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A preliminary study on the arbuscular mycorrhizal fungal (AMF) association in the the rhizosphere soils of the *Calamus* species of Pattighat Reserve Forest, Sampaje, Kodagu district of Karnataka

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Abstract
Mycorrhiza is found to play a vital role in the growth of many plant species and can also be used as one of the tools for crop improvement. The present work was undertaken to study Arbuscular Mycorrhizal (AM) association in three rattan species namely *Calamus thwaitesii* Becc. & Hook.f., *Calamus nagabettai* Fernandez & Dey and *Calamus prasinus* Lakshmana & Renuka of Pattighat Reserve Forest, Sampaje, Kodagu district of Karnataka, India. A total of 40 species of AM fungi belonging to five genera namely *Acaulospora, Entrophospora, Gigaspora, Glomus* and *Scutellospora* were isolated from the rhizopshere soils of rattans.

Keywords: AM fungi, calamus, rhizopshere, root colonization, vesicular colonization

1. Introduction
Rattans is one of the important forest products forms an integral part of rural and tribal population of many of the tropical countries, particularly of the Asian and African territory. Rattans is a general term used for the spiny climbing palms. Rattans are belonging to the subfamily Calomoideae of the family Arecaceae. There are 13 genera of rattans and 600 species (Uhl & Dransfield, 1987; Raj & Bisht, 2014) [14]. South-East Asia is having the largest number of rattan genera and species. Among the 13 genera of rattans, *Calamus* is the largest genus with about 370 species (Renuka, 1991) [16]. The woody stem of rattans provides the traditional raw material for furniture and novelty items.
There are about 60 species of rattans are found in India belonging to four genera, viz., *Calamus, Daemonorops, Korthalsia* and *Plectocomia*. They are mainly occurring in three major geographic regions, such as the Western Ghats, Sub-Himalayan hills and valleys of eastern and northeastern India and Andaman & Nicobar Islands. Among the Indian Palms, more than 50% are rattans (Basu, 1992) [2].
Among the Indian rattans, 25 species are considered to be endemic (Lakshmana, 1993) [21]. Evergreen forests of the Western Ghats form the largest natural home of rattans in south India. This includes the only one genus of rattan i.e., *Calamus*. There are 19 species of *Calamus* that have been reported from the Western Ghats. Of these, 17 species are endemic to these regions.
Among the states of the Western Ghats jurisdiction, Karnataka is having a maximum number of rattan (*Calamus*) species, i.e., 13 species. The five species of *Calamus* are endemic to the Western Ghats region of Karnataka. They are *Calmus karnatakensis, Calmus lacciferus, Calmus lakshmanae, Calmus prasinus*, and *Calmus stoloniferus* (Lakshmana, 1993) [7]. In Karnataka, the *Calamus* species are distributed in the districts such as Kodagu, Dakshina Kannada, Chikmagalur, Hassan and Uttara Kannada. The maximum number species of rattans are found in Kodagu District (Lakshmana, 1993) [21].

Mycorrhizae
There's an increasing awareness among biologists, microbiologists and ecologists about mutualistic interactions of organisms that play a key role in natural communities. One such example is the mycorrhizal symbiosis formed between soil fungi and approximately 90% of all land plant species. Basically, seven types of mycorrhizae are recognized, namely Endomycorrhizae (Arbuscular mycorrhizae), Ectomycorrhizae, Ectendomycorrhizae, Arbutoid mycorrhizae, Monotropoid mycorrhizae, Ericoid mycorrhizae and Orchidaceous mycorrhizae (Barman *et al* 2016) [1]. The Endo and Ecto types are widespread and most abundant types of mycorrhizae.
Mycorrhiza is found to play a vital role in the growth of many plant species and can also be used as one of the methods for crop improvement. Gong *et al* (1994 & 1997) [5] studied the VA mycorrhizae in the rhizosphere soils of four rattan species (*Daemonorops margaritae, Calamus simplicifolius, C. tetradactylus, and C. tetradactyloides*). Zakaria (1991) [27] did the preliminary studies on growth dependency of in vitro micro propagated *Calamus manan* on VA mycorrhiza (VAM) prior to transplanting to the field.
The present study was undertaken to know the endo-mycorrhizal association in three species of *Calamus* namely viz., *Calamus thwaitesii, Calamus nagabettaiI* and *Calamus prasinus* of Western Ghats region of Karnataka.

