Investigation on Arbuscular Mycorrhizal Alliances of Some Therapeutic Herbs of Lamiaceae Family in Hooghly District, West Bengal, India

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ABSTRACT

An investigation was carried out to determine the arbuscular mycorrhizal status in some common medicinal herbs of Lamiaceae family like, Ocimum sanctum Linn, Leucas plukenetii (Roth.) Spreng., Mentha spicata L., Ocimum canum Sims of Hooghly District, West Bengal, India. From Grid-line intersect method it was evidenced that all the plants under investigation were colonized by the vesicular arbuscular mycorrhizal fungi as both the vesicles and arbuscules were present in the roots. The percentage of mycorrhizal colonization was highest in the roots of Ocimum sanctum Linn (79%), followed by Ocimum canum Sims. (73.3%), Mentha spicata L. (70.5%) and lowest in Leucas plukenetii (Roth.) Spreng. (37%). Variations noticed in root infection and spore density were statistically significant. The vesicular arbuscular mycorrhizal fungi (VAM)/arbuscular mycorrhizae fungi (AM) which were observed in the present study mostly belong to the species group of Glomus.

Key words: *Vesicular arbuscular mycorrhizae (VAM), arbuscular mycorrhizae (AM), Glomus, Ocimum sanctum Linn, Leucas plukenetii (Roth.) Spreng., Mentha spicata L., Ocimum canum Sims.*

INTRODUCTION

"Mycorrhiza"- the term used to describe the symbiotic association between fungus and root of higher plant (Frank, 1885). Majority of flowering plants have the lively association of VAM [fungi]. It represents one of the nature's unsurpassed offerings to the mankind. Vesicular-arbuscular mycorrhizae (VAM) / Arbuscular mycorrhizae (AM) are symbiosis created among the roots of most angiospermic plants and fungi (Order: Glomales under the Sub-Class Glomeromycota). Glomales are presently placed in the class Zygomycetes (Rosendahl and Dodd, 1995).Despite the multidimensional importance of mycorrhizae in agriculture and forestry, little works have been done regarding their distribution, diversity and association with the host plants in India.

Traditional herbal medicines are increasingly being used by the health conscious people throughout the world for their primary health care. A major part of the total population in developing countries still uses traditional folk medicine obtained from plant sources (Farnsworth, 1994 and Srivastava *et al*., 1996). Medicinal plants are the rich sources of antimicrobial agents (Srivastava *et al.,* 1996). And an increasing reliance on the use of medicinal plants in the industrialized societies has been traced out to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies (UNESCO, 1998). Therefore, the purpose of the present study was to investigate the extent of VAM/AM association in some common medicinal plants of Hooghly district, West Bengal, India. Hooghly district is one of the districts of the state of West Bengal in India. It can alternatively be spelt Hoogli or Hugli. The district is named after the Hooghly River. The headquarters of the district are at Hooghly-Chinsura (Chuchura). There are four subdivisions: Chinsura Sadar, Serampore, Chandannagar, and Arambag. The latitude for Hooghly, West Bengal, India is: 22” 39’ 32’’ N - 23” 01’ 20’’ N and the longitude is: 87” 30’ 20’’ E - 88” 30’ 15’’ E with an altitude of 9-10 meters. The district is a rich source of medicinal plants and these important plants grows insides the railway tracks, road sides, river banks or other abundant places as common seasonal weeds. But due to the gradual boost of the population and continuous search for new lands for infrastructure development and for set up industry these type of natural medicinal plants are in danger, if proper care and steps is not taken to conserve these naturally occurring medicinal plants may became extinct in the near future. So there must be continuous effort to save these types of natural medicinal plants for our better future.

Thus, consequently, the present investigation was aimed to determine the endomycorrhizal colonization status in some medicinal plants of Lamiaceae family like, *Ocimum sanctum* Linn, *Mentha spicata* L., *Leucas plukenetii* (Roth.) Spreng., *Ocimum canum* Sims., still earlier reports of VAM association in members of Lamiaceae was inadequate.

MATERIALS AND METHODS

Selection of Plants:

Medicinal plants of Lamiaceae family growing mostly in Chandannagar subdivision of Hooghly District, West Bengal, India have been selected for the study which include *Ocimum sanctum* Linn, *Mentha spicata* L., *Leucas plukenetii* (Roth.) Spreng., *Ocimum canum* Sims.

