

Diversity, Abundance, and Richness of Arbuscular Mycorrhizal Fungi associated with some Solanaceous medicinal plants of Ballavpur Wildlife Sanctuary in West Bengal

SOURAV DE AND NANDLAL MANDAL*

Department of Botany, Visva-Bharati University, Santiniketan- 731235, West Bengal.

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Three well-known medicinally important plants of Solanaceae viz., *Solanum nigrum*, *Solanum torvum* and *Nicotiana plumbaginifolia* of Ballavpur Wildlife Sanctuary (BWLS) were assessed for determination of mycorrhizal association in terms of % root colonization, spore density, species richness and relative abundance. All three selected plants showed sufficient internal hyphae; however, the number of vesicles and arbuscules varies from plant to plant. Maximum root colonization percentage was recorded in *Solanum nigrum* (74%), followed by *Solanum torvum* (72%) and the lowest (67%) was noticed in *Nicotiana plumbaginifolia*. Of all three plants, spore density and species richness were noticed maximum in *S. torvum*, followed by *S. nigrum* and minimum in *N. plumbaginifolia*. Altogether, 15 different AM fungal species belonging to 6 genera viz., *Glomus*, *Acaulospora*, *Scutellospora*, *Claroideglomus*, *Diversispora* and *Gigaspora*, were isolated from all three selected plants; however, their isolation frequency, relative abundance and species richness were found to vary from plant to plant. Among all AM fungi, three species of *Glomus* viz., *G. intraradices*, *G. fasciculatum*, and *G. mosseae* and three species of *Acaulospora* viz., *A. nicolsonii*, *A. laevis* and *A. mella* are commonly present in all three selected plant with high relative abundance (above 30) and is considered as dominant AM species of BWLS. The present investigation reveals that *Glomus* and *Acaulospora* predominate over *Scutellospora*, *Claroideglomus*, *Diversispora* and *Gigaspora*. A significant positive correlation between root colonization, spore density, and species richness was recorded, and the maximum mycorrhizal association supports better plant growth promotion.

Keywords: Arbuscular mycorrhizal fungi, Ballavpur wildlife sanctuary, relative abundance, root colonization, species richness

INTRODUCTION

In India, national parks and wildlife sanctuaries are essential for environmental protection and biodiversity conservation. Out of 15 Wildlife Sanctuaries in West Bengal, Ballavpur Wildlife Sanctuary is the oldest one and was established in 1977 at Bolpur sub-division of Birbhum district. The sanctuary is spread over 200 hectares, and a fence protects the entire area. Besides the conservation of spotted deer and Indian antelope, this sanctuary is also enriched with floral diversity as it contains three large water bodies inside the area. The lateritic soil of this sanctuary harbors tropical dry deciduous-type forests, in which

many medicinally important plants are grown naturally (Ganguli *et al.* 2016). Plant of Solanaceae is commonly known as the nightshade family and contains various types of annual and perennial herbs and trees many of which are naturally grown wild; some of them are cultivated for various purposes such as ornamental, vegetable, spices, and medicine. This family contains diverse phytochemical compounds, including alkaloids, to combat various diseases and attract ordinary people for its application as traditional medicine. Of all plants of Solanaceae, *S. nigrum*, *S. torvum* and *N. plumbaginifolia* are widely occupied in this sanctuary in wild conditions. Due to the non-application of chemical fertilizers and pesticides and minimum anthropogenic activity, the soils of this area are undisturbed and provide a better

*Correspondence: nandlal.mandal@visva-bharati.ac.in

habitat for soil microbiota (Karmakar and Padhy, 2019; Kumar *et al.* 2019).

