IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 10, Issue 5 Ver. I (May. 2016), PP 99-106
www.iosrjournals.org

Assessment of Three Representative Species of Portulacaceae with Ambiguity about Mycorhizal Status

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Abstract: *Due to ambiguity about mycorrhizal status of Portulacaceae and pharmaceutical potential of three representative plants of this family viz., Portulaca grandiflora Hook., Portulaca oleracea L. and Portulaca quadrifida L. present work was undertaken for detailed microscopic assessment of roots with reference to mycorrhizal structures, colonization percentage and identification of extracted spores of Arbuscular mycorrhizal fungi (AMF) from rhizosphere soil. Overall colonization percentage (OCp) of AMF was 58.71±1.4, 69.86±1.7 and 72.07±1.8 respectively in roots of these three plants. We also determined the mean colonization percentage (MCp) for each fungal structure (V: vesicles, A: arbuscules and H: hyphae) separately. P. grandiflora and P. oleracea showed presence of VH type of colonization whereas it is VAH type in P. quadrifida. Eleven species of AMF under four families of Glomeromycetes such as: Acaulosporaceae Diversisporaceae, Gigasporaceae and Glomeraceae were identified in the rhizosphere soil of three Portulaca spp. scattered over seven genera. Based on spore density and relative abundance, Scutellospora verrucosa was dominating in P. grandiflora rhizosphere. Glomus geosporum* and *G. macrocarpum were dominating rhizosphere of P. oleracea; whereas, Rhizophagus intraradices found dominating in rhizosphere soil of P. quadrifida. In this paper genus Portulaca of Portulacaceae with ambiguity about mycorrhizal nature was studied for the first time and confirmed its mycorrhizal status.*

Keywords: *Arbuscular mycorrhizal fungi (AMF), Glomeromycetes, mycorrhizal colonization,* Portulacaceae, *Portulaca, P. grandiflora P. oleracea* and *P. quadrifida.*

I. Introduction

Arbuscular mycorrhizal fungi (AMF) are universally present in all soils and in association with a great variety of plants of different taxonomic groups. AMF favours plant growth and increase phosphate uptake [**1**]. The role of AMF is mainly to increase the root capability in absorbing nutrients and water from the soil. Higher intensity of AMF colonization would increase the ability of plant roots to absorb nutrients and water consequently better is the plant growth [2]. In recent years, there has been substantial interest noticed in understandings of the interaction between AMF and plants in particular region or in a natural environment.

Earlier studies proposed that plant species belonging to Commelinaceae, Portulacaceae and Zygophyllaceae families are non-mycorrhizal [3] [4]. According to Lafferriere and Koske, [5], the non mycorrhizal nature of some species could not be considered as conclusive proof because they might become mycorrhizal under certain conditions. Nevertheless, inadequate research work has been done at global context in general particularly in India to investigate mycorrhizal status of those plants or plant families which were previously considered as non mycorrhizal in nature. Review of literature showed Family Portulacaceae is thought to be non mycorrhizal in status by many researchers [6] [7] [8]. However, some reports are in favour of mycorrhizal nature of Portulacaceae [9] [10] [11]. Thus by keeping in mind this ambiguity about mycorrhizal or non mycorrhizal nature of Portulacaceae, in present work we emphasized on roots screening of three representative plants belonging to genus *Portulaca viz., P. grandiflora* Hook., *P. oleracea* L. and *P. quadrifida* L.

Based on recent Phylogenetic analyses of Portulaceae, *P. quadrifida* which is opposite leaved (*OL clade*) plant distributed almost exclusively in the Old World; *P. oleracea* possessing pseudoopposite leaves is placed under *subclade Oleracea* of alternate leaved taxa (*AL clade*) where as *P. grandiflora having* conspicuous leaf axillary hairs is under *subclade Pilosa* [12] [13] [14]. These three plants have been recognized for their pharmaceutical potential (Table 1). Due to ambiguity about mycorrhizal status of Portulacaceae and pharmaceutical potential of three representative plants of this family present work was undertaken for detailed account on microscopic observation of roots of *P. grandiflora*, *P. oleracea* and *P. quadrifida* with reference to mycorrhizal structures, colonization percentage and identification of AMF spores extracted from rhizosphere soil.

