

# Diversity of arbuscular mycorrhizal fungi in different salinity of mangrove ecosystem of Odisha, India

## Abstract

A study was carried out to assess the species diversity of arbuscular mycorrhizal (AM) fungi in different salinity zones of Bhitarkanika mangroves of Orissa, India. Sixteen sites of Bhitarkanika mangrove areas were surveyed for the collection of roots and soil samples. Seedlings of mangrove species were tested for AM colonization through root clearing and staining technique. Soil samples were treated separately for the mineral analysis through wet oxidation techniques and spore multiplication by pot culture methods. Wet sieving and decantation technique was followed for the isolation of AM spores from soil. AM spores were identified on the basis of morphological characteristics by following the INVAM manual. The physio-chemical analysis of soil indicated its deficiency in phosphorus which decreases (9.47Kg/ha) with increase in salinity. The genus *Glomus* is most dominant and has presence across all saline zones. A total of 45 AM species belonging to five genera namely, *Glomus*, *Acaulopspora*, *Gigaspora*, *Scutellospora* and *Enteropspora* were recorded from three salinity zones of Bhitarkanika mangrove ecosystem. The soils of lower salinity contained maximum number of AM species (21nos.) than the high salinity zones (9 nos.). The decreased number of AM species in high salinity may be due to low phosphorus content and lack of suitable host plant also. Among eighteen mangrove species from different salinity zones analyzed for mycorrhizal colonization in their root system, *Sonneratia apetala*, *Heritiera fomes*, *Excoecaria agallocha*, *Derris heterophylla*, *Bruguiera gymnorhiza*, *Avicennia officinalis*, *Aglaia cucullata*, and *Aegiceras corniculatum* were found to be mycorrhizal. This is first report on diversity of AM species in different salinity zones of Bhitarkanika mangroves of Orissa, India.

**Keywords:** Arbuscular mycorrhiza; Mangroves; Salinity; Bhitarkanika

## Introduction

Mangrove creates habitats for diverse floral and faunal communities including numerous mangrove dependent micro organisms.<sup>1</sup> The interaction between various organisms and higher species in mangrove ecosystem has been subject of investigation and drawn interests on account of atypical habitat in which the biotic elements of the unique habitat compete and/or compliments to survive and grow. The role and functions of Arbuscular mycorrhizal fungi (AMF) in relation to assimilation and translocation of soil nutrients in wetland,<sup>2,3</sup> have received increased attention over last decade. Past works have reported that AM fungi predominate soils with high salinity or alkalinity and low nutrient.<sup>4,5</sup> Their adaptability for such difficult and extreme habitat is believed to help the colonized host plants in establishing in different conditions.

Although AMF require oxygen to thrive and assumed to be of little relevance in aquatic anaerobic conditions, recent studies proves that AMF survive and colonize many halophytes.<sup>6</sup> The association of AM fungi with mangrove has also been reported from Pichavaram forest and the ganges river estuary and recently from China.<sup>7-9</sup> The objective of this study is to analyze the diversity of AM fungi in Bhitarkanika mangrove ecosystem of Odisha, India.

## Materials and methods

Bhitarkanika mangrove forests situated on the east coast of Orissa (20°4' - 20°8' N; 86° 45' - 87° 50' E) is India 's second largest mangrove ecosystem (627sq km.) both in terms of area, species diversity and distribution. The area is inundated with high tide and

low tides twice a day at an interval of 12hours, the tidal amplitude ranging from 2-3.5m upstream to 3.5-6m near the river mouths. Sixteen sites of Bhitarkanika mangrove areas were surveyed during the late winter season for the collection of roots and soil samples of all accessible species in each site.

Seedlings were uprooted together with some soil adhering to the roots. Samples were brought to laboratory and roots were separated from the adhering soil, washed gently under the tap water and fixed in FAA (Formalin-acetic acid-alcohol) for analysis of AM colonization. Root adhering or rhizosphere soil of each individual was air dried at room temperature, sieved and divided and. used for pot culture multiplication in order to multiply the spores existing in respective soil samples.

## Estimation of AM fungi colonization

AM infection in roots was assessed by root clearing and staining technique.<sup>10</sup> Root samples were cleared with 10% KOH and autoclaved for 15-20minutes at 15p/i; the autoclaved root samples were treated with 6NHCl for 5minutes. The cleared roots were then stained with 0.05% cotton blue and mounted in polyvinyl alcohol lactoglycerol (PVLG) and observed for % colonization

## Determination of soil characteristics

Soil pH, salinity, electrical conductivity, available Nitrogen, Phosphorus, Potassium, dissolved oxygen and total dissolved solids were measured through portable water analyser and following the methods of Tandon.<sup>11</sup>