2. Materials and Methods
The location selected for the study was Pattighat Reserve forest, Sampaje, Kodagu district in the Western Ghats of Karnataka. The location is confined between 12º 27' 08" to 12º 25' 77" N latitude and 075º 38' 63" to 075º 41' 83" E longitude, situated at an elevation of 980m, about 120 km from Mangalore in Kodagu District of Karnataka.

For the study of Arbuscular mycorrhizal association and Physico-chemical parameters, the following rattan (*Calamus*) species were selected. They are

1. *Calamus thwaitesii* Becc. & Hook.f.: It's a high climbing and robust cane with clustering stem. The fully grown cane may reach 20 m or more in length, the girth of the stem is about 18 cm with sheath and without sheath is about10 cm.

Distribution: In all most all parts of Western Ghats especially in moist deciduous, semi-evergreen forests of Goa, Karnataka, Kerala and Tamil Nadu.

2. *Calamus nagabettai* Fernandez & Dey: A climbing cane with clustering stems, reaching up to 25 m or more; clumps with 10-15 rhizomes; stems with leaf sheath up to 12-16 cm girth, without sheath up to 10-12 cm girth.

Distribution: Found in evergreen forests of Kodagu and Dakshina Kannada districts of Karnataka. Endemic to Western Ghats (Lakshmana, 1993) [7].

3. *Calamus prasinus* Lakshmana & Renuka: A high climbing cane, the stem is solitary with sheaths up to 9 cm girth, without sheath up to 3 cm.

Distribution: Usually found in evergreen forests of Mangalore and Sampaje divisions of Karnataka. Endemic to Western Ghats (Lakshmana, 1993) [21].

The rhizosphere soil samples of each of *Calamus* species were collected after removing one to two cm topsoil from a depth of 10-15 cm close to the plant. The collected samples were air-dried, labelled and stored at 4ºC. Non-rhizosphere soil samples were collected and processed in the same manner. The soil temperature was measured by using soil thermometer in the field itself.

The young growing roots were collected from different plants of each species along with the soil samples. About 1 to 2 grams (fresh weight) of root samples were fixed in small plastic vials containing FAA (Formalin-Acetic-Alcohol).

The wet sieving and decanting method developed by Gerdemann and Nicolson, 1963 [3] was followed for isolating the Arbuscular Mycorrhizal spores. The spores were identified following key of Schenck and Perez (1990) [20]. The method developed by Phillips & Hayman (1970) [12] was used for processing root samples and for colonization assessment was done according to the method of Giovannetti and Mosse, 1980 [4].

3. Results and Discussion

A total of 40 AM species have been isolated from the rhizosphere soils of three rattan species (Appendix 1). All the three rattan species (*Calamus nagabettai*, *Calamus nagabettai* and *Calamus prasinus*) showed mycorhizal association but the number of AM species varied from species to species. In the present study maximum number of AM species were recorded from the rhizosphere of *Calamus nagabettai* with 26 species followed by *Calamus thwaitesii* with 23; *Calamus prasinus* with 19 species and least number of species (10) were isolated from Non-rhizosphere soil (Fig. 1).

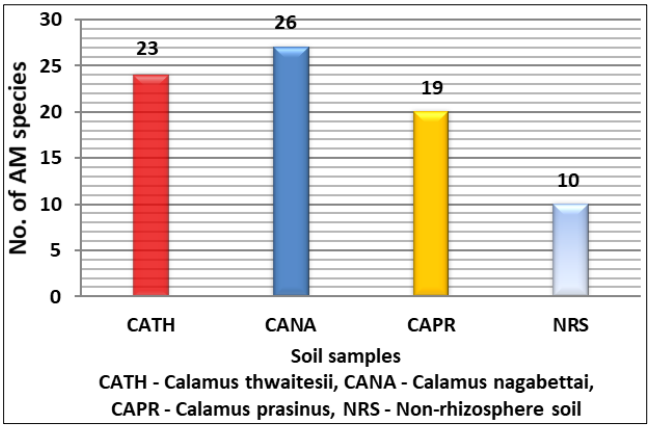

Fig 1: Total number of AM species recorded from rhizosphere and Non-rhizosphere soils

The recorded AM species are belonging to six genera namely *Acaulospora, Entrophospora, Gigaspora, Glomus* and *Scutellospora.* The dominant genus is *Glomus* with 25 species (63% of total species recorded) followed by *Acaulospora*, *Gigaspora*, *Scutellospora* and *Entrophopora* with 9, 3, 2, 1 species respectively (Fig. 2).