Table 1. Efficacy of *Portulaca* species

***Portulaca* spp**	**Ethnobotanical and Pharmaceutical potential**
***Portulaca grandiflora* Hook.**	In oriental traditional medicine to cure sore throat, skin rashes and for detoxification as a putative immune-stimulant [**15**]. Effective on hepatitis-B surface antigen (anti-HBsAg) [**16**] Aerial parts contain various diterpenoids like portulal, portulenone, portulenol, portulene [**17**] and portulene acetal a minor diterpenoid [**18**].
***Portulaca oleracea* L**	For blood purification leaf of *P.oleracea* juice is orally administered; paste of fruits and seed is applied on the teeth and gum against dental problems in Nepal [**19**]. In Unani and Ayurvedic medicines for ailments such as: bleeding piles, diarrhea, dysentery, fever, kidney, liver, skin disorders, and spleen diseases [**20**]. For anti-rheumatic and anti-fungal potential; pharmacologically anti-diabetic, analgesic and wound healing properties [**21**]. Rich source of omega- 3 fatty acids, and protein dietary antioxidant vegetable [**22**]**.** Traditional vegetable in Java & Indonesia used for dysentery, diarrhoea, inflammation, appendix, breast inflammation, constipation, haemorrhoids & worms etc [**23**].
***Portulaca quadrifida* L.**	As a vegetable useful in asthma, cough, urinary discharges, inflammations and ulcers. A poultice of the plant is applied in abdominal complaints, erysipelas and haemorrhoids [**24**]. For antifungal activity against *Aspergillus fumigates* and *Candida* albicans [**25**]. Traditional weedy and famine leafy vegetable in most African countries such as Uganda, Kenya, Ghana, Nigeria, Zambia, South Africa, Botswana, and Tanzania[**26**]**.**

II. Materials And Methods

2.1 Soil sampling:

Total ten plants of each species were sampled for soil collection. The plants were removed carefully from the natural habitats in Maharashtra region such as: Bhavans College premises (19.1254° North, 72.8362° East) for *P. grandiflora*; Arnala, Virar waste-land (20° 25' 0" North and 73° 5' 0" East) for *P. oleracea* and barren agriculture field at Shriram Nagar, Takali Road, Pandharpur (17°39'31" North and 75°18'38"East) for *P. quadrifida*. The plants along with the rhizosphere soil samples and roots were collected in different collection bags and transported to laboratory and immediately refrigerated at 4°C subsequent to arrival. The roots were processed immediately. All the ten rhizosphere soil samples for each plant were homogenized prior to remove coarse roots segments, stones and adhered particles through sieving procedure (2 mm mesh size). Subsamples of ten soils were air dried and used for estimation of physico-chemical properties.

2.2 Physico-chemical properties of soil:

Soil texture and moisture was estimated gravimetrically [**27**]. Soil pH was analysed on 1:2.5, soil: water suspension. Organic carbon was analyzed by Walkley and Black [**28**] method. Available Olsen's phosphorus was determined by extraction with 0.5M sodium bicarbonate for 30 min [**29**].

2.3 AMF colonization in Roots:

To determine the colonization percentage, root samples were processed and stained following the method of Phillips and Hayman [**30**]. Randomly selected 100 root segments were assessed microscopically for colonization percentage using the intercept method **[31]** using Olympus compound microscope. Photomicrographs were taken with the help of Canon IXUS 155 digital Camera. A root piece was considered for counting as colonized by AMF when any mycorrhizal structure such as hyphae, vesicles or arbuscules was observed. All the three AMF structures were interpreted for occurrence intensity (*OI*) *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. The pattern of AMF colonization for all the three plants was determined. Any other colonizing structures present in root piece were also recorded.

2.4 AMF spore extraction:

Spores were extracted from the100g of rhizosphere soil samples with the help of different size of sieves ranging from 25-250 μ by using sieving and decanting technique **[32]**. Total spore numbers of AMF in the soil sample were estimated following Gaur and Adholeya **[33].** The spore densities were expressed as the number of spores per 100g of soil. The spores retained on each sieve were transferred to filter paper and subsequently picked up with the help of or needle under dissecting microscope for assessment. All spores were mounted in a polyvinyl alcohol/ lactic acid/glycerol (PVLG; **[34]** and a mixture of PVLG and Melzer's reagent (1:1, v/v) **[35]**. Spores were crushed to varying degrees by applying pressure to the coverslip and then stored at 65°C for 24h to clear their contents of oil droplets. These were examined under stereomicroscope (Olympus 003421) at 100X and 400X magnifications.