Arbuscular mycorrhizal fungi (AMF) are filamentous fungi of phylum Glomeromycota. They are widely known for their mutualistic association and plant growth-promoting activity in terms of mineralization of P, N₂, Na, K and Ca by secretion of various enzymes and their mobilization through fungal hyphae (Franco-Ramirez *et al.* 2021; Schubler *et al.* 2001). AMF is also helpful for host plants as it can increase root absorption areas through its mycelium for better water uptake, boosting disease resistance, enhancing salt and draught tolerance ability, and also improving soil quality by maintaining soil microbiota (Bhale and Sawant, 2017; Muthukumar and Sathya, 2017). More than 80% of the plant root is associated with different mycorrhizal fungi and benefits plants in multiple ways; however, not all AMFs are known for equal contribution to plant growth promotion (Zhang *et al.* 2022). Each plant has its own mycorrhizal fungal dynamics and species richness, which influence the overall growth of a particular plant. This phenomenon attracts scientists to explore the mycorrhizal diversity of each plant in various soil systems and regional bases (Gao and Guo, 2010). Several earlier workers reported AM fungal association with several plants; however, reports on selected solanaceous plants are meager on a regional basis and also to such type of protected area and require attention (Lee *et al.* 2013; Gupta *et al.* 2014; Muthukumar and Sathya, 2017). Keeping the above facts, the present study aimed to explore the AMF diversity of this protected area along with their relative abundance and species richness to understand their symbiotic association with the selected plants of this region.

MATERIALS AND METHODS

Collection of rootlets and rhizosphere soil

Rhizospheric soil and rootlet samples were collected in triplicate from three different sites and three different plants of Solanaceae viz., *S. nigrum*, *S. torvum* and *N. plumbaginifolia* of Ballavpur Wildlife Sanctuary. All the selected plants are in the flowering stage and were

collected from late March to July from unique places where a single plant population grew luxuriant. For a collection of Rhizospheric soil and rootlet samples, bulk soil was dug out up to 10 cm in depth from one side of each selected plant and properly mixed. About 200g of rhizospheric soil was taken in sterile polythene bags and brought to the laboratory for storage in the refrigerator at 4°C for further study. About 1cm of finer rootlet was cut from the main root and was washed with tap water to remove the soil debris and finally preserved in a solution of formaldehyde, glacial acetic acid, and ethanol with a volume ratio of 12.5: 12.5: 200 ml for further study. (Yang *et al.* 2019).

Assessment of root colonization by AM Fungi

Phillips and Hayman (1970) method was followed to study root colonization by AM fungi. For this, previously preserved rootlets were taken out, washed with tap water twice, and boiled in a 10 % KOH solution at 90°C in a water bath for about 1 hour or until the roots softened. After cooling, the KOH solution was decanted and was acidified by mixing sufficient 2 N HCl for 10 minutes. After decanting, rootlets were further treated overnight with 0.05 % trypan blue for staining. The root colonization percentage of individual plants was calculated with the help of the following formula:

$$\% \text{ Root Colonization} = \frac{\text{No. of infected rootlets}}{\text{The total number of rootlets observed}} \times 100$$

Isolation and identification of AM Spore

For the isolation of AM Spores from rhizosphere soil, the wet sieving and decanting method of Gerdemann and Nicolson (1963) was followed. For this, previously collected samples of rhizosphere soil were air-dried, and about 25 g of soil of each plant was taken in a 500 ml beaker separately. In each beaker, about 250 ml of lukewarm water was added and stirred properly with the help of a magnetic stir. The soil water solution was then passed from different mesh-size sieves (500 to 40) arranged from bottom to top to separate the AM spores. For debris-free separation of AM spores, spores from all sieves were taken out in a beaker in a mixed way, and finally, they were centrifuged with 10% sucrose

solution at 3,000 rpm. Centrifuged spore solution was again sieved, and residues of each respective sieve were collected separately and observed under a ZEISS Primo Star Binocular Microscope with 1000 magnification for their identification. Several characteristics feature of AM spore, such as color, size, shape, surface, wall layers and structure, nature of the spore contents and hyphal attachment, were recorded, and final identification was made by the information available at www.invam.caf.wvu.edu website and manual of Schenck and Perez, (1990) specified for identification of AM fungi. Spores wall character was observed under gently pressed slide mounted with PVLG (Ploy-Vinyl-Lacto-Glycerol) solution by following the INVAM manual.

