INTERNATIONAL JOURNAL FOR INNOVATIVE RESEARCH IN MULTIDISCIPLINARY FIELD ISSN: 2455-0620 Volume - 7, Issue - 11, Nov – 2021
Monthly, Peer-Reviewed, Refereed, Indexed Journal with IC Value: 86.87 Impact Factor: 6.719
Received Date: 13/11/2021 Acceptance Date: 28/11/2021 Publication Date: 30/11/2021

Comparative mycobiota of terrestrial orchids *Eulophia nuda* Lindl. and *Geodorum densiflorum* (Lam.) Schltr.

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Abstract: In this present work, the mutually associated mycobiota of two terrestrial orchids- *Eulophia nuda* Lindl. and *Geodorum densiflorum* (Lam.) Schltr were screened. Since orchid seeds require fungal association for their germination, which means these microbes are the sources of generating suitable environment for proper growth and nutrition. *E. nuda* is also known as Amarkand due to its medicinal properties especially reported from its corm. Similarly, the corms of *G. densiflorum* also possess several medicinal roles. These associated fungal isolates may contribute in enhancing the pharmaceutical potentials of the orchids. Several methods were followed to isolate, identify, and culture the fungal species. A dominant fungal diversity reported during the growing months of the orchids, emphasizing towards their role in orchid growth.

Key Words: Orchid, Amarkand, Rhizosphere, Rhizoplane, Phyllosphere, Phylloplane, PDA, SA, MEA, CzA, CMA, PSA.

1. INTRODUCTION:

The two taxa *Eulophia nuda* and *Geodorum densiflorum* were studied during the mycological investigation which belongs to the family orchidaceae, representing diverse type of flowers among the angiosperms.

a) *E. nuda* Lindl. is a terrestrial orchid growing in shady and marshy areas of the forest. Its corm has tremendous therapeutic potentials due to deposition of various bioactive compounds. Few workers have reported anticancerous, antioxidant and antibacterial activity from the corm extracts (**Bhandari *et al*, 2001**).

b) The another orchid *G. densiflorum* (Lam.) Schltr. with terrestrial habit mostly prefers rainforests, marshy vegetation areas. As like *E. nuda* their corms also possess anticancerous, antioxidant, antibacterial, cytotoxic activities (**Akter *et al*, 2010**). The morphology and histochemistry of *E. nuda* and *G. densiflorum* corm has similarity.

Orchids require fungal association for their seeds to germinate and during the later stages of growth. The host specificity of the fungal species creates a noble environment for the nourishment and deposition of ergastic metabolites in orchids; this enhances orchid plant endemic character in wild. Various types of methods are designed by the workers for fungal isolation; the samples collected for analysis includes rhizosphere, rhizoplane, phyllosphere and phylloplane (**Nagamani *et al*, 2006**). Rhizosphere is plant associated soil aggregates inhabited by microorganisms, rhizoplane is the inner surface region of the root in contact with the soil aggregates, phyllosphere is the above ground surface area of inhabited by microbial spores and phylloplane is the inner leaf surface mycobiota. Mycorrhizal symbiont also boosts up the growth and mobilization of essential nutrients in orchid as reported by certain researchers like **Parihar *et al*** (**2013**).

2. LITERATURE REVIEW:

Mycorrhizal role in germination of orchid seeds were discovered by **Bougoure *et al*** (**2005**). One of the pre-requisite factors behind the orchid seed germination in nature was their association with a specific fungal partner. **Dearnaley *et* al** (**2012**) reported that the associated fungal genera with Orchidaceae family like *Tulasnella*, *Ceratobasidium* are mostly ectomycorrhizal fungi, root endophytes, saprotrophs, few are also known as parasites and plant pathogens. Some endophytes contribute towards the orchid mycorrhizal symbiosis, as analysed by **Sneh *et al*** (**1991**) and grouped as nearby genera of *Rhizoctonia* genus.

Cormick *et al* (**2018**) carried the work of restoration of some endangered orchid species via studying the beneficial role of fungal isolates on the growth of these endangered orchids and their indirect link with the habit and nutrient specificity of the orchids. Fungal partners are also helpful in deposition of essential secondary metabolites in the orchid tissues. Some workers like **Dawande V** and **Gurav R** (**2017**) studied the quantitative parameters of the deposited secondary metabolites from the extracts of *Eulophia* species.

3. MATERIALS:

Collection of root and soil samples

Rhizospheric soil, root and leaf samples of *Eulophia* were collected from Kolhapur district and that of *Geodorum* from Rawanwadi and Godazadi forest respectively. The samples were subsequently placed in zip-loc polythene bags, brought to the laboratory for further processing.