2.5 AMF species identification:

AMF spores and sporocarps were identified up to species level based on spore size, colour, wall layers and hyphal attachments after comparison with type or authenticated specimens with the help of using bibliographies by Schenck and Perez **[36]** and by matching descriptions of AMF species available at International Culture Collection of Vesicular and Arbuscular Endomycorrhizal Fungi [http://www.invam.wvu.edu/]. Voucher slide specimens were assigned accession codes '*BCA:MH*$_{AJP}$n' [where, *BCA:MH* Bhavan's College Andheri: Mycological Herbarium*;* $_{AP}$ initials of second Author and *'n'* is number assigned] and deposited 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 **[37]**. AMF species were considered as dominant if spore density was more than forty percent (*S40*) and relative abundance (RA) greater than 6%. 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 Physico-chemical properties of soil:

Since soil requirement for any plant and associated microbes changes from species to species and therefore physico-chemical properties of soil should be taken into consideration. It helps to understand optimum requirements of microhabitats in addition to plant species to sustain under natural conditions. Physicochemical properties of the rhizosphere soil of all the three *Portulaca* spp are presented in **Table 1.**

Table 1: Physico-chemical properties of soil for *Portulaca spp.*

Sr. No.	Parameters	Physicochemical Status		
		P. grandiflora	***P. oleracea***	***P. quadrifida***
1.	**Soil texture**	Clay	Sandy	Coarse gravel
2.	**Soil moisture (%)**	15%	18%	11%
3.	**Ph**	6.8± 0.01	8.1± 0.01	8.7± 0.01
4.	**Organic Carbon**	0.97 %	0.18%	0.21%
5.	**Phosphorus**	12.3±0.02mg.kg^{-1}	7.6±0.01mg.kg^{-1}	14.7±0.04mg.kg^{-1}

(±) Standard error of mean

P. oleracea and *P.quadrifida* were inhabitant to alkaline soil (pH ranging 8.1-8.7), organic carbon 0.18-0.21%, Olsen's Phosphorus content 7.6-14.7 mg.kg^{-1}; whereas, *P.grandiflora* found in acidic soil, with 0.97% organic carbon and12.3 mg.kg^{-1} Phosphorus. In general, soils were low in organic carbon and available phosphorus content.

3.2 AMF colonization in roots of *Portulaca spp.*:

In present investigation overall colonization percentage of AMF was 58.71±1.4, 69.86±1.7 and 72.07±1.8 respectively for *P. grandiflora, P. oleracea* and *P. quadrifida* roots **Table 2.** Recently Ramos-Zapata, *et. al.* **[11]** investigated AMF colonization in two *Portulaca* species *viz., P. oleracea* and *P. pilosa* L. According to them overall AMF colonization was 54.77±3.7 and 22.14±12.6 respectively. Our findings also make general agreement with Ramos-Zapata, *et al.* **[11]** that *P. oleracea* had good percentage of colonization *i.e.* in the range of *50-75%*. We also determined the mean colonization percentage (*MCp)* for each fungal structure (*V*: vesicles, *A*: arbuscules and *H*: hyphae) separately for all three plants. The *P. grandiflora* had *MCps*: 83.06±1.5 (V**)**, 12.62±0.7 (*A*), 80.45±2.1 (*H*)**;** *P. oleracea* showed *MCps*: 92.54±2.1 (V), 17.33±1.3 (A), 96.72±1.7 (H) and *P. quadrifida* had *MCps:* 89.43±1.6 (*V*), 32.58±1.5 (*A*) & 94.22±2.4 (*H*) **(Table 2).** Compared with recent findings [**11**] on *P. oleracea MCps:* 17.76 ±3.8 (*V*), 0.33 ±0.3 (*A*) & 50.59 ±5.4 (*H***)** we found *MCp*>75% *i.e.* excellent occurrence intensity (*OI*) for vesicular and hyphal colonization but poor (*OI*: 1-25%) arbuscular colonization. In present study we also reported type of mycorrhizal structures colonization is *VH* type in *P.grandiflora* and *P. oleracea;* whereas it is *VAH* type in *P.quadrifida.*

