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The rhizosphere is a micro ecological zone in direct proximity of plant roots. The German microbiologist, Hiltner (1904) introduced the concept of rhizosphere. The rhizosphere is defined as the zone closer to plant roots with particulate matter and microbes. The zone away from the plant root system, not directly associated with the plant is non-rhizosphere. Theoretical extent of the rhizosphere is dependent on the zone of influence of the plant roots and associated microorganisms. The rhizosphere only extends a few millimeters from the root surface, and may thus appear as an insignificant proportion of the soil environment. When considering the total root length estimated to be about 700mm for a single wheat plant (Bolton & Fredrickson 1993), Khairnar & Saler (1985) found high fungal population in rhizosphere under natural habitat. The highest number of microfungi in rhizosphere soil in some angiosperms has been reported by Sharma & Bohra (2001) and Basumatry et al. (2005). Yasmeen & Saxena (1990) explored rhizosphere and rhizoplane mycoflora of *Adiantum incisum* Forsk. Bohra & Sharma (2007) have studied the rhizosphere and non-rhizosphere mycoflora of some ferns from Rajasthan. Bharati & Pravesh (2012) explored rhizosphere and non-rhizosphere mycoflora associated with *Lygodium flexuosum* (L.) Sw. and *Ampelopteris prolifera* (Retz.) Copel. from Ranchi District of Jharkhand.

In Kolhapur District, *Anogramma leptophylla* (L.) Link and *Hypodematum crenatum* (Forssk.) Kuhn. are only restricted to small pockets at Panhala Fort (Images 1–3). The present paper deals with studies on rhizosphere and

## RHIZOSPHERE AND NON-RHIZOSPHERE MYCOFLORA OF TWO FERNS FROM PANHALA FORT, KOLHAPUR, MAHARASHTRA, INDIA

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non-rhizosphere mycoflora associated with these ferns.

**Materials & Methods:** Site: Panhala is one of the famous forts in Maratha Empire history, located in Panhala Village, 20km northwest of Kolhapur in Maharashtra, India. It is situated between 16°48'31"N & 74°6'29"E and a maximum elevation of 968m. It is a well-known hill station. The annual temperature ranges between 20–32 °C and annual rainfall is around 261mm.

**Sampling:** The rhizosphere and non-rhizosphere soils of both the ferns were collected in August 2013, by the method suggested by Bohra & Sharma (2007). The plants were uprooted and slightly shaken to remove bulk soil after which rhizosphere soil was collected in new zip lock polythene bags by tapping. Non-rhizosphere soil was collected in separate bags with the help of a sterilized trowel. The bags were then sealed, labeled and brought to the laboratory. They were kept at 4°C until the analysis.

**Isolation:** A microbial suspension was used to study the non-rhizosphere and rhizosphere mycoflora. The method adopted for this work was according to Johnson et al. (1959). To eliminate bacterial growth and better fungal separation, rose Bengal dye (66.7mg/L) and streptomycin (30mg/L) were added to potato dextrose agar (PDA) medium (Smith & Dawson 1944). The

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Image 1. Study site: Panhala Fort

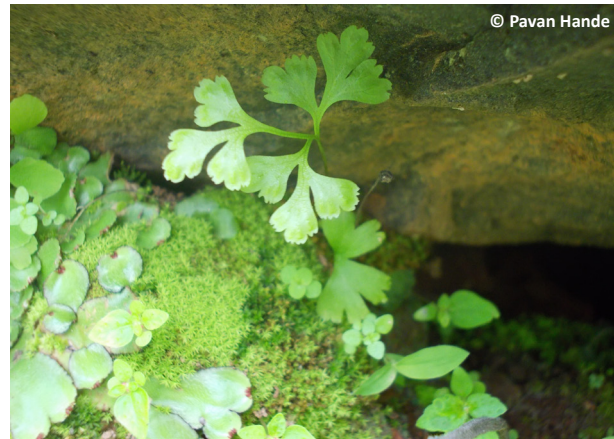


Image 2. *Anogramma leptophylla* (L.) Link.

autoclaved cool medium was poured in autoclaved petriplates. 1g of soil was weighed and three dilutions viz., 1:100, 1:1000 and 1:10000 were prepared in sterile distilled water. The soil solutions were shaken well to homogenize and after settling down, 1ml aliquot was pipetted out in separate sterilized petriplates containing PDA medium. The aliquot was spread evenly on agar surface in each petriplate. Three replicates of each dilution were taken and were incubated at  $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$  for 7–10 days. The plates were then examined for plate count. Colony forming unit and rhizosphere effect were calculated as suggested by Nagamani et al. (2006) and Starkey (1938).

Identification of fungi: The microscopy and micrometry was done. The fungi were identified after Gilman (1945), Barnett & Hunter (1973), Watanabe (2002), Nagamani et al. (2006).

