Section A-Research paper
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Comparative analysis of physiochemical characteristics of rhizosphere soil for selected orchids of India

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ABSTRACT

There are 1256 species of orchids in India, of which 447 are terrestrial. The rhizosphere, which is located around 2 mm from the root surface, is an important part of the orchids' environment. It controls the chemistry of orchid nutrients and has an impact on plant development. Studies on the physiochemical characteristics of rhizosphere soil for terrestrial orchids are lacking in India. We used standard methods to study physicochemical and chemical analysis of rhizospheric soil like determination of pH (soil reaction), electrical conductivity, organic carbon, and available macro and micronutrients (P, K, Zn, Fe, Cu, Mn) were carried out using standard procedures. We found that rhizosphere soil has a sufficiently acidic pH and good electrical conductivity. The rhizosphere soil samples of *Calanthe sylvatica*, *Malaxis rheedei* and *Satyrium nepalense* collected from Kemmangundi showed low Zn concentrations indicating low availability of soluble zinc. The other two orchids from Shimoga showed higher Zn values than standard soil. The soil samples analyzed contained adequate to high amounts of available organic carbon, potassium, Fe, Cu, Mn, but were deficient in micronutrient phosphorus. Hence, there is a need for micronutrient fertilizers rich in P and Zn to avoid inadequate absorption of trace elements by plants.

Keywords: Orchid, Electrical conductivity, organic carbon, micronutrients, phosphorus

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1. INTRODUCTION

Mycorrhiza term describes the fungus-infested roots. Symbiotic relationships between specific soil fungi and plants are known as mycorrhizae. They are crucial for one or both couples and are largely in charge of nutrition transport. The majority of plants in natural environments have mycorrhizal associations, which occur when symbiotic fungi successfully colonize healthy tissues of terrestrial plants' roots. The rhizosphere is a place where soil components, plants, and microorganisms interact with each other (Pinton et al., 2000). Such interactions may affect nutrient dynamics, plant development and growth, and susceptibility to disease and abiotic stress.

Orchids (Orchidaceae) are monocotyledonous plants that make up the biggest angiosperm family with the highest species diversity. Orchids' mycorrhizal relationship is known for more than 125 years, when he observed that the roots were frequently colonized by fungi (Srivastava et al., 2015). There are 1256 species of orchids in India, 447 of which are terrestrial. Some terrestrial orchids are achlorophyllous and myco-heterotrophic, while the majority are green and photosynthetic. All orchid species go through a protracted seedling period where they are unable to photosynthesize and are dependent on mycorrhizal fungus for an external supply of carbohydrates. These fungi take up nutrients, particularly phosphate, nitrogen, and organic carbon in a variety of ways (Xiong et al., 2022). It is believed that myco-heterotrophs get minerals and carbohydrates throughout the plant's existence. As a result, it is thought that the relationship between these orchids and fungi is more parasitic than symbiotic, with the fungi being used by the achlorophyllous orchids (Djordjević & Tsiftsis, 2022).

The rhizosphere, which is located around 2 mm from the root surface, is an important part of the orchids' environment. It controls the chemistry of orchid nutrients and has an impact on plant development. Rhizosphere microorganisms also take care of the pH of the soil to keep at the proper level. pH guards plants against diseases and poisonous chemicals (Lambers et al., 2009). Beyond this, the soil is the source of all the critical minerals that a plant needs. Of these, 14 are largely obtained from the soil solution. Rhizosphere bacteria and fungi control the availability of nutrients for plants and the soil microbial population by taking part in the geochemical cycling of nitrogen, phosphorus iron, manganese, zinc, and copper as well as micronutrients (N, K, P, S, Mg, and Ca) (Kumar et al., 2016). The majority of these elements are typically ingested in their ionic form from the soil solution. The availability of necessary components has the potential to hinder plant development. Plant roots interact with other species in their rhizosphere to get vital mineral nutrients and prevent the buildup of harmful substances (Yadav et al., 2015). The present work is the first attempt to study rhizosphere soil analysis of five terrestrial orchid species of Karnataka for determining the impact of mycorrhizal association between microbes and orchids on soil.