Well developed vesicles were observed in linear position in root tissue of *P. grandiflora* **(Figure 1b)** as well as *P. quadrifida* **(Figure 3c, 3g)** and were referred as moniliform vesicles (*mV*). In *P. quadrifida* well defined vesicular reticulum *(vr)* was established by cluster of moniliform vesicles. The stages in development of vesicular reticulum structure in *P. quadrifida* root cortex are illustrated in **Figure 3g-i.** Root cortex cells were also exhibited other form of vesicles such as, aggregated (*aV*) in *P. oleracea* **(Figure 2d**); rod shaped (*rV),* oblong (*oV),* clustered *(cV)* and grape bunch like (*gV*) etc. in *P. quadrifida* (**Figure 3d-f**).Thus various forms of vesicular growth eventually lead to improvement in colonization area, which in turn may provide nutritional benefits to these plants. Chlamydospores (*ch*) and extra-radical hyphae (*eh*) were also recorded on root surface of all the three species **(Figure 1c, 2a, 3b**). Recorbet, *et al.* **[38]** proposed that, such extra-radical hyphal web of AMF developed on root surface performs various key tasks in symbiosis including nutrient uptake from the environment, dispersal of propagules and interactions with other soil biota.

Table 2: Status of AMF colonization in roots of *Portulaca spp.*

Particular	***AMF colonization in plant roots***								
	P. grandiflora			***P. oleracea***			***P. quadrifida***		
Fungal structures	***V***	***A***	***H***	***V***	***A***	***H***	***V***	***A***	***H***
MCp (%)	83.06 *±1.5*	12.62 *±0.7*	80.45 *±2.1*	92.54 *±2.1*	17.33 *±1.3*	96.72 *±1.7*	89.43 *±1.6*	32.58 *±1.5*	94.22 *±2.4*
OI	*Excellent*^{e}	*poor*^{p}	*Excellent*^{e}	*Excellent*^{e}	*poor*^{p}	*Excellent*^{e}	*Excellent*^{e}	*Moderate*^{m}	*Excellent*^{e}
OCp **(%)**	58.*71±1.4*			69.*86±1.7*			72.07*±1.8*		
OCI	*good*^{g}			*good*^{g}			*good*^{g}		
Smc Features	*Formation of : Mv, ch; eh*			*Formation of : ch, eh, aV*			*Formation of : ch, eh, cV, Mv, rV, oV, gV; Ivr, vr*		
Pmc	*VH*			*VH*			*VAH*		

(±) Standard error of mean; **(*MCp)*** mean colonization percentage*;* **(*V*)** Vesicles; **(*A*)** Arbuscules; **(*H*)** Hyphae; **(*OI*)** Occurrence intensity **[*(p) 1-25%, (g) 50-75%, (e) >75%*], (*Smc*)** Structures of Mycorrhizal colonization**; (*OCI*)** Overall colonization intensity**[**range of values is same as ***OI***] **; (*OCp*)** Overall colonization percentage*;* **(*Pmc*);** Pattern of Mycorrhizal colonization; **(*eh*)** Extraradical hyphae; **(*ch*)** Chlamydospores; **(*aV*)** Aggregated vesicles, **(*cV*)** Clustered vesicles; **(*mV*)** Moniliform Vesicle/s; **(*rV*)** Rod shaped vesicles*;* **(*oV*)** Oblong vesicles*;* **(*gV*)** Grape bunch like vesicles; **(*Ivr*)** Initial vesicular reticulum*;* **(*vr*)** well defined vesicular reticulum*;* **(*VH*)** *vesicular-hyphal type;* **(*VAH*)** *vesicular-arbuscular-hyphal type.*

3.2 AMF species identification:

Eleven species of AMF under four families of Glomeromycetes such as: Acaulosporaceae Diversisporaceae, Gigasporaceae and Glomeraceae were identified in the rhizosphere soil of three *Portulaca spp.* scattered over seven genera *viz.*, *Acaulospora*, *Diversispora*, *Entrophospora*, *Glomus*, *Rhizophagus*, *Scutellospora* and *Septoglomus*. Amongst the seven genera, *Glomus* represented five species. The identified spores of all 11 AMF species are presented in **Table 3.**

Table 3 Identified AMF with their spore density (S) and relative abundance (RA) in *Portulaca spp.* rhizosphere (dominant species are in bold).