1. *Ocimum sanctum* Linn., Local name in Bengali: Tulsi

Habit: Aromatic herbs.

Medicinal uses: In Ayurveda *Ocimum sanctum* Linn has been well documented for its therapeutic potentials and described as antiasthmatic and antikaphic drugs (Sirkar, 1989).

Fig.1. *Ocimum sanctum* Linn.

2. *Mentha spicata* L., Local name in Bengali: Pudina.

Habit: Herbs.

Medicinal uses: Folkloric medicine as a carminative, antispasmodic, diuretic, antibacterial, antifungal, and antioxidant agent (Snoussi *et al*, 2015).

Fig.2.*Mentha spicata* L.

3. *Leucas plukenetii* (Roth.) Spreng., Local name in Bengali: Dandokolos

Habit: Annual herbs.

Medicinal uses: For curing throat troubles (Abraham *et al*, 1986).

Fig.3.*Leucas plukenetii* (Roth.) Spreng.

4. *Ocimum canum* Sims , Local name in Bengali: Bontulsi.

Habit: Herbs.

Medicinal uses: antimicrobial, antiemetic, antidiabetic, antifertility,

antiasthmatic, antistress andanticancer activity(Makker,2007).

Fig.4. *Ocimum canum* Sims

Study site:

Plant materials were mostly collected from Chandannagar subdivision region and its surrounding areas of Hooghly district.

Identification of the plants:

The plants were identified by using published literature and the herbarium of Botany Department, Burdwan University, Burdwan, and arranged according to Bentham and Hooker's system of classification.

Collection of root samples:

For each species, the feeder roots were collected directly from the plants by digging and tearing the roots up to the base of the main stem.

Preservation of roots:

The root samples after collection were thoroughly washed in running tap water and rootlets were selected, cut into small pieces and fixed in formaldehyde/acetic acid solution (Johanson, 1940) and were preserved in refrigerator at 4°C temperature.

Collection of soil samples:

Soil sample of about 10 g was collected from the root region (rhizosphere) of each of the plant species by digging the soil up to a depth of 10 cm and collected into polythene bags, labeled and stored at 4°C until analysis in refrigerator.

Preparation of root samples:

For each specimen, 100 feeder root pieces were thoroughly washed in water and boiled at 95°C temperature for different durations (like 10, 15, 20, 25 and 30 min) in 10% KOH. The segments were washed in distilled water, acidified with 1 (N) HCl and were stained with 0.05% Trypan blue in lactophenol. The excess stain was removed by washing with lactophenol. Root segments were mounted temporarily on slides in acetic acid, glycerol (1:1 V/V) and the edges of the cover slips were sealed with DPX and observed under microscopes (Leica, Model No. DMLB 3000, Lebomed, Model no.LX300).

Assessment of VAM fungal association in roots:

The VAM association in the roots of each of the specimens was examined following the method of Phillip and Hayman (1970) and the percentage of mycorrhizal association was calculated.

Collection of mycorrhizal spores from soil samples:

At first 10 g, soil sample was taken and dissolved in 100 ml distilled water in a conical flask. The conical flask was then shaken for 30 min after which the flask was kept undisturbed for 30 minutes. The soil particles precipitated at the bottom of the flask and the spores were being floated on the surface of the liquid. Mycorrhizal spores were obtained by wet sieving and decanting technique (Gerdemann and Nicolson, 1963). The solution was then passed through

250,150, 53 and 45 µm pore size sieve and the spores were collected from the residue of 53 µm sieve. This residue was dissolved in distilled water and filtered. The residue present in the filter paper was taken and mounted on a slide in lactophenol and cotton blue and were examined under microscopes (Leica, Model No. DMLB 3000, Lebomed, Model no.LX300).

Spore count

VAM fungal spores were extracted from replicates of 50 g soil by wet sieving and decanting technique (Gerdeman and Nicolson, 1963). The decantant were filtered through a filter paper with grid lines. The filter paper was then spread on a glass slide under a dissecting microscope and the number of spores was counted and expressed as spores per 100 g of dry soil.