## Characterization and identification of AM fungi

As density of AM spores in mangrove soil was very low, soil samples from different locations/sites were first inoculated in sterilized sorlite- soil mix under pot culture with *Cenchrus ciliaris* in order to multiply the existing AM spores (if any). Wet sieving and decanting method was followed to isolate the AM spores from the soil.<sup>12</sup> About 100g of representative soil samples of each location (in triplicate) was suspended in sufficient quantity of water and stirred thoroughly. The resulting soil suspension was sieved through meshes of sizes 400, 300, 200, and 100um and placed one below the other in the same order. The residues, after sieving, were filtered (Whatman no. 1) and examined under stereomicroscope for spore (Meiji, Japan). The soil salinity was measured by portable soil water analyzer (Sanco make). Diagnostic slides with spores/sporocarps were prepared

**Table I** Physio-chemical properties of mangrove soil

Salinity	Low	Medium	High
pH	6.32±1.02	6.28±1.04	6.1±0.52
O.C (%)	1.46±0.54	1.39±0.40	1.51±0.82
Conductivity(mS)	1.53±0.46	1.72±0.61	2.33±0.64
TDS(ppt)	1.01±0.38	1.01±0.45	1.11±0.28
N(kg/ha)	418.80±58.14	444.80±44.10	437.5±140.77
P(kg/ha)	11.72±7.61	10.92±6.54	9.47±2.96
K (kg/ha)	2479.22±437.70	1801.265±745.43	1513.75±242.87

O.C, organic content; TDS, total dissolved solid

## Mycorrhizal colonization

Eighteen mangrove species (representing 1 fern, 2 shrubs, 1 climber, 1 succulent, 1 herb and 12 trees) of different salinity zones were analyzed for mycorrhizal colonization in their root system. The percentage colonization of AM fungi in the roots differed among species, having no distinct trend in AM colonization across salinity zones. Figure 1 represents the status of AMF in infected mangrove species. The mycorrhizal colonization ranged from 18.51 % to 73.33 %. The highest percentage observed in case of *Agalai a cculata* (73.33 %) a rare species found in low salinity zone followed by *Heritiera fomes* (52.74 %) and *Sonneratia apetala* (47.91 %) (Figure 2). The lowest AM colonization was found in case of *Agiceras corniculatum*. Species such as *Kandelia candel*, *Sonneratia casuularis*, *Rhizophora mucronata*, *Tamarix troupii*, *Acanthus ilicifolius*, *Postulaca quadrifida*, *Sesuvium portulacastrum* and *Xylocarpus granatum* did not demonstrate AM colonization in their roots systems. Arbuscular mycorrhizal invasion containing vesicles and arbuscules within plant roots was seen in species such as *Sonneratia apetala*, *Derris heterophyla*, *Agalai a cculata* and *Heritiera fomes* (Table 2) (Figure 2).

## Diversity of AMF

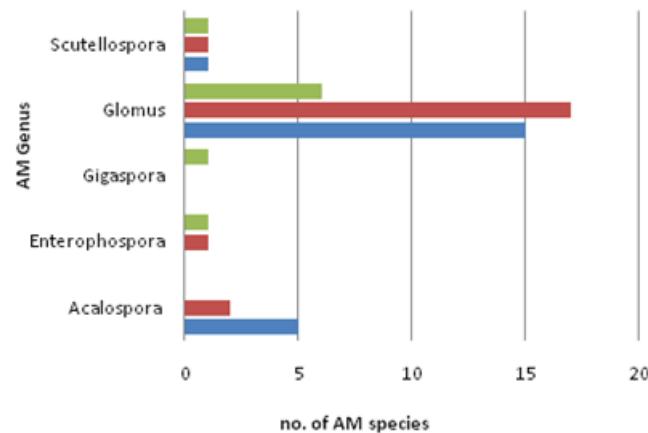
A total of 45AM fungal species representing five genera, including 6 species of *Acaulopsora*, 2 of *Entrophospora*, 1 of *Gigaspora*, 32 of *Glomus* and 3 of *Scutellospora*, were isolated from mangrove soils of different salinity zones. Out of these, 21 species occur in low and medium salinity zones and only 9 species' in high salinity area (Figure 3). The genus *Glomus* is most dominant and diverse and *Glomus* and

using polyvinyl alcohol lacto glycerol as mountant. AM spores were analyzed for their morphological characteristics like shape, size, stalk, wall layers. Identification of AM fungi was done using relevant INVAM guidelines.<sup>13</sup>

## Results and discussion

The physio – chemical characteristics of the soils of different salinity zones indicated that the soils were neither totally acidic nor alkaline having distinct salinity gradient. Soil texture analysis indicated that, mangrove soils were mostly a clayey in nature. The soil was deficient in phosphorus content, which decreases with increase in salinity level (Table 1). It was found enriched in Organic Content. Total dissolved solid was more or less similar in mangrove soils of different salinity whereas the salinity level did not influence soil pH.