Sr. No.	**AMF Species & Code**	***P. grandiflora***		***P. oleracea***		***P. quadrifida***	
		S	***RA***	***S***	***RA***	***S***	***RA***
	Acaulosporaceae						
1.	*Acaulospora rehmii* Sieverd. & S. Toro : *BCA:MH* $_{AJP}{}^{01}$	-	-	-	-	*2*	*2.78*
2.	*Entrophospora infrequens* (I.R. Hall) R.N. Ames & R.W. Schneid : *BCA:MH* $_{AJP}{}^{03}$	-	-	-	-	*8*	*11.12*
	Diversisporaceae						
3.	*Diversispora epigaea* (B.A. Daniels & Trappe) C. Walker & A. Schüßler : *BCA:MH* $_{AJP}{}^{02}$	-	-	-	-	*9*	*12.50*
	Gigasporaceae						
4.	***Scutellospora verrucosa*** (Koske & C. Walker) C. Walker & F.E. Sanders : *BCA:MH* $_{AJP}{}^{11}$	***22***	***64.70***	-	-	-	-
	Glomeraceae						
5.	***Glomus geosporum*** (T.H. Nicolson & Gerd.) C. Walker : *BCA:MH* $_{AJP}{}^{04\text{-}06}$	-	-	***19***	***59.38***	-	-
6.	*Glomus luteum* L.J. Kenn., J.C. Stutz & J.B. Morton : *BCA:MH* $_{AJP}{}^{07}$	*12*	*35.30*	-	-	-	-
7.	***Glomus macrocarpum*** Tul. & C. Tul. : *BCA:MH* $_{AJP}{}^{08}$	-	-	***9***	***28.12***	-	-
8.	*Glomus sinuosum* (Gerd. & B.K. Bakshi) R.T. Almeida & N.C. Schenck : *BCA:MH* $_{AJP}{}^{10}$	-	-	*4*	*12.50*	-	-
9.	*Glomus versiforme* (P. Karst.) S.M. Berch : *BCA:MH* $_{AJP}{}^{09}$	-	-	-	-	*4*	*5.55*
10.	***Rhizophagus intraradices*** (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler : *BCA:MH* $_{AJP}{}^{09*}$	-	-	-	-	***35***	***48.61***
11.	*Septoglomus constrictum* (Trappe) Sieverd., G.A. Silva & Oehl: *BCA:MH* $_{AJP}{}^{12}$	-	-	-	-	*14*	*19.44*
	Total	*34*	*100*	*32*	*100*	*72*	*100*
	S40% = (Total spores of AMF spp ÷ 10)× 4	*13.6 ≈ 14*		*12.8 ≈ 13*		*28.8 ≈ 29*	
	AMF species: 11	*02*		*03*		*07*	

The AMF species identified were *viz.*, *Acaulospora rehmii* Sieverd. & S. Toro, *Diversispora epigaea* (B.A. Daniels & Trappe) C. Walker & A. Schüßler, *Entrophospora infrequens* (I.R. Hall) R.N. Ames & R.W. Schneid, *Glomus geosporum* (T.H. Nicolson & Gerd.) C. Walker, *Glomus luteum* L.J. Kenn., J.C. Stutz & J.B. Morton, *Glomus macrocarpum* Tul. & C. Tul., *Glomus sinuosum* (Gerd. & B.K. Bakshi) R.T. Almeida & N.C. Schenck, *Glomus versiforme* (P. Karst.) S.M. Berch, *Rhizophagus intraradices* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler, *Scutellospora verrucosa* (Koske & C. Walker) C. Walker & F.E. Sanders and *Septoglomus constrictum* (Trappe) Sieverd., G.A. Silva & Oehl. Based on spore density and relative abundance,

one dominant species *i.e. Scutellospora verrucosa* (S > 14, RA > 6%) was dominating *P. grandiflora* rhizosphere; two dominant species *viz., Glomus geosporum* and *G. macrocarpum* (S > 13, RA > 6%) were dominating *P. oleracea* rhizosphere; whereas, *Rhizophagus intraradices* (S > 29, RA > 6%) was a dominating AMF in rhizosphere soil of *P. quadrifida*. The morphological characteristics of some dominant AMF species are illustrated in **Figure 3.**

