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Mycorrhizal Status In Endemic Pipewort: *Eriocaulon eurypeplon* Körn (Fam: Eriocaulaceae)

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Abstract: *Present paper deals with, detailed assessment of mycorrhization establidhed by Arbuscular Mycorrhizal Fungi with Eriocaulon eurypeplon Körn. It is endemic pipewort to the Western Ghats of India. Here, sixteen species of AM fungi viz., Acaulospora denticulate, Aculospora radilatata* Morton., *Acaulospora elegans, Acaulospora rehmii, Acaulospora spp., Acaulospora thomii; Ambispora granatensis, Diversispora epigaea, Gigaspora margarita, Gigaspora rosea, Glomus dimorphicum, Glomus fasciculatum, Glomus maculosum, Glomus pallidum, Scutellospora dipurpusescrns and Septoglomus constrictum were identified in association with Eriocaulon eurypeplon. Based on spore density and relative abundance only Acaulospora rehmii was found as dominant AM fungal species. In this paper Eriocaulon eurypeplon is proposed as mycorrhizal endemic pipewort.*

Keywords - *Arbuscular mycorrhizal fungi, AM fungi, endemic plant, Eriocaulon eurypeplon Körn*

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I Introduction

Family Eriocaulaceae is commonly known as Pipeworts family. It comprises eleven genera distributed in tropical and sub-tropical regions of the world. In India, the genus *Eriocaulon* is well represented with many endemic species. So far, ninety two species of *Eriocaulon* are known in India [1]. *Eriocaulon eurypeplon* Körn. is frequently distributed throughout Western Ghats of India especially at Konkan-Malabar plains. In Konkan region of Maharashtra it is abundant on wet rocks in ephemeral flush vegetation of lateritic plateaus. According to recent assessment of *Eriocaulon eurypeplon* in IUCN Red List of Threatened Species it is placed at Category: Least Concern ver 3.1 [2].

ArbuscularMycorrhizal (AM) Fungal symbiosis is the most widespread plant–microbe symbiosis [3]. Ecological functions attributable to AM fungi are well established which include increased plant tolerance in adverse soil conditions, influencing response to severe climatic conditions and increasing productivity in natural plant communities [4]. Besides, AM fungi modify the structure and function of plant communities and are useful indicators of ecosystem change [5]. In view of these beneficial roles played by species of AM fungi, they are considered as vital for conservation of rare, endemic and endangered plant species [6]. Earlier studies regarding mycotrophic status in only one member of Eriocaulaceae from Brazil was conducted by Trappe [7] which did not show any colonization in roots. Thereafter, Silva, et al., [8] acclaimed first report of mycorrhizal association in Eriocaulaceae species from Brazil with reference to *Tonina fluviatilis* roots colonization. However, review of literature showed that different species of genus *Eriocaulon* were reported to be both nonmycorrhizal [9, 10, 11, 12] and mycorrhizal [13, 14, 15, 16, 17, 18]. In present paper in-depth assessment of mycorrhization established by AM fungi associated with *Eriocaulon eurypeplon* is discussed.

II Materials And Methods

2.1 Site description and sample collection

The study area is geographically Lateritic plateau falls under Hativale (Vikhare Gothane), 12 km from Rajapur, and located in Ratnagiri district of Konkan region Maharashtra. It is situated at geographical coordinates of about 16.6572^0 North and 73.5211^0 East. The *Eriocaulon eurypeplon* samples were collected during September 2016 (MMK & SSL) and valid identification was made by consulting with expert Dr. Chandore A. N. (Dept of Botany Abasaheb Marathe ASC College Rajapur, Ratanagiri district, Maharashtra). Authentically identified plant specimens were dry preserved in herbarium and deposited in department.

2.2 Sample collection

As *Eriocaulon eurypeplon* is an ephemeral plant and found on lateritic rocky plateau having very thin layer of a soil. Therefore soil sampling was done from about 20-30 selected plants to make a figure of around 150g soil. It was done very carefully because of less availability of soil on rocks. The soil samples were collected for AM fungal spore extraction and for estimation of physicochemical properties.

