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ECTOMYCORRHIZAL FUNGAL ASSOCIATION WITH SOME PLANTATIONS OF ARAKU VALLEY AREA, ANDHRA PRADESH, INDIA

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Ectomycorrhiza is a specialized root organ and is the result of complex interaction with trees belonging to Pinaceae, Fagaceae, Betulaceae and others. In the present investigation the ectomycorrhizal association of *Casuarina equisetifolia* L.*, Dalbergia sissoo* Roxb. ex DC*, Eucalyptus globules* Labill and *Pinus sylvestris* L. plantations located in Araku valley Visakapatnam District have been found associated with *Amanita muscaria* (L.) Lam. *Boletus elatus* Pers. *Laccaria laccata* (Scop.) Cooke. *Pisolithus tinctorius* (Pers.) Coker & Couch. and *Scleroderma verrucosum* (Bull.) Pers. The experiments conducted with *Pisolithus tinctorium* (Pers.) Coker & Couch on the establishment of *Eucalyptus globules* Labill. and *Dalbergia sissoo* Roxb. ex DC. resulted to in higher seedling establishment than control.

Keywords: *Boletus, Dalbergia*, Ectomycorrhiza, Plantations.

Ectomycorrhizae is an association of fungus and feeder roots (root hairs) in which the fungus grows intercellularly in cortical region penetrating the epidermis by secreting proteolytic enzymes and developing extensively outside the root forming a network of hyphae called as 'Fungal sheath' (Harting net) or 'Fungus Mantle' which is of variable thickness and color. Harting net is the distinguishing feature of Ectomycorrhizae and is a hyphal network that extends into the root penetrating between the epidermal and cortical cells of ectomycorrhizal plants. This network is a site of nutrient exchange between the fungus and the host plant (Harley and Smith 1983).

Ectomycorrhizae mostly belongs to Phyla Basidiomycota and Ascomycota. It differs in their ability to associate with different host species. Some fungal species are restricted to specific genera. Some fungi may be able to form ectomycorrhizae with wide range of host species which may be limited in distribution by habitat requirements. It absorbs and stores plant nutrients like nitrogen, phosphorus, potassium and calcium etc in their mantle thereby help in better forest stand, establishment of high yielding forests, land reclamation and establishment of exotic plant species. It benefits host plants by faster growth, increased uptake of essential nutrients such as phosphorus and inorganic nitrogen. It improves tolerance to biotic and abiotic stress, and also to toxic metals than the non- mycorrhizal plants. Ectomycorrhizal association helps plants to overcome different kinds of stress such as soil salinity, alkalinity and acidity. Ectomycorrhizal diversity with important trees of India is still in the exploratory phase.

However more information is available on Ectomycorrhiza associated with various plants but very little information is available on *Casuarina, Dalbergia, Eucalyptus* and *Pinus* (Bagyaraj 2014, Dubey and Ginwal 1997, Elumalai and Raaman, 2009, Itoo and Reshi 2014, Kamal Prasad 2010, Lakhanpal 2000, Manoharachary *et al* 2009, Mohan 2015, Natarajan 2005, Pande *et al* 2004, Sandeep *et al* 2014, Sharma *et al* 2009, 2010).

Further no such studies are made from Eastern Ghats. Therefore an attempt is made to study ectomycorrhizal association with plantations of *Casuarina equisetifolia* L.*, Dalbergia sissoo* Roxb. ex DC.*, Eucalyptus globules* Labill. and *Pinus sylvestris* L. located in Araku valley, Visakapatnam District, Andhra Pradesh, India.

Area of Sample Collection:

Araku Valley is a Hill station in Visakhapatnam

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district located in the state of Andhra Pradesh in India. It is a valley in the Eastern Ghats inhabited by different tribes. Araku is located at 18.3333ºN 82.8667 ºE. It has an average elevation of 911 meters (2,989 ft). It is located 114 km from Visakhapatnam, close to the Odisha state border. The Anantagiri and Sunkarimetta Reserved Forest which are part of Araku Valley are rich in biodiversity. This valley is surrounded by mountains like Galikonda, Raktakonda, Sunkarimetta and Chitamogondi. These hills are endowed with rich quality Bauxite ore. Galikonda hill rising to a height of 5,000 feet (1,500 m) is one of the highest in Andhra Pradesh. The average rainfall is 1700 mm, bulk of which is received during June–October. Plantations of *Casuarina, Dalbergia, Eucalyptus* and *Pinus* are abundant in this region. (Figures 1&2).