Determination of AM diversity, relative abundance and species richness

To determine the mycorrhizal dependency of each plant, spore density (SD), relative abundance (RA), species richness (SR) and Isolation frequency (IF) were determined by using the following formula (Chahar and Belose, 2018).

$$RA = \frac{\text{Number of VAM spores of a particular genus or species}}{\text{Total number of spores isolated}} \times 100$$

Total number of spores isolated

$$IF = \frac{\text{No. of soil samples in which a species occurred}}{\text{Total no. of soil samples examined}} \times 100$$

Similarly, spore diversity in terms of Shannon–Wiener index (H'), Simpson index (D) and evenness (E) were determined by using different formulas (Chakraborty, 2016; Gao and Guo, 2010; Songachan and Kayang, 2012; Verma and Verma, 2017).

$$H' = -\sum p_i \ln p_i$$

$$D = \frac{1}{\sum (n_i - 1) / (N - 1)}$$

$$E = H' / H'_{\max}$$

Where p_i is the proportion of the (i)th species, n_i is the number of individuals of the taxon (i), (N) is the total number of species, and H'_{\max} is $\ln S$ where S is the total number of identified species. All the triplet data were statistically analyzed using one-way ANOVA and Pearson correlation at ($p < 0.05$) level for their significance.

RESULTS AND DISCUSSION

AM fungi and root colonization

Three medicinally important plants of Solanaceae viz., *S. nigrum*, *S. torvum* and *N. plumbaginifolia* were assessed to determine mycorrhizal association in terms of % root colonization and spore density (SD). During the study of root colonization by AM fungi, the cortical region of the root was observed under the ZEISS Primo Star Binocular Microscope. All three selected plants showed variable presence of internal hyphae, vesicles and arbuscules to confirm mycorrhizal association. The occurrence of characteristic arbuscular structure was less common in roots of *N. plumbaginifolia* but was sufficiently present in *S. nigrum* and *S. torvum*. Similarly, number of vesicles was noticed maximum in *S. torvum* and *S. nigrum* as compared to *N. plumbaginifolia* and above 60% rootlets contain internal hyphae in all selected species (Table 1). Plants of the Solanaceae family are commonly associated with mycorrhizal fungi, and varied AM diversity in terms of root colonization, relative abundance and species richness was documented by several earlier workers (Koul *et*

Table 1: AM fungal root infection type of selected medicinal plant species of solanaceae and their infection type

Plant species	Root infection type		
	Hyphae	Arbuscules	Vesicle
<i>Solanum nigrum</i>	++++	++	+++
<i>Solanum torvum</i>	++++	++	+++
<i>Nicotiana plumbaginifolia</i>	+++	+	++

+, ++, +++, +++++ indicate presence of desired character above 20%, 40%, 60% and 80% respectively among 50 rootlets studies.

al. 2012; Sawant and Bhale, 2016; Muthukumar and Sathya, 2017; Verma *et al.* 2019). The highest % root colonization percentage was recorded in *S. nigrum* (74%), followed by *S. torvum* (72%), and the lowest (67%) was noticed in *N. plumbaginifolia* (Table 2). Saranya and Nagarajan (2017) found a similar pattern of root colonization of *S. torvum*. The present study clearly showed that all three selected plants of Solanaceae are well colonized with mycorrhizal fungi, and their mean colonization (71%) is much higher than earlier reports of Haider *et al.* (2015) 64%,

Table 2: Root Colonization, Spore Density and Species diversity of AM fungi on selected plant species of Solanaceae

Sl.No.	Plant species	Root Colonization (%)	Spore Density	Species richness
1	<i>Solanum nigrum</i>	74 ± 2.63	95± 7.25	12
2	<i>Solanum torvum</i>	72 ± 2.16	103 ± 6.64	13
3	<i>Nicotiana plumbaginifolia</i>	67 ± 0.81	77 ± 2.62	9

± Standard error of mean among 50 rootlets studies

Table 3: Isolation of different AMF genera from selected plant with their isolation Frequency, Relative abundance and Species richness.