Those plants which were sparingly grown and not overcrowded with other associate plants were carefully chosen to avoid AM fungal flora of unwanted vegetation. This helps to prevents misleading results of AM fungal spores extraction.

The plants along with the soil samples and roots were collected in different collection bags, and transported from field to laboratory which immediately refrigerated at 4°C subsequent to arrival. The roots were processed immediately. All the rhizosphere soil samples were homogenized prior to remove coarse roots segments, stones and adhered particles through sieving procedure (2 mm mesh size). Subsamples of soil were air dried and used for estimation of physicochemical properties.

2.3 Physicochemical parameters of soil

Soil texture and moisture was estimated gravimetrically [19]. Soil pH was analyzed on 1:2.5, soil : water suspension [20]. Organic carbon was analyzed by WB rapid titration method [21] using 1N potassium dichromate and back titrated with 0.5N ferrous ammonium sulphate solution. Carbonate was estimated by Piper's rapid titration method [22] and available Olsen's phosphorus in soils was determined by extraction with 0.5M sodium bicarbonate for 30 min [23].

2.4 Status of AM fungal colonization in roots

It was determined by assessing roots for (a) percentage of AM fungal colonization and (b) occurrence intensity of three mycorrhizal components as given below:

2.4a *AM fungal percentage colonization*

The mycorrhizal colonization percentage, was determined by following rapid method of Phillips and Hayman [24]. The intercept method [25] was followed for microscopic observations of stained root segments under a Magnus Binocular Microscope. A root piece was considered for counting as colonized by AM fungi where any mycorrhizal components such as hyphae, vesicles or arbuscles was observed. The overall colonization percentage (*OCp)* was calculated on the basis of observed values for mean colonization percentage (*MCp)* associated with vesicles, arbuscules and hyphae etc.

2.4b *AM fungal Occurrence Intensity*

All the three components of AM fungi were interpreted for occurrence intensity*viz., poor* (1-25%), *moderate* (25-50%), *good* (50-75%) and *excellent* (>75%) which was denoted as '*p*, *m*, *g* and *e'* respectively. To interpret occurrence intensity (*OI*) of fungal structures, mean colonization percentage (*MCp) for* each fungal structure (*V*: vesicles, *A*: arbuscules and *H*: hyphae) was determined separately. Based on microscopic observations of randomly selected 25 root segments pattern of AM fungal colonization for *Eriocaulon eurypeplon* was determined. Any other special structures of mycorrhizal colonization (*Smc*) if present in root piece was also recorded.

2.5 AM fungal spore extraction

Spores were extracted from the10g of rhizosphere soil with the help of different size of sieves ranging from 25-250 µ by using sieving and decanting technique [26]. Total spore numbers of AM fungi in the soil sample were estimated following Gaur and Adholeya [27]. The spores isolated were mounted in a polyvinyl-lactoglycerol (PVLG) and PVLG solution mixed with Melzer's reagent 1:1 (v/v) ratio[28]. Only spores that appeared to be healthy were recorded, counted examined under stereomicroscope (Olympus 003421) and photo-micrographically documented with the help of Canon IXUS 155 digital Camera.

2.6 AM fungal species identification

Taxonomic placements of AMF spores and sporocarps up to species level was based on spore size, colour, wall layers and hyphal attachments using bibliographies by Schenk and Perez [29] after comparison with type or authenticated specimens. The identification is purely based on the synaptic keys [30,31,32] and also after consultation with descriptions of AM fungal species provided by International Culture Collection of Vesicular and Arbuscular Endomycorrhizal Fungi [http://invam.caf.wvu.edu/Myc_Info/Taxonomy/species.htm]. The species codes were followed after Schenk and Perez [29]. Voucher slide specimens were assigned accession codes '*BCA:MH*$_{YRY}$n' [where, *BCA:MH is Bhavan's College Andheri: Mycological Herbarium; YRY:* initials of second Author and n is number assigned'] and preserved in Mycorrhizal Research Laboratory of Department.

