively. The most abundant fungi of the wasrsak (2) were *Aspergillus* with CFU (14), followed by *Penicillum* CFU value (6), *Aspergillus niger* with CFU (5) while *Rhizopus* was with least CFU (4) (Table 4.7).

Comparison among soil samples

Data shown in Figure 4.1 and 4.2 bestowed hat all soil isolates from different areas was contaminated with various fungi *i.e Aspergillus niger, Aspergillus flavus, penicillium, Rhizopusetc* with varied number of CFU values.

Table 4.1
Soil Sample Tarnab Farm

Species names	**Colony color**	**Surface**	**Colony shape**	**CFU**	**Nature of hyphae**	**Conidia shape**
Aspergillus flavus	Green	Quietly spherical	Flate	5	Septate	globose
Aspergillus niger	Black	Smooth walled	Raised	3	Septate	Rough and irregular
Rhizophus	White	Smoth and striated in texture	Raised	4	Aseptate	Globose
Pinicellium	yellowish	Smooth / rough	Round	6	Septate	oval

Table 4.2
Soil Sample Jindi River

Species name	**Colony color**	**Surface**	**Colony shape**	**CFU**	**Nature of hyphae**	**Conidia shape**
Aspergillus flavus	Green	Quietly spherical	Flat	19	Septate	globose

Table 4.3
Soil Sample Haryana

Species names	Colony color	Surface	Colony shape	CFU	Nature of hyphae	Conidia shape
Aspergillus flavus	Green	Quietly spherical	Flate	14	Septate	globose
Aspergillus niger	Black	Smooth walled	Raised	5	Septate	Rough and irregular
Rhizophus	White	Smoth and striated in texture	Raised	2	Aseptate	Globose
Pinicellium	yellowish	Smooth / rough	Round	1	Septate	Oval

Table 4.4
Soil Sample Warsak

Specie names	**Colony color**	**Surface**	**Colony shape**	**CFU**	**Nature of hyphae**	**Conidia shape**
Aspergillus flavus	Green	Quietly spherical	Flat	14	Septate	globose
Aspergillus niger	Black	Smooth walled	Raised	5	Septate	Rough and irregular

Rhizophus	White	Smooth and striated in texture	Raised	4	Aseptate	Globose
Penicillium	Yellowish	Smooth / rough	Round	6	Septate	oval

Table 4.5
Soil Sample Sufaid Sang

Species names	**Colony color**	**Surface**	**Colony shape**	**CFU**	**Nature of hyphae**	**Conidia shape**
Aspergillus flavus	Green	Quietly spherical	Flat	1	Septate	globose
Aspergillus niger	Black	Smooth walled	Raised	2	Septate	Rough and irregular
Rhizophus	White	Smoth and striated in texture	Raised	9	Aseptate	Globose
Pinicellium	Yellowish	Smooth / rough	Round	6	Septate	oval

Table 4.6
Soil Sample Warsak Garhi Sherdad

Species names	**Colony color**	**Surface**	**Colony shape**	**CFU**	**Nature of hyphae**	**Conidia shape**
Aspergillus niger	Black	Smooth walled	Raised	10	Septate	Rough and irregular
Rhizophus	White	Smooth and striated in texture	Raised	6	Aseptate	Globose

Table 4.7
Soil Sample Zandai Tarnab

Species names	**Colony color**	**Surface**	**Colony shape**	**CFU**	**Nature of hyphae**	**Conidia shape**
Aspergillus flavus	Green	Quietly spherical	Flat	3	Septate	globose
Aspergillus niger	Black	Smooth walled	Raised	2	Septate	Rough and irregular
Rhizophus	White	Smooth and striated in texture	Raised	4	Aseptate	Globose
Pinicellium	yellowish	Smooth / rough	Round	2	Septate	oval
Fusarium	Whitish yellow	Smooth	Slender	4	septate	Sickle