MATERIALS AND METHODS

1. Sample collection:

Soil samples were collected from different ecological niches so that diversity in the root system and mycorrhizal association was reflected therein. Orchids were explored in their natural habitats. The plant was carefully dug to avoid damage to the root system. The underground rhizosphere soil samples attached to roots were collected in clean polythene bags and labeled (Jyothsna & Purushothama, 2013).

Five orchids' root soil samples were collected from two regions of Karnataka, India. *Nervilia crociformis, Pecteilis susannae* were collected from Linganmakki Reservoir and Kogar of Shimoga respectively. *Calanthe sylvatica, Malaxis rheedei,* and *Satyrium nepalense* were collected from Tiger Shola, Z- point and Kemmangundi of Chikmagalur respectively (Table 1).

2. Soil analysis

Rhizosphere soil analysis was carried out to study the soil quality. Both physicochemical and chemical analysis like determination of pH (soil reaction), conductivity (EC), organic carbon, and available macro and micronutrients were carried out using standard procedures as outlined by Ryan *et al.* (2001) laboratory manual.

2.1 pH

The pH scale measures the activity of hydrogen or hydroxyl ions in the soil-water system. It shows whether soils are neutral, acidic, or alkaline. Standard acidic, neutral and basic buffer solutions of pH 4.0, 7.0, and 9.2 respectively were prepared in pure water. 100g of soil sample was mixed with 500ml of distilled water over a period of 1-2 minutes to create a saturated soil paste. The dirt mixture was left to stand in the beaker for an hour until it reflected. The pH meter was calibrated with two buffers—one acidic and the other alkaline or neutral—and set to an appropriate temperature. Each sample's pH was measured.

2.2 Electrical conductivity (EC)

Electric current (EC) can flow through ions just like it does through metals. As a result, as the amount of soluble salts in the soil increases, correspondingly rises the EC of the soil-water system. As a result, measuring EC will reveal the amount of soluble salts present in the soil at any given temperature. The conductivity meter was calibrated using common 0.01N potassium chloride. The soil sample was measured in a predetermined amount of distilled water and shaken. Until clear supernatant liquid was produced, it was let to stand. In the liquid supernatant, EC was measured after the conductivity meter was calibrated (Corwin and Lesch, 2005).

2.3 Organic carbon

The source of nitrogen in the soil is called soil organic matter (SOM), and measuring SOM serves as a gauge of how much nitrogen is available. The colorimetric approach was used to quantify organic carbon (Chambers et al., 2011). Chromic acid (potassium dichromate and sulfuric acid) was used to oxidize the organic material. A colorimetric measurement was made of the chromic acid's reduced green color's intensity. The quantity of organic carbon in the soil directly correlates with this intensity.

2.4 Available phosphorus

Phosphorus can be found in soil as a variety of orthophosphates. The plants have access to just a limited portion of resources at any given moment. Calcium, aluminum, and iron-phosphorus fractions make up the majority of the soil's available phosphorus concentration.

Particularly calcium-phosphorus components are prevalent in neutral and alkaline soils. For alkaline soils, the Bray and Kurtz P-1 technique (Sims, 2000) was utilized. Using a colorimeter, the amount of phosphorus removed using the aforementioned techniques was calculated by observing the color intensity at 660 nm.

2.5 Available potassium

Water soluble, exchangeable, non-exchangeable (fixed), and lattice potassium are all forms of potassium found in soil. Since ammonium ions are similar in size to potassium ions and may therefore substitute for the latter, the ammonium acetate technique proposed by Rao et al., (2000) was adopted. Acetate burns smoothly and leaves no residue on the flame photometer's burner during estimating.

2.6 Available micronutrients (Zinc, Iron, Copper and Manganese)

Zinc (Zn), Iron (Fe), Copper (Cu) and Manganese (Mn) were isolated from rhizospheric soil and normal soil. Neutral ammonium acetate, chelating agents like EDTA (Ethylene diamine tetra acetic acid) DTPA (Diethylene triamine pentaacetic acid) were used for extraction of like Zn, Mn, Fe and Cu. These chelating agents combine with free metal ions in solution to form soluble complexes. The colorimetric method was used for the estimation of available Magnese (Reisenauer, 1988). We estimated the Cu contents using a method by Maftoun et al., (2005). Sun et al., (2017) reflectance spectroscopy method was used to estimate zinc contents. Tarafder and Thakur (2005) method was used to the spectrophotometric determination of Iron.