*Scutellospora* have presence across all saline zone. *Acaulospora* did not occur at high salinity level. Six species of *Glomus* were found in areas of high saline inundation (Figure 4). Most of *Acaulospora* species occurs in low salinity except *A. delicata* that preferred a higher salinity. We isolated single species of *Gigaspora* and *Enterophospora* from high salinity zone. However, *E. colombiana* was present in muddy soil of medium salinity zone.



**Figure I** AM species distribution in different salinity zones (no. of species).

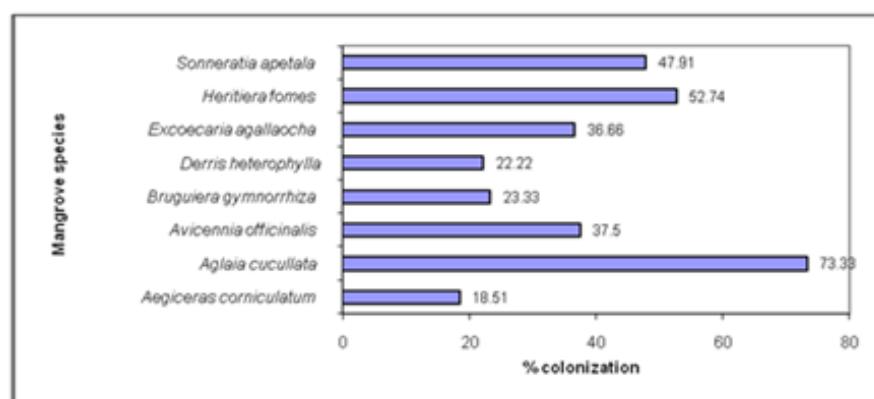
The study exhibited rich diversity of arbuscular mycorrhizal fungi in mangrove soil and species richness, with reference to salinity zones and physiochemical properties of soil. Occurrence of arbuscular mycorrhizal fungi in saline soils were earlier considered negligible or

minimal.<sup>14</sup> However, recent studies have established the ubiquitous behavior of AM fungi in saline and coastal soils. Our study makes further invades on the subject by listing mycorrhization of *Aegiceras corniculatum*, *Aglaia cucullata*, *Avicennia officinalis*, *Bruguiera gymnorhiza*, *Derris heterophylla*, *Excoecaria agallocha*, *Heritiera fomes*, *Sonneratia apetala*. Occurrence of mycorrhizal colonization in

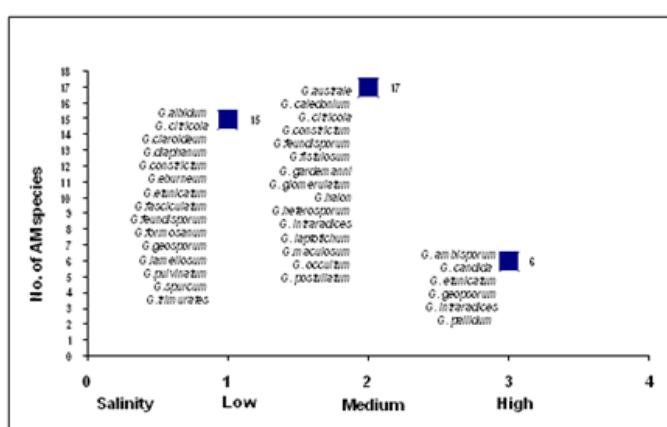
these mangrove plants strengthen the significance of AMF association in wet land ecosystem.<sup>15,16</sup> *Agalaia cuculata*, *Excoecaria agallocha* and *Avicennia officinalis*, *Aegiceras corniculatum*, *Bruguiera gymnorhiza*, *Heritiera fomes* were reported as mycorrhizal in Bhitarkanika mangroves, and thus the present study confirms the earlier reports on mangroves forests of different sea coasts of India.<sup>2,3,8</sup>