Spore density (S) was considered as the number of spores in 100 g soil. Relative abundance (RA) was defined as the percentage of spore numbers of a species divided by the total spores observed [33]. The dominant AM fungal species was determined according to relative abundance (RA > 6%) and spore density (S ≥ 40 spores). Statistical data processing for percentage colonization in roots, spore density and relative abundance of AMF species was performed for standard errors of means by using Microsoft excel 2007.

III Results

3.1 Physicochemical parameters of soil

As soil requirement varies with plant and associated microbes varies from species to species and hence Physicochemical properties of soil associated with any plant should be taken into consideration. It helps to understand optimum requirements of microhabitats in addition to plant species for sustaining under natural conditions. Physicochemical properties of soil associated with *Eriocaulon eurypeplon* are presented in Table 1.

Table 1: Physicochemical properties of soil associated with endemic pipewort: *Eriocaulon eurypeplon*

Sr. No.	Parameters	Status
1.	Colour	Red
2.	Soil texture	Lateritic rough
3.	pH	6.40 ± 0.02
4.	Organic Carbon	5.28%
5.	Organic Matter	9.08 %
6.	Carbonate	143.44± 0.01 $mg.kg^{-1}$
7.	Phosphorus	7.48 ± 0.02$mg.kg^{-1}$

(±) Standard error of mean

The soil of *Eriocaulon eurypeplon* is nearly acidic pH 6.4; organic carbon 5.28%, and calculated organic matter 9.08% is higher in soil. Whereas, carbonate content 143.44 $mg.kg^{-1}$, Olsen's Phosphorus content 7.48 mg.kg-1. In general, soil is slightly red, laterite rough in texture, slightly acidic in reaction; high in organic matter, carbonate content and available phosphorus level.

3.2 AM fungal percentage colonization in roots of *Eriocaulon eurypeplon*:

The roots of *Eriocaulon eurypeplon* from lateritic plateau of Rajapur area were examined for presence of AM fungi and denoted by *MCp i. e.* mean colonization percentage for individual components. The root colonization status of AM fungi is presented in Table 2. Our study revealed that the roots of all the plant samples are colonized in the range of 76- 100% by AM fungal components. The degree of AM colonization found varied in all specimens. The Overall colonization percentage denoted by *OCp* in *Eriocaulon eurypeplon* is 58.67%.

3.3 *AMF Occurrence Intensity*

The occurrence intensity is also presented in Table 2. Analysis of mychorrhizal roots suggests variation in occurrence intensity of individual AM Fungal components. All the three AM Fungal components such as vesicles, arbuscles and hyphae were interpreted for occurrence intensity if it lies in the range of 1-25%: Poor; 25-50%: Moderate; 50-75%: good; more than 75%: Excellent (Table 2). It is evident that vesicular colonization found 100% in all the plant samples analyzed during study. Besides the regular components of mycorrhiza like hyphae and vesicles, other structures such as chlamydospore *(ch)*, linearly formed chlamydospores *(lch);* intraradial spore *(S)* and moniliform vesicles *(mV)* are also recorded (Figure 1A-E).