Table 1: Collection sites in India for different orchid species

S.N.	Collection Sites	Orchid Plants
	Shimoga	
1.	Linganmakki Reservoir	*Nervilia crociformis*
2.	Kogar	*Pecteilis susannae*
	Chikmagalur	
3.	Tiger Shola	*Calanthe sylvatica*
4.	Z-Point	*Malaxis rheedei*
5.	Kemmangundi	*Satyrium nepalense*

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3 RESULTS

The analysis showed that the soil quality varied from one collection site to the other. Soil pH was observed to be acidic at both the locations. pH of soil samples of five orchids *Calanthe sylvatica, Nervilia crociformis, Pecteilis susannae* rhizosphere were 5.9, whereas *Malaxis rheedei* and *Satyrium nepalense* were 5.5 and 5.2. It was significantly lower than normal soil pH (6.3-8.5).

Electrical conductivity (EC) is in the normal limits in all the six samples studied. Ions allow electric current to pass through them and hence EC of soil-water system rises with increasing content of soluble salts in the soil. Measurement of EC gives the concentration of soluble salts in the soil at a particular temperature. The rhizosphere soil samples of *Calanthe sylvatica*, *Malaxis rheedei* and *Satyriumnepalens*e collected from Kemmangundi showed low EC values 0.01, 0.02 and 0.04 respectively, indicating low availability of soluble salts. Other two orchids from Shimoga showed higher EC values (0.08 and 0.11).

In the present study, the rhizosphere soil was found rich in organic carbon in all the samples. *Pecteilis susannae* and *Nervilia crociformis* orchids of Shimoga had maximum organic C (3.40% and 2.11%) which was 4-5 times more than normal soil. Among Kemungundi orchids *Satyrium nepalense* had minimum organic carbon content (0.85%), which was also higher than normal range (0.5-0.75).

The rhizosphere soil was noted scanty in orthophosphorus. Their value ranged from 4- 7 (kg/ac), which is significantly lesser than normal soil range (9-22 kg/ac).

Available potassium exists in the form of water-soluble, exchangeable, non-exchangeable (fixed), and lattice-K. The first two forms constitute a small part, considered to be easily available to plants. The soil samples analysed contained an adequate to the high amount of available potassium.

S.N.	**Orchid Species**	**Ph**	**EC (ds)**	**Organic Carbon (%)**	**Phosphorus (kg/ac)**	**Potash (kg/ac)**	**Zn (ppm)**	**Fe (ppm)**	**Cu (ppm)**	**Mn (ppm)**
1	**Normal soil**	**6.3-8.5**	**<1**	**0.5-0.75**	**9-22**	**60-120**	**1**	**2**	**0.2**	**2**
2	***Calanthe sylvatica***	**5.9**	**0.04**	**1.76**	**4**	**104**	**0.64**	**2.1**	**0.6**	**8.4**

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3	***Malaxis rheedei***	**5.5**	**0.02**	**1.58**	**7**	**74**	**0.80**	**63.1**	**0.7**	**44.5**
4	***Nervilia crociformis***	**5.9**	**0.08**	**2.11**	**5**	**152**	**1.7**	**37**	**1.1**	**47.3**
5	***Pecteilis susannae***	**5.9**	**0.11**	**3.40**	**5**	**130**	**1.26**	**35**	**1**	**50**
6	***Satyrium nepalense***	**5.2**	**0.01**	**0.85**	**6**	**126**	**0.70**	**25.4**	**0.6**	**43**

The rhizosphere soil samples of *Calanthe sylvatica*, *Malaxis rheedei*, and *Satyrium nepalens*e collected from Kemmangundi showed low Zn concentrations (0.64, 0.8, and 0.7 respectively), indicating low availability of soluble zinc. The other two orchids from Shimoga showed higher Zn values than standard soil (1.7 and 1.26 respectively).

Available micronutrients (Cu, Fe and Mn) which are essential and required in minor quantities for plant growth were found to be sufficient in all six samples.