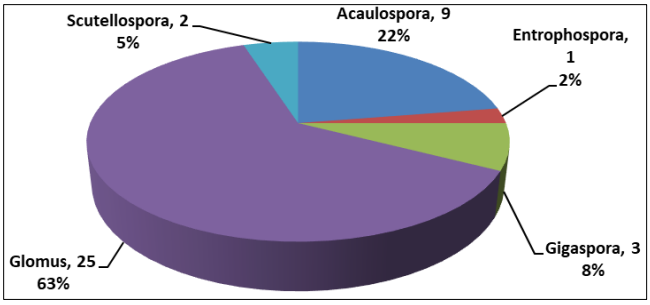

Fig 2: Generic level of Total AM species distribution in rhizosphere and non-rhizosphere soils

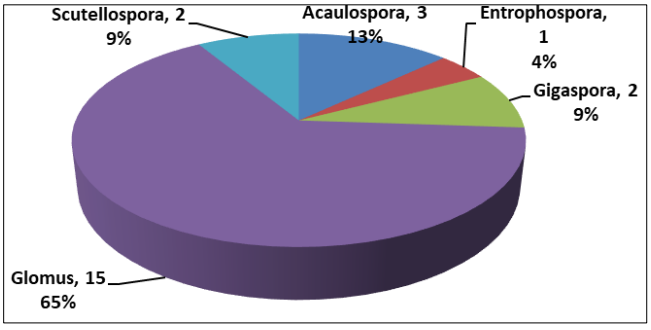

Fig 2a: Generic level of AM species distribution in rhizosphere of *Calamus thwaitesii*

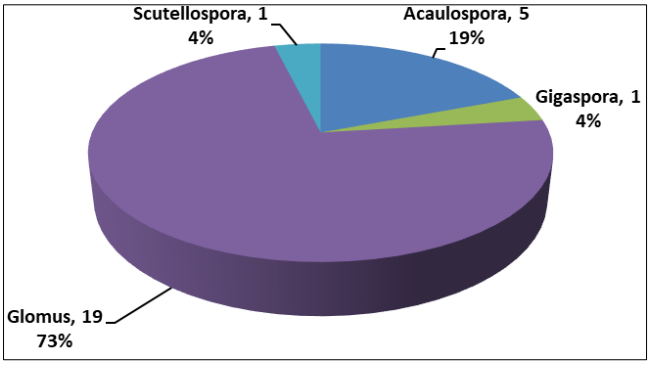

Fig 2b: Generic level of AM species distribution in rhizosphere of *Calamus nagabettai*

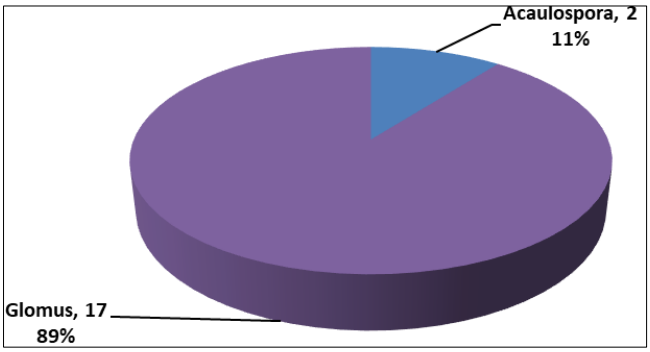

Fig 2c: Generic level of AM species distribution in rhizosphere of *Calamus prasinus*

In the study, the genus *Glomus* was found to be dominant in all the three rattan species (Figure 2). Similar results were obtained by Sastry and Johri, 1999 [19]; Rodrigues and Jaiswal, 2001 [18]; Rani and Manoharachary, 1994 [15]; Prasad, 1999 [13]; Nagabhushanam *et al*., 1999 [10]; Uniyal and Uniyal, 2000 [25]; Shwetha and Lakshamana (2011) [10], Marati and Devadiga (2018) [8].