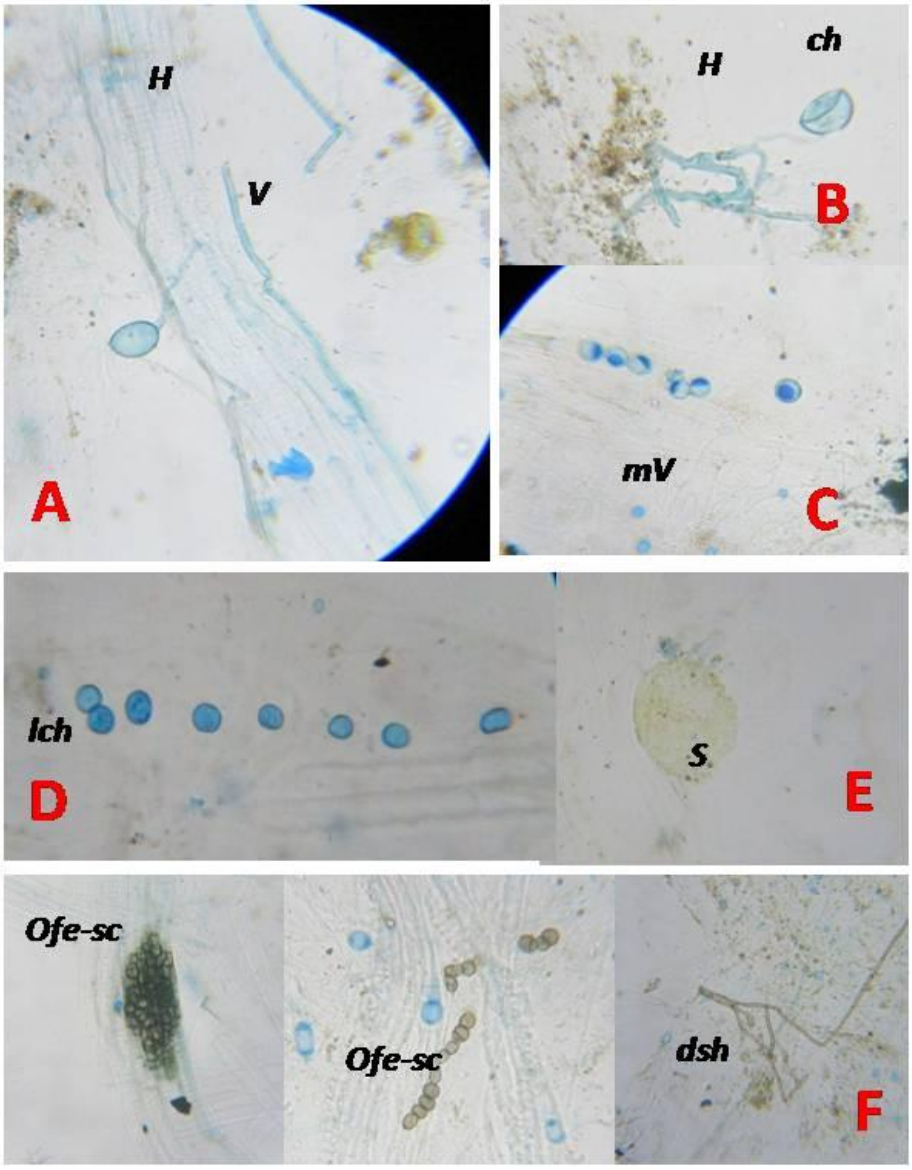

FIGURE: 1

Figure 1: (A-E) Mycorrhizal components colonizing the roots of endemic pipewort: *Eriocaulon eurypeplon* [*ch*: Chlamydospore; *H:* Hyphae, *S*: Intraradial spore, *V*: Vesicles, *mV:* moniliform vesicles; *lch*: Linearly formed chlamydospores, etc; **(F) Other fungal endophyte** [*ofe*] showing dark septate hyphae [*dsh*] and sclerotia [*sc*].

Table 2: Status of AM fungal colonization in roots of endemic pipewort: *Eriocaulon eurypeplon*

Particulars	***AM fungal colonization in plant roots***		
Fungal structures	***Vesicles***	***Arbuscules***	***Hyphae***
MCp (%)	*[e]100*	*[p]00*	*[e]76*
OCp (%)	*[g]58.67*		
OI	*Excellent*	*Poor*	*Excellent*
OCI	*Good*		
Smc Features	*Formation of ch, H, S, V, mV & lch.* (Fig. 1A-E)		
Pmc	*VH*		
Other fungal endophytes (Ofe)	*Present : dsh* & *sc* (Fig. 1F)		

(*MCp)* mean colonization percentage*;* **(*OI*)**Occurrence intensity **[*(p) 1-25%, (m) 25-50%, (e)>75%*]; (*Smc*)**Structures of Mycorrhizal colonization [***(ch)*** Chlamydospore, ***(H)***Hyphae, ***(S)***Intraradial spore, ***(V)***Vesicles, ***(mV)***moniliform vesicles, ***(lch)***Linearly formed chlamydospores]**; (*OCI*)**Overall colonization intensity**[**range of values is same as ***OI*]; (*OCp*)**Overall colonization percentage*; (Pmc)***Pattern of Mycorrhizal colonization; ***(VH)*** *vesicular-hyphal type; **(ofe)**Other fungal endophytes,* ***(dsh)***dark septate hyphae and ***(sc)***sclerotia.