4. METHOD:

The samples were further processed for fungal isolation as mentioned by **Aneja** (**2003**) and below is brief note on isolation steps followed:

1. Rhizosphere mycobiont isolation:
The soil aggregates associated with the orchid corm and rootlets were washed under running tap water, sun dried and spread over the culturing media on petriplate.

2. Rhizoplane mycobiont isolation:
Corms and rootlets of the orchids were surface sterilized with double distilled water, cut into one-centimetre-long pieces and inoculated into petriplate containing culture media.

3. Phyllosphere mycobiont isolation:
Leaf samples were plugged, washed through double distilled water, one-centimetre-long pieces transferred into ten millilitre distilled water and pipette out one millilitre into culture media containing petriplate.

4. Phylloplane mycobiont isolation:
Leaves were sterilized with double distilled water, cut into one-centimetre-long pieces and transferred into culture plate.

Culturing and sub-culturing of pure isolates:
The surface sterilized roots and leaves were inoculated in sterilized nutrient media. To obtain pure culture of the fungal species six media PDA (Potato Dextrose Agar, Himedia), SA (Sarbourd's Agar, Himedia), CMA (Corn Meal Agar, Himedia), CzA (Czapex Dox Agar, Himedia), PSA (Potato Sucrose Agar, Himedia) and MEA (Himedia) were used.

Morphological identification and digitalization of mycobiont isolates:

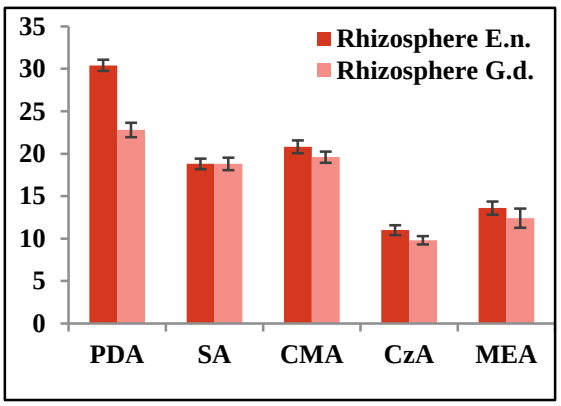
Fungal isolates were identified on the basis of their morphological characters via some recognized manuals like Manual of Soil Fungi (**Gilman, 1945**). Digital documentation performed by Zeiss Axio Star PlusTrinocular microscope using Canon power digital camera.

5. DISCUSSION:

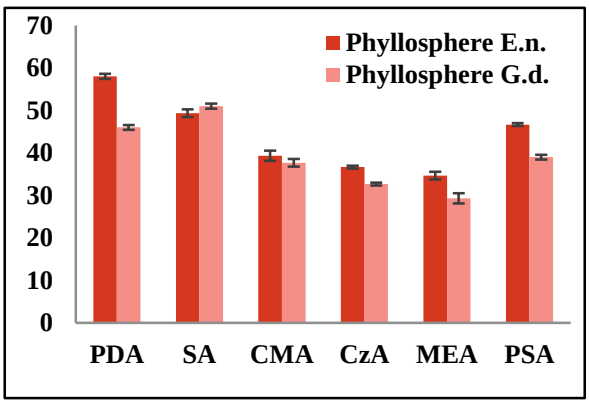
The entire analysis for mycological symbiont of both the orchids showed similarity among their tabulated data which contribute towards their corm's morphological similarities and secondary metabolites deposition; finally helps in its various medicinal properties. Media specific results shows that different fungal genus require different specific substrate for their normal growth and activity in association with the host. This specificity of the fugal partner may contribute towards the endemicity of the orchids. The fugal association may enhance the nutrient uptake of the orchids because their diversity increases during the growing periods of the orchid i.e. around August to September.

6. RESULT:

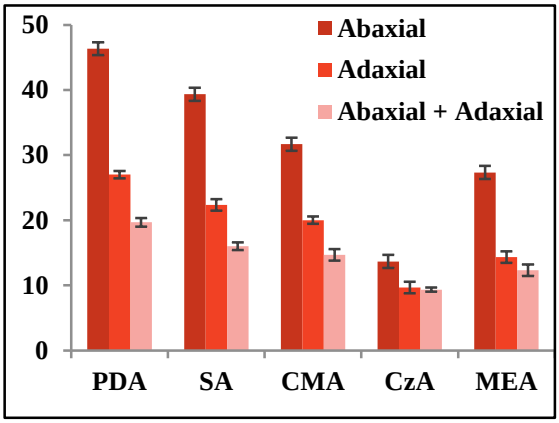
The fungal colonies isolated from rhizosphere, rhizoplane, phyllosphere and phylloplane under different media were tabulated and compared for both the orchids. A comparative account of colonies isolated in rhizosphere soil analysis of both the orchids shown in Graph1.1, followed by the comparative graphical representation of rhizoplane (Graph 1.3), phyllosphere (Graph 1.2) and phylloplane (Graph 1.4) of the orchids.