IV. Discussion

Earlier studies proposed that plant species belonging to Commelinaceae, Portulacaceae and Zygophyllaceae families are non-mycorrhizal **[3] [4]**. According to Wang and Qiu **[8]**, *P. oleracea* is a non-mycorrhizal plant. Likely, Vatovec *et al.* **[7]** placed *P. oleracea* under non-host plant category for mycorrhizal root colonization. Muthukumar and Udayan **[6]** also noticed lack of VAM association in *P. oleracea* studied from soil (pH 8.8, P 0.83 $mg.kg^{-1}$) of Western Ghats of Southern India. Thus, most studies suggested Portulacace as non mycorrhizal. However, few reports exceptionally showed poor colonization in Portulacaceae in general particularly in *Portulaca* spp. Previously Parthipan *et al.* **[9]** showed *P. oleracea* had 20% AMF colonization and presence of *Glomus claroideum* in soil of Tamil Nadu state of India. Similarly, Raja *et al.* **[10]** also showed 5 % VAM infection in *P. oleracea* grown in Nilgiri Hills of same Indian State. However, recently Ramos-Zapata, *et. al.* **[11]** found that several ruderal species present in a traditional agricultural system with maize (Yucatan, Mexico) belonging to non mycorrhizal families including Portulacaceae had their roots colonized by AMF, and hence act as temporal hosts of AMF species.

By consulting with literature on AMF colonization in Portulacaceae, it was observed that investigations were carried with reference to *P. oleracea* [9] [10] [11] and *Portulaca pilosa* L **[11].** Other than *Portulaca, Claytonia megarhiza* (Gray) Parry and *Lewisia pygmaea* (Gray) Robinson were also studied for mycorrhizal status from 4298m and 3488m elevation respectively which were found as non-mycorrhizal plants **[39]**.Thus it is revealed that, most findings were related with *P. oleracea* and there were mycorrhization in some cases while other were non-mycorrhizal. Other species remained unfocused from exploration point of view globally. Hence present report proved distinct mycorrhization in other two species *i.e. P.grandiflora* and *P. quadrifida* along with *P. oleracea* which would help to take initiatives to study mycorrhizal status of family Portulacaceae with reference to other genera.

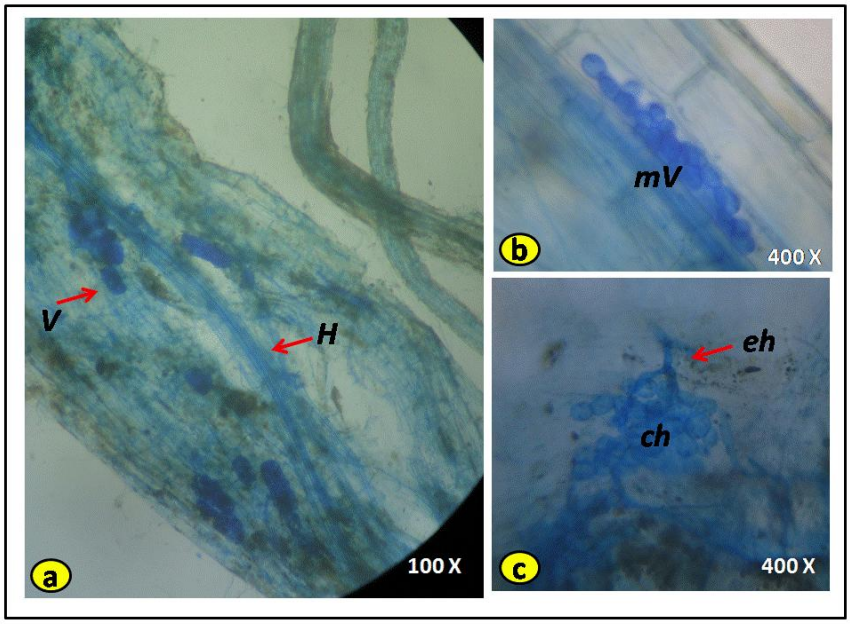

Figure 1. Structures of AMF colonization in roots of *Portulaca grandiflora.* Hook showing: a.typical colonization [Hyphae (*H);Vesicle/s(V)];* **b.** moniliform vesicles (*mV);* **c.** extra-radical hyphae (*eh*) bearing cluster of young chlamydospores (*ch).*