3.4 Assessement of AM fungal species

In present study total sixteen species of AM fungi under five families of Glomeromycetes such as: Acaulosporaceae, Ambisporaceae, *Diversisporaceae*, Gigasporaceae and Glomeraceae are identified from the soil samples of *Eriocaulon eurypeplon* scattered over 7 genera *viz., Acaulospora, Ambispora, Diversispora, Gigaspora, Scutellospora, Glomus* and *Septoglomus.* The spores of all 16 species are presented in Table 3. Amongst the sixteen species, genus *Acaulospora* represents six species (37.5%); *Gigaspora* represents two species (12.50%); *Glomus* represents four species (25%); whereas, remaining four genera such as *Ambispora, Diversispora, Scutellospora* and *Septoglomus* represents single species (6.25%). These AM fungal species are identified as *viz., Aculospora denticulate* Sieverding & Tora., *Aculospora radilatata* Morton., *Acaulospora elegans* Trappe & Gerd., *Acaulospora rehmii* H. Magn., *Acaulospora* spp1, *Aculospora thomii* Blaszkowski; *Ambispora granatensis* Palenzuela, Ferrol & Oehl; *Diversispora epigaea* (B.A. Daniels & Trappe) C. Walker & A. Schüßler*; Gigaspora margarita* (Becker & Hall), Bentivenga & Morton, *Gigaspora rosea* Nicolson & Shcenck; *Glomus dimorphicum* Boye Tchko & Tewari., *Glomus fasciculatum* (Thaxtex) Gerd & Trappe Emend Walker, *Glomus maculosum* Miller & Walker, *Glomus pallidum* Hall; *Scutellospora dipurpusescrns* Mortan & Koske; and *Septoglomus constrictumi* G. A. Silva & Oehl.

3.5 Spore density and relative abundance

The total number of AM fungal spores recovered from soil samples of *Eriocaulon eurypeplon* are 141 and encountered at the rate of 1-9 spores 10^{-1} g soil as shown in Table 3. The spore density (S) of all 16 AM fungi is determined and expressed as number of spores per 10g of soil of *Eriocaulon eurypeplon* and is presented in Table 3. Among the 16 species following three species *viz., Acaulospora rehmii* (S=74), *Gigaspora rosea* (S=12) and *Aculospora denticulate* (S=9) are apparently dominating the soil sample. However, based on spore density and relative abundance, only one species is dominant (S ≥ 40 spores 10 g-1 soil, RA > 6%) *i. e. Acaulospora rehmii*. Thus in present investigation more number of AM fungal species are recovered from soil associated with *Eriocaulon eurypeplon.* More AM fungi were belonging to Acaulosporaceae (44.44%) followed by Gigasporaceae (33.33%).Thus, it is concluded that, all the soil samples of *Eriocaulon eurypeplon* showed establishment of multi-sporic pattern of AM fungal colonization.

Table. 3 Identified AM fungi with their spore density (S) & relative abundance (RA) in soil sample of endemic pipewort: *Eriocaulon eurypeplon*