Identification of VAM fungi:

The arbuscular mycorrhizal fungi were identified by using manuals of Trappe (1982), Morton and Benny (1990), Schenck and Perez (1990) and Mukerji (1996).

Statistical methods:

All the data were taken in ten replicates and the standard error of mean (SEM) value (±) was calculated. Each of the data was checked for interpretation whether they were statistically significant or not. The data were analyzed by using the statistical method like, analysis of variance (ANOVA), and critical difference (CD) at 5% level was calculated.

RESULTS AND DISCUSSION

It was evident from the present study that all the plants under investigation exhibited root colonization by the vesicular arbuscular mycorrhizal fungi as both the vesicles and arbuscules were present in the roots. The percentage of mycorrhizal colonization was highest in the roots of *Ocimum sanctum* Linn (79%), followed by *Ocimum canum* Sims. (73.3%), *Mentha spicata* L. (70.5%) and lowest in *Leucas plukenetii* (Roth.) Spreng. (37%). The VAM fungi found in this study were identified using standard manual and the synoptic key of Schenck and Perez (1987), Morton and Benny (1990) and Trappe (1982). The VAM/AM fungi recorded in the present study mostly belong to the species group of *Glomus*. The genus includes both sporocarpic and non-sporocarpic species.

The results (Table: 1) had revealed that mycorrhizal association was very satisfactory in *Ocimum sanctum*. Both the vesicles and arbuscules were observed in the roots of *Ocimum*

sanctum so; the endophyte was a vesicular arbuscular mycorrhiza (VAM). So, *Ocimum sanctum* has been established to be a suitable host plant for AM fungi.

Percentage of total root colonization attained in peak level during rainy season (79%± 2.28). However, extraradical spore count reached in peak during winter (310 /100g of soil sample) and turned down during rainy season (122/100g of soil sample).

Table 1. Mycorrhizal colonization in *Ocimum sanctum* Linn., Family: Lamiaceae

Plant	Season of Collection	Presence or absence of mycorrhizal association	Vesicles	Arbuscules	Percente of Root colonization (%)*	No.Extraradical spores/100 gm of soil*
Ocimum sanctum	Winter	+	+	+	57 ± 2.30	310 ± 4.10
	Summer	+	+	+	70 ± 1.05	226 ± 1.97
	Rainy	+	+	+	79 ± 2.28	122± 1.30

*Data are the mean values of ten replicates

The results (Table: 2) had revealed that mycorrhizal association was there in the roots of *Mentha spicata*. Vesicles and arbuscules were viewed in the roots, so, the endophyte was vesicular arbuscular mycorrhiza (VAM).

Percent root colonization was maximum in rainy season (70.5% ± 0.65) whereas, extraradical spore count was highest during winter (394 /100g of soil sample) and lowest during rainy season (264 /100g of soil sample).

Table 2. Mycorrhizal colonization in *Mentha spicata L. Syn-M. spicata var. viridis L.; M. viridis Linn.*,Family: Lamiaceae/Labiatae

Plant	Season of Collection	Presence or absence of mycorrhizal association	Vesicles	Arbuscules	Percente of Root colonization (%)*	No.Extraradical spores/100 gm of soil*
Mentha spicata L.	Winter	+	+	+	34 ± 0.71	394± 4.99
	Summer	+	+	+	43 ± 0.76	328 ± 7.71
	Rainy	+	+	+	70.5 ± 0.65	264 ± 7.2

*Data are the mean values of ten replicates

The results (Table: 3) has confirmed that mycorrhizal association was there in *Leucas plukenetii*. Both the vesicles and arbuscules were observed in the roots of *Leucas plukenetii*, though the number of arbuscules was less. Hence, the endophyte was vesicular arbuscular mycorrhiza (VAM).

Total root colonization was poor in winter (18%). Extraradical spore count was highest during winter (418 /100g of soil sample) and lowest during rainy season (200/100g of soil sample).