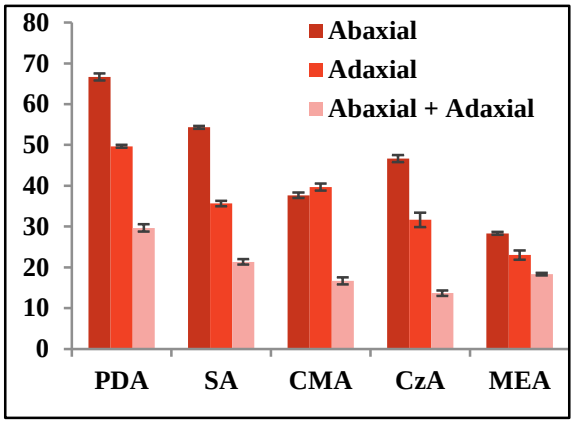
Graph 1.1: Comparative rhizosphere colonies of both orchids. * Mean values ± error bars.



Graph 1.2: Comparative phyllosphere colonies of both orchids.



Graph 1.3: Comparative rhizoplane colonies of both orchids.



Graph 1.4: Comparative phylloplane colonies of both orchids.

7. CONCLUSIONS:

An overall tabulated data of the total fungal colonies reported from both the orchids during their dry (non-growing months) and wet (growing months) seasons highlight a tremendous increase in fungal diversity that might enhance the orchid growth and their nutrient solubilisation. The total fungal colonies isolated from *E. nuda* and *G. densiflorum* during their non-growing months (dry periods) represented in Table 1 while those fungal genera isolated during their growing months (wet periods) in Table 1.1

Table 1: Total tabulated colonies of fungal genera isolated from *E. nuda* and *G. densiflorum* during their non- growing seasons. * Mean values ± standard error bars.

Fungal genera	*Eulophia nuda*			*Geodorum densiflorum*		
	PDA	CMA	MEA	PDA	CMA	MEA
Aspergillus	21±0.1	20±0.7	22±1.6	30±0.5	21±0.22	18±0.4
Penicillium	10±0.22	13±0.56	10±1.1	20±1.1	14±0.83	10±0.71
Cladosporium	10±0.91	8±0.83	8±0.5	4±0.7	5±0.8	5±0.8
Fusarium	6±0.88	7±0.11	10±0.43	5±0.61	7±0.55	7±0.33
Mucor	5±0.32	7±0.32	6±0.33	4±0.3	9±0.11	5±0.42
Rhizopus	8±0.54	5±0.73	2±0.04	11±0.22	7±0.23	7±0.6
Curvularia	9±0.7	6±0.09	14±0.81	8±0.9	9±0.04	9±1
Alternaria	8±1.1	10±0.5	15±1	7±0.08	11±0.8	8±1.1

Fungal genera	*Eulophia nuda*			*Geodorum densiflorum*		
	PDA	**CMA**	**MEA**	**PDA**	**CMA**	**MEA**
Aspergillus	48±1.2	30±0.08	23±1	37±0.9	25±0.05	20±0.16
Penicillium	30±0.5	21±0.65	15±1.2	26±0.7	20±0.05	15±0.71
Cladosporium	27±0.07	10±0.83	9±0.9	28±0.55	16±1.2	11±0.43
Fusarium	11±1	20±0.91	22±0.83	8±0.85	17±1	12±0.1
Mucor	14±0.46	6±0.11	5±0.55	7±0.82	3±0.6	4±0.31
Rhizopus	5±0.32	4±0.32	1±0.32	8±0.22	7±0.4	3±0.28
Curvularia	16±0.02	11±0.4	19±0.87	10±0.21	15±0.34	13±0.84
Alternaria	10±0.88	15±0.32	15±0.76	9±0.17	11±0.80	12±0.88

Table 1.1: Total tabulated colonies of fungal genera isolated from *E. nuda* and *G. densiflorum* during their growing seasons.

8. RECOMMENDATIONS:

Till now detail works on plant's fungal partners did not explain the exact role in mineralization and plant growth thus further detail study on this regard can improvise the view. As orchid plants depend upon microbial population for their growth periods.

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