

Influence of different Arbuscular mycorrhizal fungi (AMF) as growth , biomass yield and N and P uptake of *Syzygium cumini* (L.) Skeels.

S.S. Kamble¹,

H.C Lakshman²

P.G. Department of studies in Botany, Shivaji University,
Kolhapur – 416004, Karnataka, INDIA.

Abstract:

AM fungal spores distribution and percent of colonization was studied in 12 places of Dharwad district. Physicochemical character of the soil showed with low phosphorus content. The distribution of different AM fungal spores from the rhizosphere of *Syzygium cumini* showed most dominated AM fungal spores. Green house pot experiments were undertaken on *Syzygium cumini* with the inoculation of different AM fungi. The result revealed variedly, the percent of colonization clearly promotes the establishment of below ground. The different AM fungi influences with moderately higher root colonization and spore number which are correlated each other. *Glomus bagyarajii*, *Glomus geosporum*, *Glomus mosse*, *Rhizophagus fasciculatus*, *Sclerocystis calospora*, *Sclerocystis clavispora*, *Scutellospora calospora* and *Scutellospora nigra* etc. The growth response of *Syzygium cumini* showed significantly improved plant height, root length, stem diameter, leaf area and plant biomass. *Scutellospora erythropha* was most influenced AM fungi on *Syzygium cumini* and it is followed by *Glomus bagyarajii*, *Rhizophagus fasciculatus* respectively. The percent of root colonization, spore number, N and P content was higher among mycorrhizae inoculated plant compare to non-inoculated (control plant). It was apparent that this symbiotic association become integral component of the plants. Communities in both natural and agricultural ecosystem and improved mineral nutrition.

Key words: Arbuscular mycorrhizal fungi (AMF), *Syzygium cumini*, *Sclerocystis calospora*, *Glomus bagyarajii*, *Glomus geosporum*, *Rhizophagus fasciculatus*, percent colonization, plant biomass.

Introduction:

Arbuscular mycorrhizal fungi (AMF) represents an important by their ubiquity in the soil microbial biomass and their direct involvement in essential process at the plant-soil interface (Harley and Smith, 1983; Bagyaraj, 1996, Lakshman, 2005, 2009). Interest in these associations is mainly because of the manifold benefits conferred on the host by the fungus. They are sown to improve the nutritional status of plants and growth and development, protect plants against root pathogens and confer resistance to drought and soil salinity conditions (Bagyaraj and Derma, 1995). The extent of root colonization varies with several soil and climatic factors apart from the

host involved. However, these fungi show a preferential colonization to hosts, and thereby, the extent to which a host is benefited depends on the fungal species involved in the symbiosis (Abbott and Robson, 1985; Miller et al., 1987). The existence of inter and intraspecific variations among the plant species involved in relation to their phosphorus requirement and the ability of the host to translocate the native oil phosphorus further determines efficacy of these fungi (Koide, 1991; Lakshman, 1996; Lakshman, 2012, 2015). Though AM fungi are not host specific, recent studies have indicated host preference for AM endophytes (Hetrick, 1984; Sreenivas and Rajashekhara, 1989; Srinivasa and Gurumurthy, 1997). Thus, it is essential to screen for an efficient AM fungus for a particular host in order to harness the maximum benefit from the fungus. Furthermore, since AM fungi cannot be grown on laboratory media, production of a large quantity of the inoculum is difficult as is the inoculation of the soil under field conditions. Nevertheless, since most of the commercially important crops are raised under nursery conditions before being transplanted to the main field. Arbuscular mycorrhiza fungal association was observed in all the examined plants. Vesicles, Arbuscules and extrametrical spore's Characteristic of AM fungi were observed.

Materials and methods:

Field survey was conducted in Dharwad district in Karnataka where *Syzygium cumini* is growing. Dharwad district is situated in the Western sector of the northern half of Karnataka State. The District encompasses an area of 4263 km² lying between the latitudinal parallels of 15°02' and 15°51' North and longitudes of 73°43' and 75°03' East. The region The District lays approximately about 800 mts above the sea level, it has moderate and healthy climate. The District may be divided into 3 natural regions, viz., the Mainad, Semi-Malnad and Maidan. These regions, on an average, receive moderate to heavy rainfall and have dense vegetation. The selected 12 places were of different locations.