The isolated AM fungal (AMF) species were in the following order:

Glomus→Acaulopsora→Gigaspora→Scutellospora→Entrophos phora (25 9 3 2 1).

Same sequential series was documented by the earlier workers also.

Nagabhsushanam *et al*, (1999) isolated AM fungi associated with some common legume trees in the order: Glomus → Acaulospora → Gigaspora → Scutellospora → Entrophospora → Sclerocystis. Rodrigues (2006) isolated AM fungi from the iron ore mine wastelands in Goa in the order: Glomus → Acaulospora → Gigaspora → Scutellospora. Marati and Devadiga (2018) [8] isolated AM fungi from the rhizosphere soils of three rattan species in Charmady Ghats of Karnataka in the order: Glomus → Acaulospora → Sclerocystis.

Glomus species produce more spores than other AM species (Suresh & Nagaraj, 2010) [11] also Glomus and Acaulospora species require less time to produce spores, the other AMF like Gigaspora and Scutellospora species develop extensive mycelium but produce fewer spores (Wand and Jiang, 2015) [26].

The spore density (SD) ranged between 10 and 41 spores/10g of soil. The maximum SD (41 spores/10g of soil) was recorded from the rhizosphere of *Calamus nagabettai* and minimum (10 spores/10g of soil) was recorded from the non-rhizosphere soils. Similar results were obtained by other workers also. Shwetha and Lakshamana (2011) [14] recorded 11 to 36 spores/10g of soil in the members of Amaranthacaeae. Thapa, T. *et al* (2015) [23] reported spores number ranged from 53 to 197 per 100 g (= 5.3 to 19.7 spores/10g) of rhizospheric soil in some of the medicinal plants. Marati and Devadiga (2018) [8] recorded 51 spores/10g of soil in the rhizosphere of *Calamus nagabettai* from Charmady Ghats region of Karnataka. Muthukumar and Udaiyan (2001) reported the spore density ranging from 12 to 72 spores/10g soil in the medicinal plants of Western Ghats.

The AM fungal infections (Hyphal colonization, Arbuscular colonization & Vesicular colonization) varied among the rattan species. The Hyphal colonization (HC) varied from 86.54% and 93.08%. The maximum HC (93.08%) was recorded from the rhizosphere of *Calamus nagabettai* and minimum (86.54%) from the rhizosphere of *Calamus thwaitesii*. The arbuscular colonization (AC) ranged between 32.00% and 47.09%. The maximum AC (47.09%) was recorded from the rhizosphere of *Calamus nagabettai* and minimum AC (32.00%) from the rhizosphere of *Calamus thwaitesii*. The vesicular colonization (VC) ranged between 39.10% and 57.50%. The maximum VC (57.50%) was recorded from the rhizosphere of *Calamus thwaitesii* and minimum from the rhizosphere of *Calamus prasinus*. Similar results were obtained by Marati and Devadiga (2018) [8] in the investigation of AM fungal association in three rattan species from the Charmady Ghats in Karnataka. All three rattan species showed AM fungal infection with varying degree of infection. Thapar *et al*. (1992) [24] also recorded the varying degree of infection among different plants within a family. Nagabhushanam *et al*. (1999) [10] investigated the association of VAM fungi with 40 legume trees growing in the Godavari belt. All the trees showed VAM infection with varying incidence.

4. Conclusion

The rattan species namely *Calamus thwaitesii*, *Calamus nagabettai* and *Calamus prasinus* showed AM fungal infection. The degree of infection varied from species to species. The spore density, hyphal colonization, arbusucular colonization and vesicular colonization varied among the three species of rattans. Though the spore density was minimum compared to other species; maximum spore density was recorded in *Calamus nagabettai*. Highest arbuscular colonization was recorded in the roots of *Calamus nagabettai*. Glomus was found to be dominant in the rhizosphere soils of all the three species of rattans. Hence, the dominant species of Glomus can be isolated, mass multiplied and are used for the crop improvement in the future work.

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