**Results and Discussion:** In the present study, fungal species found in non-rhizosphere were more as compared to rhizosphere of both the ferns. Higher percentage of fungal species was shown by *H. crenatum* (Forssk.) Kuhn 44.5% in non-rhizosphere and 27.77% in rhizosphere. Lower percentage of fungal species was found in *A. leptophylla*, i.e., 38.88% in non-rhizosphere and 16.66% in rhizosphere. *Aspergillus* was found to be dominant among the fungal population in soil samples (Table 1, Fig. 1). Number of fungal propagules present per gram of soil (CFU) was more in non-rhizosphere of *A. leptophylla* measuring  $3.9 \times 10^5$ . Whereas CFU in non-rhizosphere of *H. crenatum* was less, up to  $1.7 \times 10^5$  (Fig. 2). While considering rhizosphere effect, i.e., R/S ratio, *A. leptophylla* had 0.71 and that of *H. crenatum* was 1.56 (Fig. 3). Clearly indicating that *A. leptophylla* has suppressive effect and *H. crenatum* has stimulatory effect on the soil fungi. Abdel-Hafez (1982) recorded greater



Image 3. *Hypodematum cernatum* Forssk. Kuhn

diversity of fungi occurring in the non-rhizosphere than in the rhizosphere. According to Sullia (1973) rhizosphere having the lowest fungal association, may be due to antifungal root exudates in higher plants. The inhibitory effect on the growth of fungi in rhizosphere may be due to some antimicrobial compounds present in the ferns (Bharti & Pravesh 2012). Yasmeen (1989), Yasmeen & Saxena (1990), Gupta et al. (1991), Bohra & Sharma (2007) have also observed almost similar results, while Khairnar & Saler (1985) found high fungal population in rhizosphere under natural habitat. Sharma & Bohra (2001), Basumatary et al. (2005), have also reported that the number of micro-fungi was highest in rhizosphere soil in some angiosperms.

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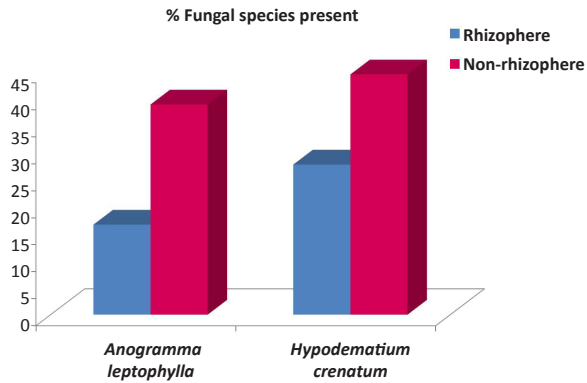


Figure 1. Percentage of fungal species present

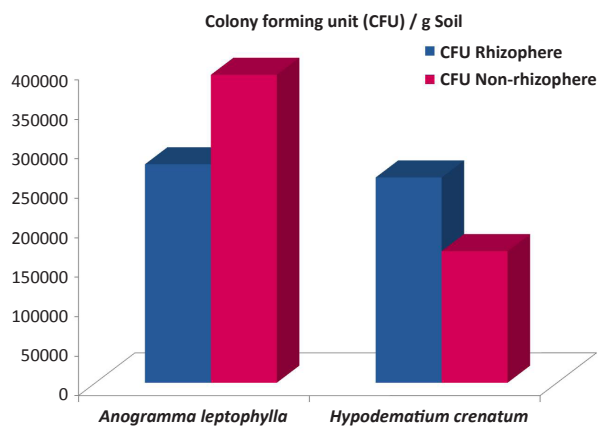


Figure 2. Colony forming units (CFU)/g soil

Table 1. Fungal population associated with *Anogramma leptophylla* and *Hypodematium crenatum*

	Fungal species	<i>Anogramma leptophylla</i>		<i>Hypodematium crenatum</i>	
		R	N-r	R	N-r
1	<i>Absidia cylindrospora</i>	-	-	+	-
2	<i>Aspergillus candidus</i>	-	-	-	+
3	<i>A. carbonius</i>	-	-	-	+
4	<i>A. flavus</i>	-	+	-	-
5	<i>A. fumigates</i>	+	+	-	-
6	<i>A. humicola</i>	-	+	-	-
7	<i>A. niger</i>	-	+	+	-
8	<i>Aspergillus</i> sp.	-	-	+	-
9	<i>Fusarium</i> sp.	+	-	-	+
10	Gray mycelium	-	-	-	+
11	<i>Mucor</i> sp.	+	-	-	-
12	<i>Penicillium reticulosum</i>	-	+	+	-
13	<i>Penicillium rugulosum</i>	-	-	-	+
14	<i>Rhizopus oryzae</i>	-	-	-	+
15	<i>Trichocladium</i> sp.	-	+	-	-
16	Unidentified	-	-	+	-
17	<i>Cuvularia</i> sp.	-	-	-	+
18	White Mycelium	-	+	-	+
% Fungal species Present		16.66	38.88	27.77	44.44

R - Rhizosphere; N-r - Non-rhizosphere

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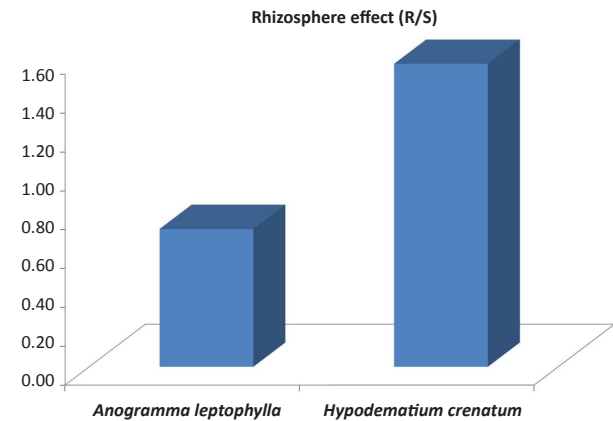


Figure 3. Rhizosphere effect (R/S)

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