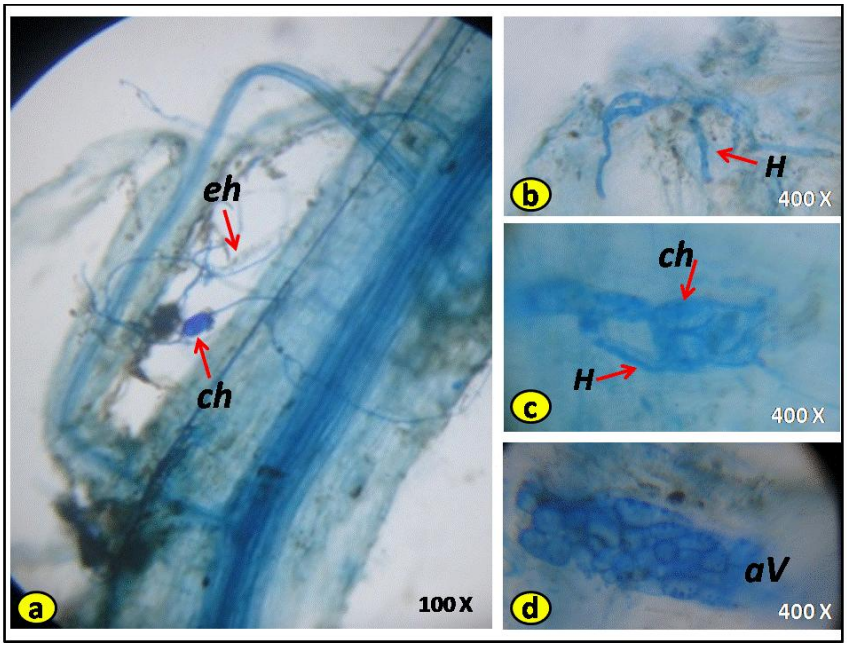

Figure 2. Structures of AMF colonization in roots of *Portulaca oleracea* Linn. showing: a. extra-radical clamydispore (*ch*) & extra-radical hyphae (*eh*); **b.** corticular thick walled hyphae (*H*); **c.** corticular hyphae & developing chlamydospores (*ch*); **d.** aggregated vesicles (*aV*) in a cortex cell.