**Table 2** Plants analyzed for mycorrhizal colonization, their habit, habitat and distribution

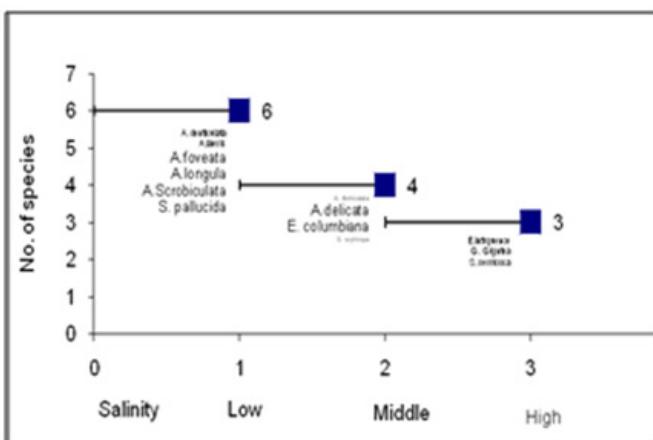
Species	Family	Habitat	Habit	Distribution
<i>Acrostichum aureum L.</i>	Adiantaceae	Low Salinity	Fern	Common
<i>Aegiceras corniculatum (L.) Blanco</i>	Myrsinaceae	Low Salinity	Tree	Common
<i>Aglaia cucullata Ker-Gawl.</i>	Amaryllidaceae	Low Salinity	Tree	Rare
<i>Avicennia officinalis L.</i>	Avicenniaceae	Low Salinity	Tree	Common
<i>Crinum defixum Ker-Gawl.</i>	Amaryllidaceae	Low Salinity	Shrub	Common
<i>Excoecaria agallocha L.</i>	Euphorbiaceae	Low Salinity	Tree	Common
<i>Heritiera fomes Buch. Ham.</i>	Sterculiaceae	Low Salinity	Tree	Common
<i>Kandelia candel (L.)Druce</i>	Rhizophoraceae	Low Salinity	Tree	Common
<i>Sonneratia apetala Buch.Ham</i>	Sonneratiaceae	Low Salinity	Tree	Common
<i>Sonneratia casuarina L.</i>	Sonneratiaceae	Low Salinity	Tree	Common
<i>Acanthus ilicifolius L.</i>	Acanthaceae	Medium Salinity	Shrub	Common
<i>Avicennia officinalis L.</i>	Avicenniaceae	Medium Salinity	Tree	Common
<i>Bruguiera gymnorhiza(L.) Savigny</i>	Rhizophoraceae	Medium Salinity	Tree	Common
<i>Derris heterophylla(Willd.)Back.&amp;Bakh.</i>	Fabaceae	Medium Salinity	Climber	Common
<i>Excoecaria agallocha L.</i>	Euphorbiaceae	Medium Salinity	Tree	Common
<i>Heritiera fomes Buch. Ham.</i>	Sterculiaceae	Medium Salinity	Tree	Common
<i>Portulaca quadrifida</i>	Portulacaceae	Medium Salinity	Succulents	Common
<i>Rhizophora mucronata Lam.</i>	Rhizophoraceae	Medium Salinity	Tree	Common
<i>Sesuvium portulacastrum</i>	Aizoaceae	Medium Salinity	Herb	Common
<i>Sonneratia apetala Buch.Ham</i>	Sonneratiaceae	Medium Salinity	Tree	Common
<i>Tamarix troupii</i>	Tamaricaceae	Medium Salinity	Shrub	Common
<i>Avicennia officinalis L.</i>	Avicenniaceae	High Salinity	Tree	Common
<i>Kandelia candel (L.)Druce</i>	Rhizophoraceae	High Salinity	Tree	Common
<i>Rhizophora mucronata Lam.</i>	Rhizophoraceae	High Salinity	Tree	Common
<i>Xylocarpus granatum Koen.</i>	Meliaceae	High Salinity	Tree	Common



**Figure 2** Mycorrhizal colonization in the root system of different plant species (%).



**Figure 3** Distribution of *Glomus* species in different salinity zones.



**Figure 4** Distribution of AM species in different salinity zones.

In contrast to previous findings, *Rhizophora mucronata* was found to be non-mycorrhizal in high saline zone of Bhitarkanika mangroves. It may be due to the differences in salinity level, as most of the mangrove species having mycorrhizal association, occupied in low salinity areas. The species *Bruigiera gymnorhiza* and *Derris heterophylla*, obtained from medium salinity, was also observed as mycorrhizal, but with low percentage of colonization, as compared to others. We did not find any AM infection in *Kandelia candel*, *Rhizophora mucronata* and *Xylocarpus granatum* collected from high salinity zone. However, these species cannot be considered as nonmycorrhizal as earlier studies reported them as mycorrhizal from mangrove soils of different conductivity<sup>9</sup> and medium range of salinity.

This study confirmed occurrence of AM spores in muddy soils of mangroves<sup>17</sup> *Glomus* was the most dominant AM found in low salinity area, similar to the coastal saline soils of west coast of India.<sup>2,3</sup> Occurrence of *Glomus intraradices* and *G. geosporum* in high salinity zones indicate possible fungal adaptation to salt tolerance.

Absence of *Sclerocystis* from Bhitarkanika mangrove commonly found in Sunderban mangrove ecosystem and western coast of India indicated complex interaction of edaphic agroclimatic factor determining growth of AM fungi from typical to a micro environment.<sup>18</sup> Among 45 species reported in the study, 15 species are found in coastal dunes of the India. The incidence of *E. infrequence*, *G. gignatea*, *G. ambisporum*, *G. candida* and *G. pallidum* in only high salinity can be attributed to their salt tolerant trait.

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## Conflict of interest

The author declares no conflict of interest.

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