Roots and rhizospheric soil samples were collected from the growing regions of *Syzygium cumini* (L.) Skeels growing region of Dharwad district. Roots and soil samples were packed in clean polyethylene bags with labeling. The physico chemical properties of soil such as nature, soil type, pH, organic carbon and available phosphorus of the soil have been determined following the procedure of Jackson (1973) (Table 1). Recovery of AM fungal spores from the rhizospheric soil sample, in the present study was used by adopting wet sieving and decanting technique (Gerdemann and Nicolson, 1963). The cleaned roots were transferred into 10% KOH solution and heated at 90°C degree for one hour and the time period was adjusted according to root bit delicacy. 10% of KOH was poured off and roots were rinsed with tap water. Root bits were taken out and acidified by placing in 2% HCl solution washed with distilled water, stained in 0.05 trypan blue in lactophenol according to the procedure (Phillips and Hayman, 1970). The percent of root colonization was calculated by the formula as proposed by (Giovannetti and Mosse, 1980).

$$\text{Percentage of root colonization} = \frac{\text{Total number of infected roots}}{\text{total number of roots}} \times 100$$

AM fungi were identified based on the keys proposed (Walker, 1983; Schenck and Perz, 1990).

Mycorrhizal analysis:

AM Fungal propagules were isolated from the rhizosphere soils collected from nurseries and plantations site. Isolation, quantification and root colonization of AM Fungi was done by using wet sieving and decanting technique (Gerdmann & Nicolson, 1963). The quantitative estimation of AM spores was done by modified method of Adholeya and Gour (1994). To study the colonization of Arbuscular Mycorrhizae, the rapid clearing and staining method by Philips and Hayman (1970) was employed. AM fungi were identified by using (Trappe 1982; Walker, 1983; Schenck and Perz, 1980).

Syzygium cumini (L.) Skeels is an ever green tropical tree belongs to the family Myrtaceae commonly called Jambula or Malabar plum. Wine and vinegar prepared from its fruits, good source of vitamin A and C. Its leaves and bark are used for controlling blood pressure and gingivitis. The fruit has a combination of sweet, mildly Sour and astringent flavour, grinded seeds powder is used to control diabetes. Its foliage provides shade and is grown just for its ornamental value. Bioinoculant studies on this plant is very meagre. Therefore, studies were undertaken at field and green house conditions.

Indian sandal wood small tropical tree. The plant is being cultivated and traded for many years. Plant wood and oil have high demand and is an important trade item in India. Its wood is used on construction material for temples. The sandal wood has a specified girth and it is used in the preparation material of soap, cosmetics and pharmaceutical industry. As literature members of the family Santalaceae lack Arbuscular mycorrhizal (AM) association. Therefore, his study was undertaken to evaluate the AM fungal dependency on this plant in the University campus.

Results:

Physico-chemical characteristic of the soil in 12 places have depicted with low phosphorus (Table 1). The percent of AM fungal colonization clearly promotes the establishment of below ground. Arbuscular mycorrhizal (AM) fungi associates with *Syzygium cumini* with neighbouring plant roots linked and enhance the nutrient absorption. And the AM fungal dependency was quite higher with spore number (Table 2). The results of the study revealed the AM fungi was recorded higher that reflects the mycorrhization was conducive in the members of Myrtaceae. There were 39 AM fungal spores were isolated and identified. The most dominated AM fungal spores were,

Glomus geosporum, *Glomus bagyarajii*, *Rhizophagus fasciculatus*, *Sclerocystis calospora*,

Sclerocystis clavispora, *Scutellospora calospora* and *Scutellospora nigra* etc. Recovered AM fungal spore number at different location range from (144-309) per 50g.soil. whereas the percent root colonization of *Syzygium cumini* was noted down. Similarly the AM fungal dependency was from (48.6-97.1) was observed.