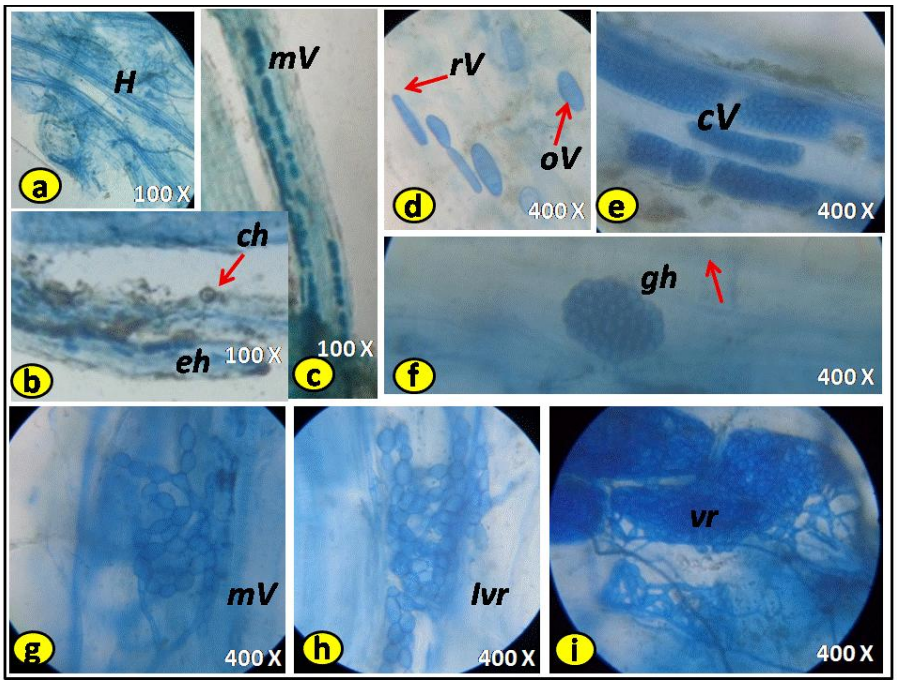

Figure 3. Structures of AMF colonization in roots of *Portulaca quadrifida* Linn showing: a. hyphal colonization (*H)*; **b.** extra-radical hyphae (*eh*) and chlamydospore (*ch*) **c.** vesicular colonization with moniliform vesicles *(mV)*; **d.** rod shaped vesicles (*rV)* & oblong vesicles *(oV)*; **e.** clustered vesicles *(cV)*; **f.** grapes bunch like vesicles (*gV*)**; g-i. *Stages in development of vesicular reticulum structure in root cortex*- g.** moniliform vesicles- clustering (*mV)*; **h.** moniliform vesicles clustered to form initial vesicular reticulum *(Ivr)*; **i.** Well defined vesicular reticulum *(vr).*

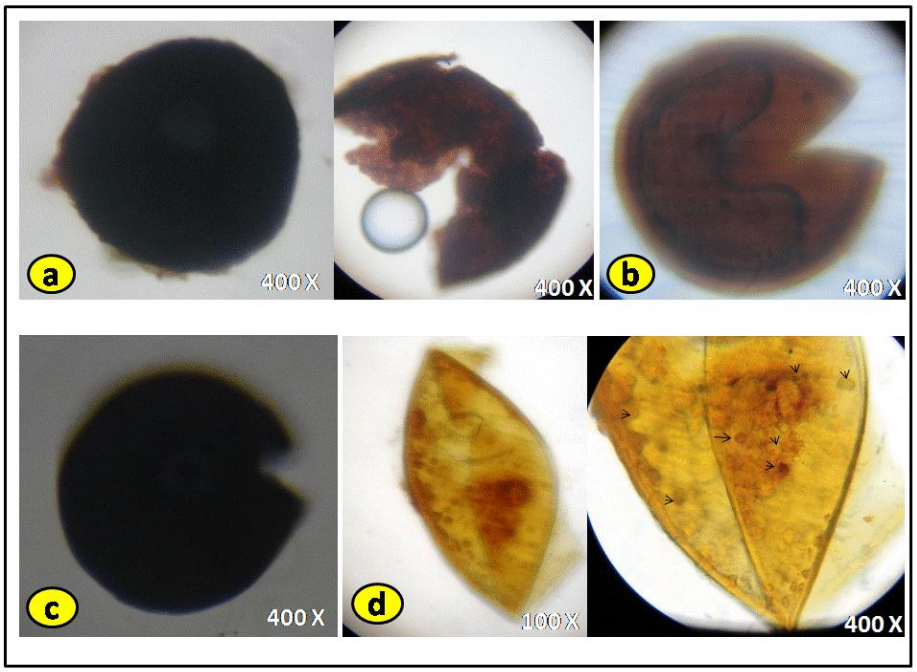

Figure 4. Morphological characteristics of some dominant AMF spores in rhizosphere soil of *Portulaca spp*: a. *Scutellospora verrucosa* (Koske & C. Walker) C. Walker & F.E. Sanders (entire spore mount in PVLG & crushed spore in PVLG + Melzer's reagent)**; b.** *Glomus geosporum* (T.H. Nicolson & Gerd.) C. Walker**; c.** *Glomus macrocarpum* Tul. & C. Tul.**; d.** *Glomus versiforme* (P. Karst.) S.M. Berch, possesing *Rhizophagus intraradices* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler (black arrow headed)**.**

V. Conclusion

We recognized, to the best of our knowledge for first time, the mycorrhizal status of *P.grandiflora P. oleracea* and *P.quadrifida*. Moreover, the first genus of Portulacaceae with ambiguity about mycorrhizal nature was studied for the first time in respect of detailed root colonization and associated AMF species in rhizosphere of it. We confirmed Portulacaceae is mycorrhizal in nature on the basis of assessment of three representative species of genus *Portulaca*. Such studies provide significant information which is considered as necessary prerequisites for tapping the potential of mycorrhizal fungi in commercial cultivation of plant species. Thus, it may facilitate the potential use of AMF in endeavours aimed at commercial cultivation of pharmacologically important plants *P.grandiflora P. oleracea* and *P.quadrifida* in near future.

Acknowledgements

Thanks are due to Dr. (Mrs.) V. I. Katchi, Principal, BCA, Mumbai for providing laboratory facility. We also thanks to unrevealed reviewers for their comments.

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