Table 3. Mycorrhizal colonization in *Leucas plukenetii* (Roth.) Spreng. , Syn. *L. aspera* Link, Family: Lamiaceae

Plant	Season of Collection	Presence or absence of mycorrhizal association	Vesicles	Arbuscules	Percente of Root colonization (%)*	No.Extraradical spores/100 gm of soil*
Leucas plukenetii (Roth.) Spreng.	Winter	+	+	+	18 ± 1.08	418 ± 5.92
	Summer	+	+	+	30.6± 0.52	316 ± 8.05
	Rainy	+	+	+	37 ± 1.45	200 ± 4.71

*Data are the mean values of ten replicates

It is found from the results (Table: 4) that mycorrhizal association was present in *Ocimum canum*. Both the vesicles and arbuscules were observed in the roots of *Ocimum canum*.Hence, the endophyte was vesicular arbuscular mycorrhiza.

Percentage of total root colonized was very good in rainy season (73.3%).Extraradical spore count was highest during winter (183 /100g of soil sample) and lowest during rainy season (84/100g of soil sample).

Table 4. Mycorrhizal colonization in *Ocimum canum* Sims., Family: Lamiaceae

Plant	Season of Collection	Presence or absence of mycorrhizal association	Vesicles	Arbuscules	Percente of Root colonization (%)*	No.Extraradical spores/100 gm of soil*
Ocimum canum Sims	Winter	+	+	+	35 ± 1.38	183 ± 0.76
	Summer	+	+	+	60± 1.22	135 ± 1.38
	Rainy	+	+	+	73.3 ± 1.07	84 ± 0.83

*Data are the mean values of ten replicates

Graph 1.Total mycorrhizal colonization of selected plants:

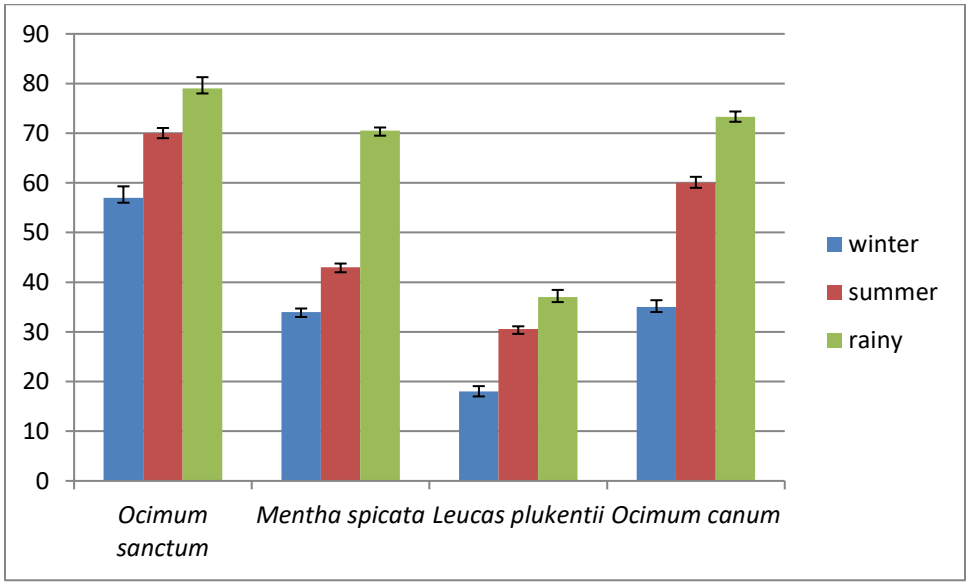
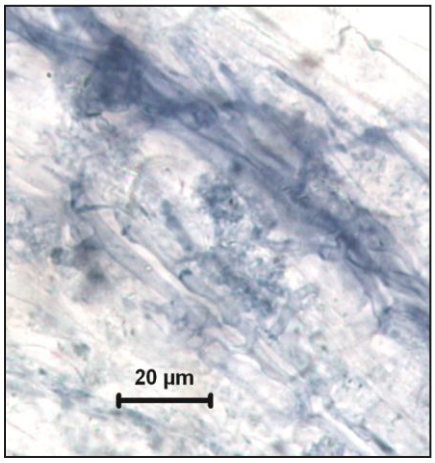
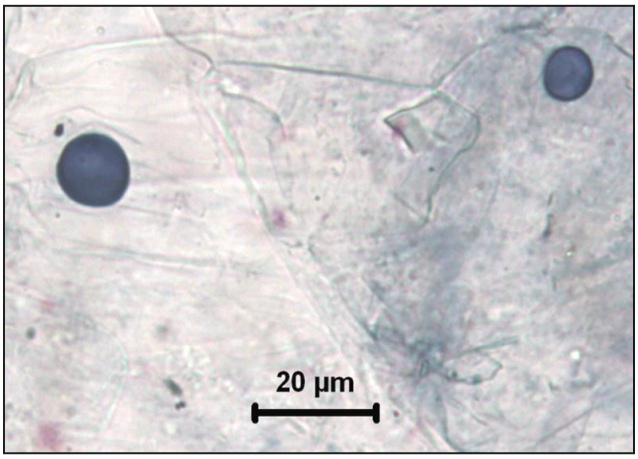