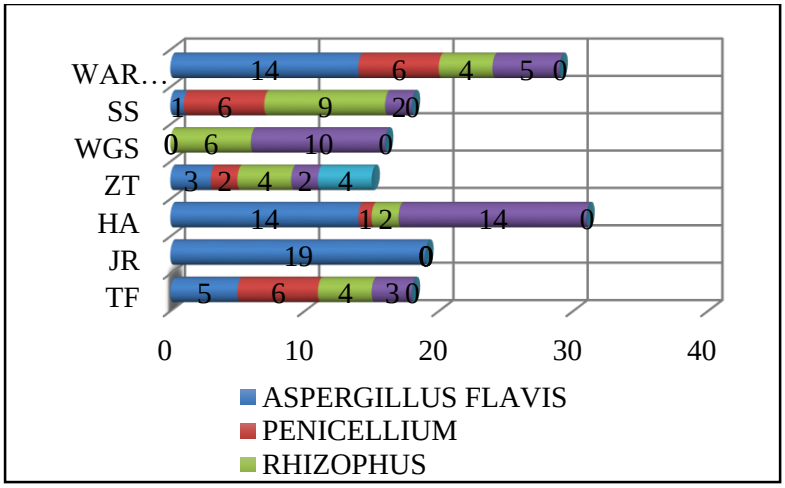

Figure No. 4.1. Comparing Species Diversity in Different Area of Soil samples

Discussion

During the study, the genus *Aspergillus* was observed to exist in almost all collected samples from different research sites with the maximum number of colonies. Several workers in different study areas have also made these observations from many parts of the world. Previous research also showed that the two most common fungi in forest soils were *Aspergillus* and *Penicillium*. According to Saravanakumar *et al*. (2012), who investigated the flora of *Penicillium* and *Aspergillus* in various habitat soils, they found 23 species of Aspergillus and 16 species of *Penicillium* in wet evergreen forest soils. They identified 20 species of *Aspergillus* and 22 species of *Penicillium* from their study of the *Aspergillus* and *Penicillium* flora in Northeast Anatolia. *Aspergillus* is more common in the summer, whereas *Penicillium* is more common in the winter. The dominant genus in the study, *Aspergillus*, was frequently isolated from soils throughout all seasons and was found on a variety of organic debris. All soil samples contain *Aspergillus* species that are common as well as to being dominant.

The current study has reported that *Aspergillus* is more common than *Penicillium* in warmer climates, which is consistent with these observations. One of the most popular techniques used by researchers is the measurement of colony-forming units, which provides a reliable estimate of the number of fungal populations in various environments, such as soil. There was variance in the percentage that each step contributed to the overall fungal population. Any soil's fungal diversity is influenced by a variety of elements, including moisture, pH, and organic

matter. Many types of fungi can grow in both acidic and alkaline soils. The type of organic content, weather, surface vegetation, and soil texture all affect where soil fungi are found. The moisture content and soil texture are found to be directly correlated. Because silt and clay soils have the highest moisture content, there is an apparent increase in the number of fungi. In the current study, the isolated fungi were identified using morphological, microscopic, and cultural traits. With a comprehensive nutritional foundation, PDA is recognised as the general medium most frequently used in the isolation of fungi.

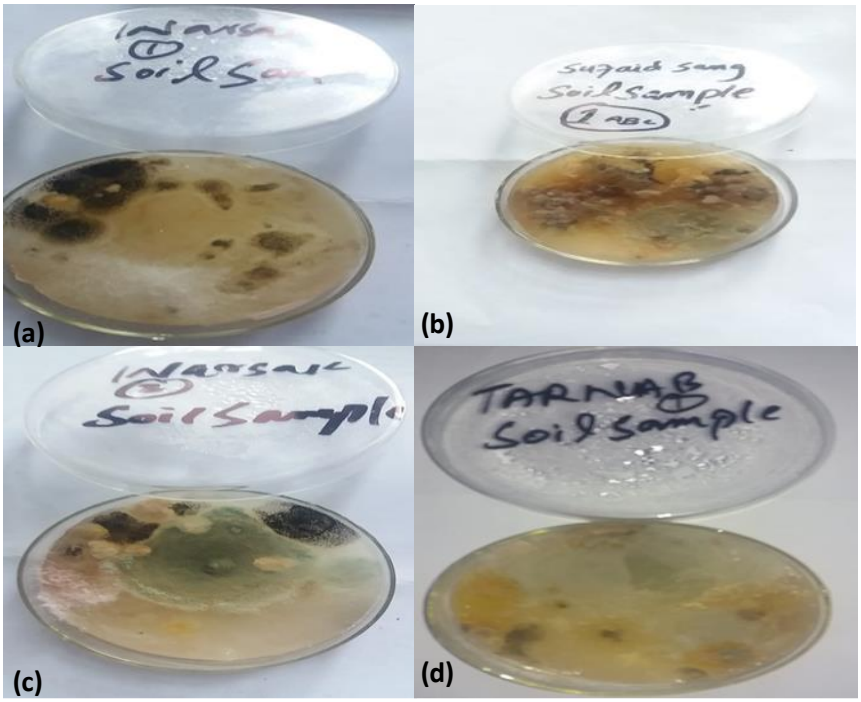

Fig. 4.2: Fungus of (a) Warsak soil sample, (b) Sufaid sang soil sample, (c) Warsak Garhi Sherdad soil sample (d) Tarnab Form soil sample

Conclusion

Most of the microorganisms that inhabit our surroundings are bacteria and fungi, which are employed in a variety of industrial processes such as the synthesis of pharmaceuticals, textiles, enzymes, and bioremediation. Microorganisms are crucial to the composting of organic waste and can contribute significantly to the best kind of agricultural waste. The diversity of microorganisms found in the habitat of agricultural soil was identified and isolated in this study.

References

Akhtar, S., Mahmood-ul-Hassan, M., Ahmad, R., Suthor, V., & Yasin, M. (2013). Metal tolerance potential of filamentous fungi isolated from soils irrigated with untreated municipal effluent. *Soil Environ*, *32*(1), 55-62.

Alsharjabi, F. A., & Al-qadasi, A. M. (2015). Mycobiota associated with weaning dried foods consumed in Taïz City, Republic of Yemen. *Journal of Basic & Applied Mycology (Egypt)*, *6*, 49-58.