4. DISCUSSION

The interaction of plant roots with the soil microbiome takes place in the rhizosphere, which is a special area. It affects the nutritional level of the plants and soil fertility. The physicochemical properties of soil are the combined reaction impacts of plants, soil, and climate (Bian et al., 2020). We found that the pH and EC of soil samples are comparatively less than normal soil. The cause of the rising levels of acidity of soil could be, that as water travels through the soil, there is a propensity for bases to be leached out and replaced by hydrogen ions; this continual leaching causes the production of acidic soils. These modifications modify the soil's chemical composition and the solubility of particular compounds. Many chemicals become more soluble in acidic environments, and compounds containing calcium and phosphorus may be leached away, leading to nutritional deficits (Yadav et al. 2015).

The maintenance of terrestrial ecosystem activities and functions, such as carbon (C) and nutrient cycling, depends heavily on the rhizosphere soil, a hub of microbial activity. Rhizospheres used to have very high C contents compared to regular soil because plant-rhizosphere interactions may be responsible for up to 50% of the total organic carbon generated from terrestrial ecosystems (Zhao et al., 2022). Another reason could be due to the strong activity of soil microbes in decomposing plant litter, the soil was rich with organic carbon in all of the samples (Dotaniya et al., 2015).

Like soil, phosphorus exists in organic form and is made available to developing plants by their decomposition. Phosphorus is usually found in soil as orthophosphates and a very small portion of these is ever accessible to plants (Jones & Oburger, 2011). We noted low phosphorus content in soil for all orchids, because of which the orchid plants formed a mycorrhizal connection with local fungus species.

There are four types of readily available potassium in soil: lattice-K, exchangeable, non-exchangeable (fixed), and soluble in water. The first two types make up a modest portion and are thought to be accessible to plants (Mouhamad et al., 2016). The soil's qualities, such as moisture content, pH, conductivity and temperature, all affect the availability of K to plants (Hou et al., 2019). The amount of readily available potassium was sufficient to high in all soil samples analyzed, showing the rhizosphere soil is suitable for the growth of orchids.

According to Malakouti, (2008), the structure of microbial communities in rhizosphere soils can be predicted by finding important micronutrient content in soil. A sufficient quantity of micronutrients also ensures efficient use of macronutrients. In our study, all six samples were found to be sufficiently high in the important micronutrients (Cu, Fe, and Mn), which confirms the presence of good orchids- microbes association in rhizosphere soil. Whereas the variation in the concentration of Zn was noted at different locations, the reason behind it can be more leaching of Zn from the soil. Hence, we conclude that rhizosphere soil is deficient in micronutrients phosphorus and zinc so there is a need for micronutrient fertilizers rich in P and Zn to avoid inadequate absorption of trace elements by plants.

CONCLUSION: The rhizosphere soil of terrestrial orchids is scanty in phosphorus, so there is a need for fertilizer rich in P for better growth of orchids in India.

CONFLICT OF INTEREST: None

5. REFERENCES

1. Bian, X., Xiao, S., Zhao, Y., Xu, Y., Yang, H., & Zhang, L. (2020). Comparative analysis of rhizosphere soil physiochemical characteristics and microbial communities between rusty and healthy ginseng root. *Scientific Reports*, *10*(1), 15756.

2. Chambers, F. M., Beilman, D. W., & Yu, Z. (2011). Methods for determining peat humification and for quantifying peat bulk density, organic matter and carbon content for palaeostudies of climate and peatland carbon dynamics. *Mires and Peat*, *7*(7), 1-10.

3. Corwin, D. L., & Lesch, S. M. (2005). Apparent soil electrical conductivity measurements in agriculture. *Computers and electronics in agriculture*, *46*(1-3), 11-43.

4. Djordjević V, Tsiftsis S (2022) The role of ecological factors in distribution and abundance of terrestrial Orchids. In: *Orchids Phytochemistry*. Biology and Horticulture, pp 3–72

5. Dotaniya, M. L., & Meena, V. D. (2015). Rhizosphere effect on nutrient availability in soil and its uptake by plants: a review. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *85*, 1-12.