Table 5. Name of the mycorrhizal fungi present in the selected medicinal plants of Lamiaceae Family:

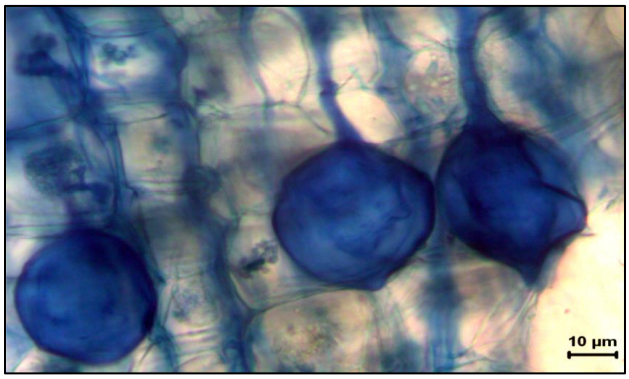
SL. No.	Name of the plants	Mycorrhizal fungi
1.	*Ocimum sanctum* Linn.	*Glomus fasciculatum, Acaulospora* sp., *Gigaspora* sp.
2.	*Mentha spicata* L.	*Glomus* sp., *Acaulospora* sp., *Gigaspora* sp.
3.	*Leucas plukenetii* (Roth.) Spreng.	*Acaulospora* sp., *Glomus mosseae*
4.	*Ocimum canum* Sims	*Glomus fasciculatum, Glomus* sp.,



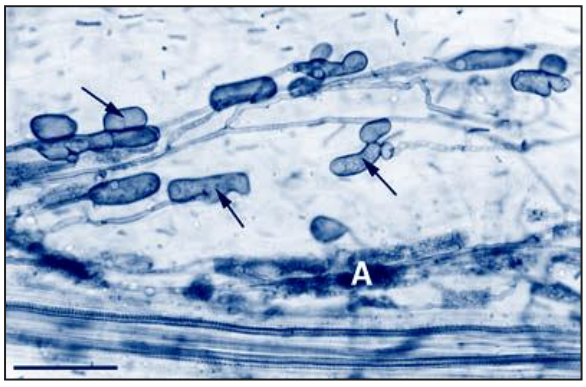
P1: Arbuscles of Ocimum *sanctum*



P2: Spore of *Glomus fasciculatum* in roots of *Ocimum sanctum*



P3: Vesicles of *Glomus* of *Ocimum canum*



P4: Vesicles found in *Mentha*

[P=Plate]

From this present investigation it is reveled that the percentage of mycorrhizal colonization was highest in the roots of *Ocimum sanctum* Linn (79%), followed by *Ocimum canum* Sims. (73.3%), *Mentha spicata* L. (70.5%) and lowest in *Leucas plukenetii* (Roth.) Spreng. (37%). Variations noticed in root infection and spore density were statistically significant. The vesicular arbuscular mycorrhizal fungi (VAM)/arbuscular mycorrhizae fungi (AM) which were observed in the present study mostly belong to the species group of *Glomus*.

CONCLUSION

From the results of the present investigation it can be concluded that there is a significant incidence of arbuscular mycorrhizal (AM) fungal associations in the medicinal plants of Lamiaceae in the study area. All the plant species examined were colonized by AM fungi though there appeared marked variation in the percentage root colonization of the selected plants. Since a significant number of medicinal and aerometric plants are present in Hooghly district region, extensive research works are required to establish a database of mycorrhizal species colonizing these plants and to determine their efficiency towards improving quality and quantity of bioactive medicinal compounds. However, it should be mentioned here that though earlier reports of VAM association in members of Lamiaceae was in adequate but the present report may certainly be a significant finding.

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