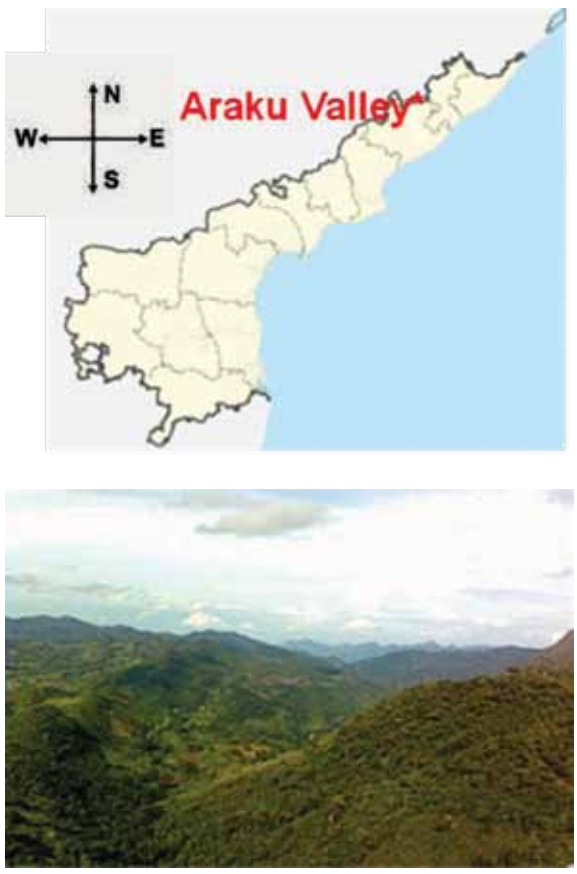


Figure 1 & 2. Study area Map and location.

MATERIALS AND METHODS

Place of collection

Regular surveys were undertaken in plantations such as *Pinus sylvestris* L., *Casuarina equisetifolia*, *Eucalyptus globulus.* and *Dalbergia sissoo* in the Araku Valley areas , Andhra Pradesh. The selected sampling areas were visited at regular intervals during April 2015 to March 2016 and recorded the occurrence and distribution of Ectomycorrhizal (ECM) fungi. The basidiomata samples were collected for pure culture production. ECM were grown on Potato Dextroze Agar and only mycelia growth was observed.

Collection and processing of Ectomycorrhizal (ECM) fungi

In the field, all the sporocarps of ectomycorrhizal fungi viz., *Amanita muscaria* (L.) Lam. *Boletus elatus* Pers. *Laccaria laccata* (Scop.) Cooke. *Pisolithus tinctorius* (Pers.) Coker & Couch. and *Scleroderma verrucosum*. occurring in the vicinity of *Pinus sylvestris*, *Casuarina equisetifolia*, *Eucalyptus globulus.* and *Dalbergia sissoo* species from the sampling sites in the Araku valley areas were collected at periodical intervals. The fruit-bodies were observed at the first sight for their connection with the roots of nearby plants for confirming their mycorrhizal nature. Notes on the general type of habit, habitat, collection number, date of collection and proper hosts were recorded for all the fungi. Morphological features of the sporocarps/fruit bodies such as shape, texture, colour etc were recorded when the fungi were in fresh condition. To provide a clear visual record of the main characteristics of the fungal sporocarps/ fruit bodies, photographs were taken in the field itself. As far as possible, detection of the hyphal connection between sporocarps/ fruit bodies and mycorrhizal roots was made. ECM fungal fruit bodies at their different stages and mycorrhizal roots were collected and kept in paper bags/ cotton cloth bags and transported to the laboratory. Spore prints were prepared

and both macroscopic and microscopic details on the fungi were recorded and identification of the fungi up to species level was made. Fungal specimens were air-dried and preserved (Mohan *et al.* 2008).

Morphological characters

The sporocarps/ fruit bodies were collected and recorded the morphological features from the field. The macroscopic characters like colour, size and shape of the pileus, gills and or tubes, stipe, attachment of gills, presence or absence of stipe, annulus and volva, presence or absence of rhizomorphs, etc. were recorded in the field.

Preservation of fruit bodies

Attempt was made to isolate the axenic cultures from fresh fruit bodies of different ECM fungi and then the specimens were air dried using an electric hot air oven. The dried specimens were preserved in polythene covers containing naphthalene balls to prevent from pests and mites attack and brought to the laboratory for further microscopical analysis for proper identification based on ectomycorrhizal morphotypes by Agerer (2002), Ingleby *et al.* (1990), Goodman *et al. (*1996) Seedling establishment study was made as per the methodology of Paul (1994), Marx (1980) and Mukerji *et al.* (2002).