Genus	Family	Isolation Frequency (%)	Relative abundance (RA)	Species richness
<i>Glomus</i>	Glomeraceae	100	53.09	6
<i>Acaulospora</i>	Acaulosporaceae	100	34.18	4
<i>Scutellospora</i>	Gigasporaceae	66.66	6.18	2
<i>Claroideglomus</i>	Claroideglomaceae	55.55	3.63	1
<i>Diversispora</i>	Diversisporaceae	33.33	2.18	1
<i>Gigaspora</i>	Gigasporaceae	11.11	0.36	1

Muthukumar *et al.* (2006) 69.94%. Higher mean AMF colonization in this area may be due to the undisturbed lateritic soil of this sanctuary.

Spore density and species richness

All three selected plants showed variable spore density per 25g of rhizosphere soil. Maximum (103 ± 6.64) spore density was noticed in *S. torvum* followed by *S. nigrum* (95± 7.25), and minimum (77 ± 2.62) was recorded in *N. plumbaginifolia* (Table 2). The degree of spore density is directly related to closer mycorrhizal association with plants, and a similar observation was recorded by earlier workers in other Solanaceae plants (Songachan and Kayang, 2012; Bhale and Sawant, 2017). Of all three plants, *S. torvum* exhibits maximum (13) species richness, out of which five species of *Glomus*, four species of *Acaulospora*, two species of *Scutellospora* and a single species of *Claroideglomus* and *Diversispora* were noticed. Similarly, the species richness of *S. nigrum* was 12, of which six species of *Glomus*, four species of *Acaulospora* and a single species of *Claroideglomus* and *Gigaspora* were noticed. Minimum (9) species richness was recorded in *N. plumbaginifolia*, where four species of *Glomus*, three species of *Acaulospora* and a

single species of *Claroideglomus* and *Diversispora* were noticed (Table 2).

AM fungal diversity and abundance

Altogether, 15 different AM fungi belonging to 6 genera of 5 families were isolated; however, their isolation frequency, relative abundance, and species diversity varied among plant species (Table 3; Fig.1). Of all isolates of AM spore, maximum isolation frequency (100%) was noticed in genera *Glomus* and *Acaulospora*, followed by *Scutellospora* (66.66%), *Claroideglomus* (55.55%), *Diversispora* (33.33%) and minimum (11.11%) was noticed in *Gigaspora*. Due to the high isolation frequency of *Glomus* and *Acaulospora*, their relative abundance is always higher than that of other genera. Chahar and Belose (2018), in their study of AM fungal diversity in Sanjay Gandhi National Park, Borivali, Maharashtra, showed similar maximum isolation frequencies of *Glomus* and *Acaulospora*. Maximum relative abundance (53.09) was recorded in *Glomus*, followed by *Acaulospora* (34.18), *Scutellospora* (6.18), *Claroideglomus* (3.63), *Diversispora* (2.18) and minimum (0.36) was noticed in *Gigaspora*. During the study of AM diversity, the genera *Glomus* contains maximum of six species (*G.intraradices*, *G.*

Table 4: AMF species diversity and their relative abundance of selected plant species of Solanaceae

Number of AM Genera and Species isolated		Plant species		
		<i>S. nigrum</i>	<i>S. torvum</i>	<i>N. plumbaginifolia</i>
1. <i>Glomus</i>		(RA 54.73, SR 6)	(RA 46.60, SR 5)	(RA 59.74, SR 4)
	i. <i>G.intraradices</i>	+	+	+
	ii. <i>G.fasciculatum</i>	+	+	+
	iii. <i>G.mossae</i>	+	+	+
Species	iv. <i>G.etunicatum</i>	+	-	+
	v. <i>G.hoi</i>	+	+	-
	vi. <i>G.multicaulis</i>	+	+	-
2. <i>Acaulospora</i>		(RA 35.78, SR 4)	(RA 33.98, SR 4)	(RA 32.46, SR 3)
	i. <i>A.nicolsonii</i>	+	+	+
Species	ii. <i>A.laevis</i>	+	+	+
	iii. <i>A.mella</i>	+	+	+
	iv. <i>A.elegans</i>	+	+	-
3. <i>Scutellospora</i>		(RA 7.60, SR 1)	(RA 9.70, SR 2)	
Species	i. <i>S.pellucida</i>	+	+	-
	ii. <i>S.persica</i>	-	+	-
4. <i>Claroideglomus</i>			(RA 5.82, SR 1)	(RA 5.19, SR 1)
Species	i. <i>C.etunicatum</i>	-	+	+
5. <i>Diversispora</i>			(RA 3.82, SR 1)	(RA 2.59, SR 1)
Species	i. <i>D.gibbosa</i>	-	+	+
6. <i>Gigaspora</i>		(RA 2.10, SR 1)		
Species	i. <i>G.margarita</i>	+	-	-