The distribution of AM fungal spores in twelve places of dharwad district, showed Dasankoppa had (360/50gsoil) higher number of spores, it was followed by Khundagol (312/50gsoil) and Mansure (217/50gsoil) respectively (Table 3).

Growth response of *Syzygium cumini* showed considerable improvement over the noninoculated (control plants). The increased plant height, root length, stem diameter, leaf area, short and root dry weight was significantly influenced by the inoculation of AM fungi, especially *Scutellospora erythropha*, followed by *Glomus bagyarajii* and *Rhizophagus fasciculatus* respectively (Table 4). Similarly, the present of root colonization, spore number, number of leaves, nitrogen and Phosphorus content among mycorrhizae inoculated plants were more compare to non-inoculated plants (Table 5). In the present study, optimum number of spores of AM fungi was isolated. However, there was a significant correlation between percent root colonization and spore number.

Discussion:

The mycorrhizal association with many tropical plants have been reported by (Brundrett *et al.*, 1996; Lakshman, 2003; Khade *et al.*, 2008; Wadder *et al.*, 2013). It is known that many tree species are highly dependent on AM fungal association (Janos, 1989; Lakshman, 1996; Onguene and Kuyper, 2001). In the present investigation, AM fungal dependency and percent of root colonization in *Syzygium cumini* had moderate to significantly higher colonization and spore number was recorded. It is apparent that these symbiotic soil fungi have become an integral component of plant communities in both natural and agricultural ecosystems. The symbiosis confers numerous benefits to host plants including improved plant growth and mineral nutrition.(Lakshman,1996; Borowicz, 2001). Green house experiments documented (Table 4-5) indicate that identity of the efficient indigenous AM fungal inoculation is extremely for determining the cultivation of *Syzygium cumini* at seedling stage (Cherdhai phosri *et al.*, 2010). Our study showed improved growth of *Syzygium cumini* seedlings in response AM fungi inoculation with indigenous AM fungi, the most influenced Am fungi *Scutellospora erythropha* followed by *Glomus bagyarajii* and *Rhizophagus fasciculatus* second and third AM fungal strains. Plant height, root length, number of leaves, leaf area, biomass yield, percent root colonization, spore number, N and P content inoculated plants over the non-inoculated plants. The increase in mycelium increases root surface area so the efficiency of the root is increased. This was observed in the roots of AM fungi inoculated seedlings, there by increasing the efficiency of roots in absorption of minerals and water, hormone production, nitrogen production, phosphorus uptake and resistance to disease (Gianinazzi and Gianinazzi, 1983; Mosse, 1991; Bagyarajii, 2006).

Arbuscular mycorrhizal fungi augment root system surface are and increases its absorption efficiency and there by AM fungi inoculation improved growth of forest tree seedling in species such

as *Artrocarpus heterophyllus* and *Terminalia bellerica* (Lakshman, 1996), *Tectona grandis* (Durga and Gupta, 1995), *Dalbergia sissoo* (Singh *et al.*, 1998), *Azardichata indica* (Suman and Bergyaraj, 2003), *Santalum album* (Binu *et al.*, 2015). The beneficial effect of AM Fungal colonization and growth may have resulted in improved growth of *Syzygium cumini* seedlings. Positive relation between growth parameters and Am fungi colonization by *Scutellospora erythropha*, *Glomus bagyarajii* and *Rhizophagus fasciculatus* colonization and AM fungal spore number may be related to these reasons. Arbuscular mycorrhizal colonization on root length is strictly linked to the amount of sunlight, which in turn will decide the production of photosynthate by the plant resulting in increased carbon allocation in to the root system which stimulates AM fungal colonization (Fitter, 1985; Aboot and Gazey, 1994; Smith and Read, 1997; Laakshman, 2008). However, it can be concluded, further research to clarify the basis physiology of this symbiotic relationship in tress is desirable to realize the full potention application of AM fungi in agro-forestry.

Table 1. Physico-chemical characteristics of soils of *Syzygium* growing places of Dharwad district.