Ciriminna, R., Fidalgo, A., Pandarus, V., Beland, F., Ilharco, L. M., & Pagliaro, M. (2013). The sol–gel route to advanced silica-based materials and recent applications. *Chemical reviews*, *113*(8), 6592-6620.

Currah, R., Smreciu, E. A., & Hambleton, S. (1990). Mycorrhizae and mycorrhizal fungi of boreal species of Platanthera and Coeloglossum (Orchidaceae). *Canadian Journal of Botany*, *68*(6), 1171-1181.

Dar, G. H. (2009). *Soil microbiology and biochemistry*. New India Publishing.

Gadde, S. S., & Kalli, V. D. R. (2020a). Applications of Artificial Intelligence in Medical Devices and Healthcare. *International Journal of Computer Science Trends and Technology*, *8*, 182-188.

Gadde, S. S., & Kalli, V. D. R. (2020b). Applications of Artificial Intelligence in Medical Devices and Healthcare. *International Journal of Computer Science Trends and Technology*, *8*, 182-188.

Gadde, S. S., & Kalli, V. D. (2021a). Artificial Intelligence at Healthcare Industry. *International Journal for Research in Applied Science & Engineering Technology (IJRASET)*, *9*(2), 313.

Gadde, S. S., & Kalli, V. D. (2021b). The Resemblance of Library and Information Science with Medical Science. *International Journal for Research in Applied Science & Engineering Technology*, *11*(9), 323-327.

Gul, M. T., Dheyab, A. S., Shaker, E. K., Muhammad, N., & Pauzi, A. N. (2020). In vitro evaluation of anti-urolithiatic properties of *Strobilanthes crispus* extracted using different solvents. *Research Journal of Chemistry and Environmentl*, *24*, 1.

Gul, M. T., Muhammad, N., Pauzi, A. N., Bakar, M. F. A., Talip, B. A., Abdullah, N., ... & Ibrahim, N. (2021). Evaluation of *Phyllanthus niruri* L. from Malaysia for In-vitro Anti-Urolithiatic Properties by Different Solvent Extraction: Properties of Anti-Urolithiatic. *Biological Sciences-PJSIR*, *64*(1), 81-86.

Jan, H. U., Saeed, A., Parveen, G., Mukhtar, N., Siraj, M., Sami, A., & Gul, M. T. (2023). Pharmacognostic Study of *Ehretia acuminata*

R. Br. *Proceedings of the Pakistan Academy of Sciences: B. Life and Environmental Sciences*, *60*(2), 267-272.

Klich, M. A. (2002). *Identification of common Aspergillus species*. CBS..

Kour, D., Rana, K. L., Yadav, A. N., Yadav, N., Kumar, V., Kumar, A., ... & Saxena, A. K. (2019). Drought-tolerant phosphorus-solubilizing microbes: biodiversity and biotechnological applications for alleviation of drought stress in plants. *Plant growth promoting rhizobacteria for sustainable stress management: Volume 1: Rhizobacteria in abiotic stress management*, 255-308.

Lowenfels, J., & Lewis, W. (2010). *Teaming with microbes: The organic gardener's guide to the soil food web*. Timber Press.

Pauzi, A. N., Muhammad, N., Sairi, N. H., Putra, T. T., Gul, M. T., Rahim, N. F. A., ... & Abdullah, N. (2019, July). The effect of different solvent extraction towards antiurolithiatic properties of *Euphorbhia hirta* and *Orthosiphon stamineus*. In *IOP Conference Series: Earth and Environmental Science* (Vol. 269, No. 1, p. 012040). IOP publishing.

Gul, R., Jan, H. U., Gul, M. T., Khan, M. A., Malik, A., Jan, S., Rafiullah, A. S. D., & Muhammad, N. (2019). In-vivo pharmacological investigation of root extract of *Myrtus communis* L. by using Animal model (Mus musculus). *In-vivo*, *4*(1).

Rahim, N. F. A., Muhammad, N., Putra, T. N. M. T., Marzuki, W. N. A. S. W., Abdullah, N., Talip, B. A., ... & Gul, M. T. (2019). Antioxidant Activity and its Interaction Effect on Polyherbal Formulations of *Nephrodium inophyllum*, *Polygonum minus Annona squamosal* L. and *Stevia rebaudiana*. *Journal of Advanced Research in Fluid Mechanics and Thermal Sciences*, *61*(1), 1-9.

Stefanis, C., Alexopoulos, A., Voidarou, C., Vavias, S., & Bezirtzoglou, E. (2013). Principal methods for isolation and identification of soil microbial communities. *Folia microbiologica*, *58*, 61-68.

Wipf, D., Krajinski, F., van Tuinen, D., Recorbet, G., & Courty, P. E. (2019). Trading on the arbuscular mycorrhiza market: from arbuscules to common mycorrhizal networks. *New Phytologist*, *223*(3), 1127-1142.

Zareef, H., Gul, M. T., Qureshi, R., Aati, H., & Munazir, M. (2023). Application of ethnobotanical indices to document the use of plants in traditional medicines in Rawalpindi district, Punjab-Pakistan. *Ethnobotany Research and Applications*, *25*, 1-29.