+ is present; - is absent and RA=relative abundance, SR= species richness

fasciculatum, *G. message*, *G. etunicatum*, *G. hoi* and *G. multicaulis*) followed by *Acaulospora* 4 species (*A.nicolsonii*, *A. laevis*, *A. Mella* and *A. elegans*), *Scutellospora* 2 species (*S. pellucida* and *S. persica*) and only single species contain by genera of *Claroideglomus* (*C.*

etunicatum), *Diversispora* (*D. gibbosa*) and *Gigaspora* (*G. margarita*) (Table4). The plant *S. nigrum* contains six species of *Glomus*, four *Acaulospora*, and one *Scutellospora* and *Gigaspora*. The plant *S. torvum* contains five species of *Glomus*, all four species

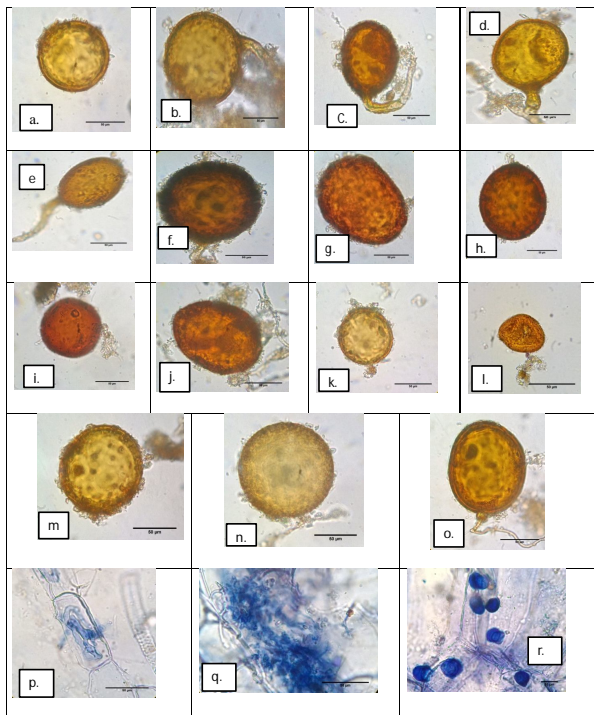


Fig. 1: Different types of isolated AM fungal spores (a-o) and root infection (p-r) from selected medicinal plant of Solanaceae. a.-*Glomus intraradices*, b.-*Glomus fasciculatum*, c.-*Glomus mossae*, d.-*Glomus etunicatum*, e.-*Glomus hoi*, f.-*Glomus multicaulis*, g.-*Acaulosporanicolsonii* h.-*Acaulosporalaevis*, i.-*Acaulosporamella*, j.-*Acaulospora elegans*, k.-*Scutellospora pellucida*, l.-*Scutellospora persica*, m.-*Claroideglomusetunicatum*, n.-*Diversisporagibbosa*, o.-*Gigaspora margarita*, p.-Fungal hyphae in cortex, q.- Arbuscule, r.-Vesicle.