Sl.No	Characteristics (%)	Sandy loam soil
1.	pH	6.8
2.	Soil moisture	27.03
3.	Organic Matter	0.96
4.	E.C. mm h _o /cm ²	0.97
5.	N	1.52
6.	P	0.27
7.	K	2.43
8.	Zn	20.2
9.	Cu	1.05
10.	Mg	1.41
11.	Pb	0.71

Elemental Concentration is in mg/kg soil. Each value is the mean of 12 samples.

Table 2. Showing the percent root colonization and spore number screened in *Syzygium cumini* growing sites of Dharwad district.

Places	Root colonization	Spore/50g soil	Am fungal dependency (%)
A	52.7	144	48.6
B	49.4	3.7	41.3
C	71.3	212	56.7
D	54.2	47	47.1
E	69.1	218	37.4
F	56.4	211	59.3
G	68.8	207	76.2
H	77.4	198	81.1
I	66.3	27	61.5
J	53.5	232	78.4
K	68.6	309	51.6
L	76.4	221	64.2

Table 3. The distribution of different AM fungal spores genera of rhizospheric soil of *Syzgium cumini* at twelve different location in Dharwad district.

Sl.No	AM fungal spores						
	<i>Glomus bagyarajii</i>	<i>Glomus geosporum</i>	<i>Glomus mosseae</i>	<i>Rhizophagus fasciculatus</i>	<i>Sclerocystis clavispora</i>	<i>Scutellospora erythropha</i>	<i>Scutellospora nigra</i>
A.	+	+	+	+	-	+	+
B.	+	-	+	-	-	+	-
C.	+	+	+	+	+	+	+
D.	-	+	+	+	+	-	+
E.	+	+	-	-	-	-	-
F.	-	+	-	-	-	+	+
G.	+	+	+	+	+	+	-
H.	-	-	+	-	+	+	+
I.	+	+	+	-	-	+	-
J.	-	+	+	+	+	+	+
K.	-	+	+	-	+	+	-
L.	+	+	+	+	-	+	+

Note: + : present; - : Absent.

A: Dharwad; B: Unakal; C: Navalore; D: Hubli; E: Adaragunchi; F: Sherwad; G-T: Yamanur; I-G: Noolvi ; J-u: Ettinagudda; K- Dasankoppa; L- Mansuru; K- Khundagol; L- Manvi.

Table 4. Showing the effect of different AM fungi on plant height, root length, biomass yield in *Syzgium cumini* for 80 days.

Sl.No	Different AM fungi	Plant height(cm)	Root length (cm)	Stem diameter (cm)	Leaf area (Sq.cm)	Shoot dry weight (g/plant)	Root dry weight (g/plant)
1.	<i>Acaulospora laevis</i>	57.3e	36.2b	0.59a	18.21d	29.5e	2.2c
2.	<i>Glomus leptonicum</i>	55.1d	34.0g	0.56c	198.0b	28.3d	2.10ab
3.	<i>Gl.macrocarpum</i>	58.2e	37.3b	0.60b	201d	29.4c	2.4d
4.	<i>Gl. mosseae</i>	54.3c	39.1c	0.64d	204d	27.4b	2.3ab
5.	<i>Gl.bagyarajia</i>	63.4a	4.2c	0.89d	2.7.5e	31.4ab	3.2b
6.	<i>Gl. calospora</i>	56.4g	38.4g	0.73d	202e	27.7c	2.06d
7.	<i>Gl.margarita</i>	53.5e	37.5d	0.77ab	204c	26.5b	2.4a
8.	<i>Rhizophagus fasciculatus</i>	62.5b	40.0e	0.90c	189.0d	31.6c	3.1c
9.	<i>Sclerocystis dussi</i>	54.0g	34.1d	0.74e	192.3b	27.1c	2.7b
10.	<i>Scutellaria erythropha</i>	67.1a	42.3b	1.00d	214.0e	32.2a	3.30b
11.	<i>Scutellaria nigra</i>	52.4g	35.0e	0.72d	197.0ab	26.1c	2.8d
12.	O control	27.3a	19.5a	0.34b	121.0b	11.5a	0.76a

Table 5. Showing the effect of different AM fungi as spore number, percent root colonization, number of leaves, Nitrogen and Phosphorus content in *Syzigium cumini* at 180 days.