Air dried *Pisolithus tinctorius*. washed with sterilized water many times and has been roughly grinded and mixed with sterile soil: sand (1:1) and seedlings of *Dalbergia sissoo* and *Eucalyptus globulus* were planted. Number of seedlings used per pot being 3 and total number of seedlings planted in different pots being 100 Percentage seedling establishment was calculated as per formula:

$$\%\text{Seeding established} = \frac{\text{No. of seedlings established}}{\text{No. of seedlings planted}} \times 100$$

Similar numbers of controls were maintained without inoculants. The fungal inoculum used being 10 gm for 100 gm of soil:sand (1:1). Seedling growth was also recorded in treated plants over control.

RESULTS AND DISCUSSION

In the present investigation the ectomycorrhizal association of *Pinus sylvestris, Casuarina equisetifolia, Eucalyptus globulus* and

Figure-3: *Amanita muscaria* (L.) Lam. 4. *Boletus elatus* Pers. 5. *Laccaria laccata* (Scop.) Cooke. 6. *Scleroderma verrucosum* (Bull.) Pers. 7. *Pisolithus tinctorius* (Pers.) Coker & Couch.

Dalbergia sissoo Plantations located in Araku valley have been investigated. These have been found associated with *Amanita muscaria, Boletus elatus Laccaria laccata. Pisolithus tinctorius* and *Scleroderma verrucosum* (Table 1, Figure 3-7). Similar results were obtained by Elumalai and Raaman, (2009), Itoo and Reshi (2014), Nagaraj *et al.* (2015), Malajczuk N. (1982), Natarajan (1987), Sharma *et al.* (2010), Zhenga 2014.

Table:1- Host plant and Ectomycorrhizal Association in study area.

Sl. No.	Name of the Host Plants	*Amanita muscaria* (L.) Lam.	*Boletus elatus* Pers.	*Laccaria laccata* (Scop.) Cooke	*Pisolithus tinctorius* (Pers.) Coker & Couch	*Scleroderma verrucosum* (Bull.) Pers.
1	*Pinus sylvestris* L.	+	+	+	+	+
2	*Casuarina equisetifolia* L.	+	–	–	+	+
3	*Dalbergia sissoo* Roxb. ex DC	+	+	+	+	+
4	*Eucalyptus globulus* Labill	–	+	–	+	+

+ =Present, — = Absent.

Interestingly *Pisolithus tinctorius* has been found abundantly associated with *Eucalyptus globulus* and *Dalbergia sissoo* plantations.

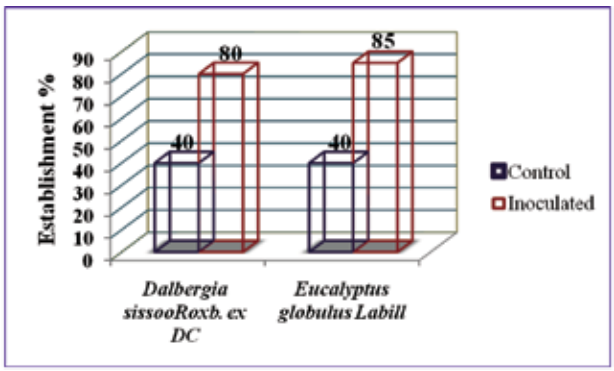
The experiments conducted with *Pisolithus tinctorius* on the establishment of *Eucalyptus globulus* and *Dalbergia sissoo* seedlings showed positive correlation. Significant differences are noted in the growth between the inoculated and control seedlings. Better seedling establishment to an extent of 80% was noted in treated over control (with no fungus) which showed around 40% establishment only (Fig. 8). Sharma *et al*. (2008) and Arenla and Ajungla (2014) have obtained similar results with reference to *Dendro calamus* sp. and Muthukumar & Udaiyan (2010) on *Casuarina equisetifolia* L. Wani and Wani (2006), Zhenga *et al.* (2014) on *Pinus sylvestris* L.

Seedling growth experimental results are presented in Table-2. From the data it is clear that root- length, shoot length and primary leaf length have increased in treated over control. Data is obtained after a period of 3 months.

Table-2. Growth of the seedlings inoculated with *Pisolithus tinctorius* on *Eucalyptus globulus* and *Dalbergia sissoo, after 3 Months.*

Growth Parameters	*Eucalyptus globulus*		*Dalbergia sissoo*	
	Control (cm)	Inoculated (cm)	Control (cm)	Inoculated (cm)
Root length	8.6	9.2	7.9	9.4
Shoot height	18.9	26	15.6	24.8
Leaf length	9.1	9.6	8.6	10.7

Figure-8: Seedling establishment ratio in *Dalbergia sissoo* and *Eucalyptus globules* with Association of *Pisolithus tinctorius.*



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