6. Hou, W., Xue, X., Li, X., Khan, M. R., Yan, J., Ren, T., & Lu, J. (2019). Interactive effects of nitrogen and potassium on: Grain yield, nitrogen uptake and nitrogen use efficiency of rice in low potassium fertility soil in China. *Field Crops Research*, *236*, 14-23.

7. Jones, D. L., & Oburger, E. (2011). Solubilization of phosphorus by soil microorganisms. *Phosphorus in action: biological processes in soil phosphorus cycling*, 169-198.

8. Jyothsna, B. S., & Purushothama, K. B. (2013) Studies on the mycorrhiza of Geodorum densiflorum (Lam.) Schltr. from Western Ghats of Karnataka, India.

9. Kumar, V., Kumar, M., Shrivastava, N., Bisht, S., Sharma, S., & Varma, A. (2016). Interaction among rhizospheric microbes, soil, and plant roots: Influence on micronutrient uptake and bioavailability. *Plant, Soil and Microbes: Volume 2: Mechanisms and Molecular Interactions*, 169-185.

10. Lambers H, Mougel C, Jaillard B, Hinsinger P. (2009) Plant-microbe-soil interactions in the rhizosphere: an evolutionary perspective.

11. Maftoun, M., Mohasseli, V., Karimian, N., & Ronaghi, A. M. (2003). Laboratory and greenhouse evaluation of five chemical extractants for estimating available copper in selected calcareous soils of Iran. *Communications in soil science and plant analysis*, *34*(9-10), 1451-1463.

12. Mouhamad, R., Alsaede, A., & Iqbal, M. (2016). Behavior of potassium in soil: a mini review. *Chemistry International*, *2*(1), 58-69.

13. Malakouti, M. J. (2008). The effect of micronutrients in ensuring efficient use of macronutrients. *Turkish Journal of Agriculture and Forestry*, *32*(3), 215-220.

14. Pinton R, Varanini Z, Nannipieri P. (2000) The rhizosphere as a site of biochemical interactions among soil components, plants, and microorganisms. InThe rhizosphere pp. 17-34). CRC Press.

15. Reisenauer, H. M. (1988). Determination of plant-available soil manganese. In *Manganese in Soils and Plants: Proceedings of the International Symposium on* 'Manganese in Soils and Plants' held at the Waite Agricultural Research Institute, The University of Adelaide, Glen Osmond, South Australia, *an Australian Bicentennial Event* (pp. 87-98). Dordrecht: Springer Netherlands.

16. Rao, C. S., Rao, A. S., & Bansal, S. K. (2000). Relationship of some forms of potassium with neutral normal ammonium acetate extractable K in mineralogically different benchmark soil series of India. *Journal of the Indian Society of Soil Science*, *48*(1), 27-32.

17. Ryan J, Estefan G, Rashid A. Soil and plant analysis laboratory manual. ICARDA; (2001).

18. Sims, J. T. (2000). Soil test phosphorus: Bray and Kurtz P-1. *Methods of phosphorus analysis for soils, sediments, residuals, and waters*, 13.
19. Sun, W., & Zhang, X. (2017). Estimating soil zinc concentrations using reflectance spectroscopy. *International journal of applied earth observation and geoinformation*, *58*, 126-133.

20. Tarafder, P. K., & Thakur, R. (2005). Surfactant-mediated extraction of iron and its spectrophotometric determination in rocks, minerals, soils, stream sediments and water samples. *Microchemical Journal*, *80*(1), 39-43.

21. Xiong, Q., Hu, J., Wei, H., Zhang, H., & Zhu, J. (2021). Relationship between plant roots, rhizosphere microorganisms, and nitrogen and its special focus on rice. *Agriculture*, *11*(3), 234.

22. Yadav BK, Akhtar MS, Panwar J. (2015) Rhizospheric plant-microbe interactions: key factors to soil fertility and plant nutrition. Plant microbes symbiosis: applied facets.:127-45.

23. Zhao, X., Tian, P., Sun, Z., Liu, S., Wang, Q., & Zeng, Z. (2022). Rhizosphere effects on soil organic carbon processes in terrestrial ecosystems: A meta-analysis. *Geoderma*, *412*, 115739.