Specimen Accession Code	AM fungal species	S	RA
	Family: Acaulosporaceae		
BCA:MH$_{YRY}$01	*Aculospora denticulate* Sieverding & Tora.	9	6.382
BCA:MH$_{YRY}$02	*Aculospora radilatata* Morton.	1	0.709
BCA:MH$_{YRY}$03	*Acaulospora elegans*Trappe &Gerd.	1	0.709
BCA:MH$_{YRY}$04	*Acaulospora rehmii* H. Magn.	74	52.482
BCA:MH$_{YRY}$05	*Acaulospora* spp1	2	1.418
BCA:MH$_{YRY}$06	*Aculospora thomii* Blaszkowski.	2	1.418
	Family: Ambisporaceae		
BCA:MH$_{YRY}$07	*Ambispora granatensis* Palenzuela, Ferrol & Oehl	6	4.255
	Family: *Diversisporaceae*		
BCA:MH$_{YRY}$08	*Diversispora epigaea* (B.A. Daniels & Trappe) C. Walker & A. Schüßler	4	2.836
	Family: Gigasporaceae		
BCA:MH$_{YRY}$09	*Gigaspora margarita* (Becker & Hall), Bentivenga & Morton	6	4.255
BCA:MH$_{YRY}$10	*Gigaspora rosea* Nicolson and Shcenck	12	8.510
BCA:MH$_{YRY}$15	*Scutellospora dipurpusescrns* Mortan & Koske	5	3.546
	Family: Glomeraceae		
BCA:MH$_{YRY}$11	*Glomus dimorphicum* Boye Tchko & Tewari.	7	4.964
BCA:MH$_{YRY}$12	*Glomus fasciculatum* (Thaxtex) Gerd & Trappe Emend Walker	2	1.418
BCA:MH$_{YRY}$13	*Glomus maculosum* Miller & Walker.	2	1.418
BCA:MH$_{YRY}$14	*Glomus pallidum* Hall.	6	4.255
BCA:MH$_{YRY}$16	*Septoglomus constrictumi* G.A. Silva & Oehl	2	1.418
Total	***16 AM fungal species***	**141**	**100**

IV Discussion

In recent study [34], *Eriocaulon manoharanii* from Kaas plateu Satara, Maharashtra showed 79.2±6.1% AM fungal colonization; 210 ±10 spores per 5g soil were belonging to *Glomus constrictum, Acaulospora x, Acaulospora y;* whereas dark septate endophytic fungi were absent. However, in present study apart from mycorrhizal components colonizing the *Eriocaulon eurypeplon* root system, the dark septate hyphae [*dsh*] and sclerotia [*sc*] of other fungal endophyte [*ofe*] are also distinctly recorded (Figure 1F). One more species *Eriocaulon robustum* Steud., is recently reported as non mycorrhizal but found colonized the roots ((1.92 ± 0.00 -25.54 ± 2.23%)) with dark septate endophyte [35].

According to Jhonson et al., [36] resources limitation is a driving factor for local adaptations in mycorrhizal symbiosis. Smith and Read [37], observed the AM fungal colonization aids in growth of the host plants to adverse condition. Present AM fungi are also colonized in adverse condition such as hard lateritic rocky plateau with very thin layer of soil. Ning and Cumming [38]**,** in their study recorded similar observation about colonization under adverse conditions, where in a grass plant *Andropogon genardii* adapts to the levels of nutrients present in the local soils due to the AM fungal association. However, in the absence of AM fungal association, growth of host is limited [39] and thus have beneficial role of AM fungal symbiosis in nutrient limiting conditions. Although *Eriocaulon eurypeplon* is an ephemeral plant our findings suggests that, presence of dormant spores or sporocarps of mycorrhizal symbiont in less amount of soil under such a harsh and stressful environmental conditions on lateritic plateau enables the pipewort species to grow luxuriantly at the monsoon shower.

Recently from Mucuge (Bahia) at eastern side of the Chapada Diamantina region of Brazil, Pereira, et al., [40] showed that seven different native AM fungal species were associated with a microendemic Eriocaulaceae plant, *Comanthera mucugensis* subsp. *mucugensis.* These native AM fungal species inoculation is undoubtedly an important biotechnological tool and encourages their use in conservation programs of endangered *Comanthera.* Similarly, present data on assessment of native AM fungi associated with *Eriocaulon eurypeplon* an endemic pipewort to Western Ghats will be useful for future conservation program.

V Conclusion

Present work has proved good colonization percentage of AM fungi in *Eriocaulon eurypeplon.* We have also confirmed the general agreement about poor arbuscular colonization is existing in *genus Eriocaulon.* This pipewort showed establishment of multi-sporic pattern of AM fungal colonization. Thus, present work makes a first report on AM fungal status in endemic pipewort species**:** *Eriocaulon eurypeplon* from Western Ghats of Maharashtra.

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