Sl.No	Different AM fungi	Spore number in 50g/soil	% Root colonization	Number of leaves /plant	% N content	% P content
1.	<i>Acaulospora laevis</i>	--	--	13.1a	1.82a	0.05c
2.	<i>Glomus leptonicum</i>	194	71.5b	28.3b	2.12a	0.27d
3.	<i>Gl.macrocarpum</i>	258	68.2c	27.5c	2.42b	0.25bc
4.	<i>Gl. mosseae</i>	271	92.1d	126.5b	2.36e	0.32d
5.	<i>Gl.bagyarajii</i>	314	87.4e	32.1ab	3.14g	0.25g
6.	<i>Gl. calospora</i>	203	59.2g	29.4d	2.12c	0.29g
7.	<i>Gl.margarita</i>	215	74.2b	34.3e	3.17ab	0.29a
8.	<i>Rhizophagus fasciculatus</i>	312	78.1c	31.0c	2.79b	0.31c
9.	<i>Sclerocystis dussi</i>	179	66.4a	29.0g	2.82c	0.24b
10.	<i>Scutellaria erythropha</i>	317	92.3b	34.0d	3.16e	0.44c
11.	<i>Scutellaria nigra</i>	288	63.7ab	26.2e	2.11d	0.24b

References:

Abbott LK, Gazey C. 1994. An ecological view of the formation of AM mycorrhizas. *Plant and Soil*. 159:69-78.

Brundrett M, Bougher N, Grove T, Malajczuuk N. 1996. Working with mycorrhizas in forestry and agriculture. ACIAR. Canderra. 374 pp.

Binu, NK. Ashokan, PK and Balasundran, M. 2015. Influence of different AM fungi and shade on growth of Sandal (*Santalum album* L.) seedlings. I. *Trop.Forest.Sci*. 27(2):158-165.

Cherdchaiphosri, Alia Rodriguez, Ian R. Sanders and Peter Jeffries. 2010. The role of mycorrhizas in more sustainable oil palm cultivation. *Agr. Ecosy and Enviorn*. 135: 185-193.

Mosse, B. 1991. Arbuscular mycorrhizal fungal research prospects for practical utilization. *Mycorrhiza News*. 3(1):1-4.

Smith, SE and Read, DJ. 1997. *Mycorrhizal Symbiosis*. Academic press. London, 605pp.

Roopa, KJ and Lakshman, HC. 2008. Screening of AM fungi on rhizosphere soil of *Carthamus tinctorius* L. (Safflower). *N.J.Life.Sci*.5(1):35-38.

Lakshman, HC. 2008. VAM fungal diversity of forest tree species industry deciduous forest of Dharwad. In : *Forest Biodiversity*. Vol II. Eds. M. Muthuchelian, S.Kannaiyan, A. Gopalan. Pp 223-230.

Lakshman, HC.1996, VA-mycorrhiza studies in some important timber tree species. Ph.D thesis. Karnataka university. Dharwad. India.349 pp.

Lakshman, HC, Inchal RF, Mulla, FI. 2003. VA mycorrhizal association and its importance in tree species. I. *J. Eco. Plan*. 178:213-237.

Krishna H. Waddar and Lakshman, H. C. 2010. Effect of AM Fungi on the Forest tree seedlings of *Tamarindus indica* L. and *Azadirachta indica* Juss. For Integrated Nursery Stock. *Inter. J. of Plant Protection*. 3(2): 248-252.

Waddar, K.H., Lakshman, H.C. and Sandeepkumar, K. 2013. Diversity of arbuscular mycorrhizal fungi on four important tree species in Dharwad district of Karnataka. In: *Perspectives of plant Biodiversity*. Ed. K.Muthuchelian. Daya Publishing house. New Delhi. Pp: 224-229.