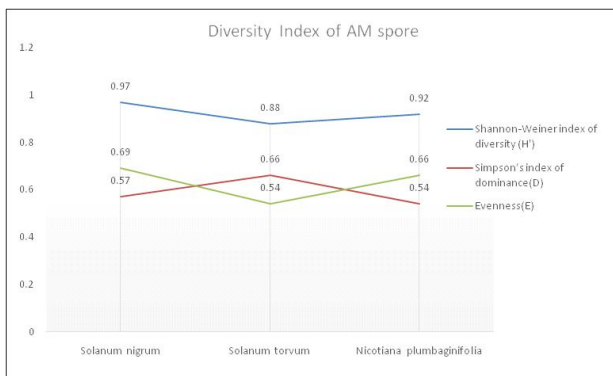


Fig. 2: Diversity indices of AM fungi on selected plant species of solanaceae

of *Acaulospora*, and all two species of *Scutellospora* with one species of *Claroideglomus* and *Diversispora*. Similarly, plant *N. plumbaginifolia* contains only four *Glomus* species, three *Acaulospora* species, and one *Claroideglomus* and *Diversispora*.

The relative abundance of each genus in different plants was also variable; however, genera *Glomus* and *Acaulospora* always

showed higher relative abundance in all three selected plants of Solanaceae than the other four genera in the same selected plants. Among all species of AM fungi, three species of *Glomus* viz., *G. intraradices*, *G. fasciculatum* and *G. mosseae* and three species of *Acaulospora* viz., *A. nicolsonii*, *A. laevis*, *A. mella* are commonly present in all three selected plant with high RA (above 30) and is considered as significant dominant AM fungi of BWLS. The RA of other genera, such as *Scutellospora*, *Claroideglomus*, *Diversispora* and *Gigaspora*, is very low (below 10) and unevenly present in soil and selected plants. Genus *Scutellospora* is more common in *S. torvum* than *S. nigrum* and utterly absent in *N. plumbaginifolia*. Genera *Claroideglomus* and *Diversispora* are present in *S. torvum* and *N. plumbaginifolia* and absent in *S. nigrum*; however, the genus *Gigaspora* is only present in *S. nigrum* and absent in the other two plants. (Table 4). The present investigation reveals *Glomus* and *Acaulospora* as a predominating genus of AM fungi in this area and agreed with the data of other solanaceous plants by earlier workers (Koul *et al.* 2012; Saranya and Nagarajan, 2017; Venkatachalapathi *et al.* 2020).

AM diversity index and statistical analysis

During the AM diversity index study, the maximum (0.97) Shannon-Wiener index (H') was recorded in *Solanum nigrum*, revealing that this plant harbors the maximum no. of diverse AM species among the three plants studied. Simpson's index of dominance (D) index showed the highest value in the case of *S. torvum* (0.66) > *S. nigrum* (0.57) > *N. plumbaginifolia* (0.54), indicating there is a balanced and healthy diversity of AM fungi in the studied plant rhizosphere; although in *S. torvum* AM fungal community is healthier than the other two plants of Solanaceae. Distribution of AM fungi was more even in *S. nigrum* as Evenness (E) was measured highest in (0.69) compared to *N. plumbaginifolia* (0.66) and *S. torvum* (0.54) (Fig. 2). It is evident from above indices that AM fungal occurrence in *N. plumbaginifolia* is poorer in comparison to those two species of *Solanum*.

The present study shows a significant positive correlation ($r=0.99$) between spore density and species richness at 0.05. Similarly, root colonization and species richness also showed

a significant positive correlation ($r=0.86$) at the same probability level (Songachan and Kayang, 2012) and also recorded a significant positive correlation between root colonization and species richness of some Solanaceae plants growing under natural conditions. A positive correlation ($r=0.99$) was also observed between relative abundance and species diversity. In contrast, a positive ($r=0.83$) but non-significantly correlation was recorded with spore density and root colonization in all three selected plants of Solanaceae (Sawant and Bhale, 2016). In general, it was found that maximum root colonization is always associated with high spore density and species diversity, and both of them influence the relative abundance of particular AM fungi, and they can vary from plant to plant.

DECLARATIONS

Conflict of interest: Authors declare no conflict of interest.

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