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STUDY OF ARBUSCULAR MYCORRHIZAL ASSOCIATION IN SOME MEDICINAL HERBS OF ACANTHACEAE FAMILY FROM DARJEELING DISTRICT, WEST BENGAL, INDIA

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ABSTRACT

A survey was carried out to determine the arbuscular mycorrhizal status in some common medicinal herbs of Acanthaceae family like, *Andrographis paniculata* (Burm.f.) Wall.ex Nees, *Hygrophila auriculata* (Schumacher) Heine, *Barleria lupulina* Lindley, *Justicia adhatoda* L. From Grid-line intersect method it was evidenced that all the plants under investigation were colonized by the arbuscular mycorrhizal fungi as arbuscules were present in the roots. The percentage of mycorrhizal colonization was highest in the roots of *Barleria lupulina* Lindley (49.5%), followed by *Hygrophila auriculata* (Schumacher) Heine (27%), *Andrographis paniculata* (Burm.f.) Wall.ex Nees (21%), and lowest in *Justicia adhatoda* L. (14%). Variations noticed in root infection and spore density were statistically considerable. The arbuscular mycorrhizae fungi (AM) which were observed in the current study typically belong to the genus *Glomus*.

KEY WORDS: **Vesicular arbuscular mycorrhizae (VAM), arbuscular mycorrhizae (AM), *Glomus, Andrographis paniculata* (Burm.f.) Wall.ex Nees, *Hygrophila auriculata* (Schumacher) Heine, *Barleria lupulina* Lindley, *Justicia adhatoda* L.**

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INTRODUCTION

Medicinal plants are well known almost all over the world for their natural therapeutic potential in modern and traditional medicine. Research on amazing curative agents from plants is very much attractive area of modern research. Medicinal plants are used for treatment of various diseases that's why quality as well as quantity improvement of medicinal plants is very essential. Acanthaceae (the acanthus family) is a family of dicotyledonous flowering plants containing almost 250 genera and about 2500 species.[1] The family contains a considerable number of medicinal plants. Among these, *Andrographis paniculata* Wall is one of the most popular medicinal plants used traditionally for the treatment of array of diseases such as cancer, diabetes, high blood pressure, ulcer, leprosy, bronchitis, skin diseases, flatulence, colic, influenza, dysentery, dyspepsia and malaria.[2] *Hygrophila spinosa* T Ander, belonging to this family, is a promising medicinal plant with great economic potential. The medicinal value of *H. spinosa* has been appreciated in the ancient medical literature. The plant contains terpenoids, alkaloids, flavonoids, and is traditionally known as an aphrodisiac, renal tonic, and for its health-promoting properties.[3] *Barleria lupulina* Lindl. an extraordinary medicinal plants with anti-inflammatory, analgesic, ulcerogenic and antiperoxidative potential.[4] A wide range of phytochemical constituents have been isolated from *J. adhatoda* which possesses activities like antitussive, abortifacient, antimicrobial, cardiovascular protection, anticholinesterase, anti-inflammatory.[5] M*ycorrhiza is* a symbiotic association between soil borne fungus and plant roots.[6] Arbuscular mycorrhizal fungi (AMF) show symbiotic association with the roots of this medicinal plants. In this association; the fungus colonizes the host plants roots, at intracellular level as arbuscular mycorrhizal fungi (AMF). AMF occur in soil, sand, in crop fields, and are found to be associated with native plant species and represents different ecological condition. In the rhizospheric soil the arbuscular mycorrhizal fungi (AM fungi) are present in the form of chlamydospores, zygospores and azygospores as resting spores.[7] AMF contribute to the phosphorus nutrition of plants by enhancing phosphorus uptake from the soil. The AM fungal association improves the growth of medicinal plants as well as increases the productivity of medicinal compounds.[8] In *A. paniculata* improved growth, biomass yield, Phosphorus nutrition and andrographolide concentration have been observed by inoculating vesicular arbuscular mycorrhizal fungi.[8] The ecological importance of AM fungi is immense as because they improve plant nutrition and growth by getting associated with the roots of the majority of land plants.[9, 10] However, many scientists reported the occurrence of VAM/AM in medicinal and aeromatic plants from India.[11, 12,13,14,15] Darjeeling District of West Bengal, India, the foothill of Himalayas is well known for its biodiversity. It is located of between latitude 27° 13' N to 26° 27' N and longitude 88°53' E to 87°59' E. A variety of wild and natural plant species are found in this region hence was the inspiration of the present study for investigation of VAM/AM association in some common medicinal herbs of Darjeeling district. The earlier reports regarding VAM/AM association in the members of Acanthaceae family is very much scanty and inadequate, so the present investigation was designed to determine the endomycorrhizal colonization status in some common medicinal plants of Acanthaceae family from Darjeeling district like, *Andrographis, Hygrophila, Barleria* and *Justicia* .Though earlier reports of VAM association in members of Acanthaceae is inadequate.

MATERIALS AND METHODS

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.[16]

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 and were preserved in refrigerator at 4°C temperature.[16]

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 root samples of each of the specimens was examined following the standard method,[17] 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.[18] 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 three replicates of 50 g soil by wet sieving and decanting technique.[19] 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 standard manuals.[20, 21, 22, 23]

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 as shown in Table 1.

Table 1
VAM/AM association with medicinal plants of Acanthaceae family

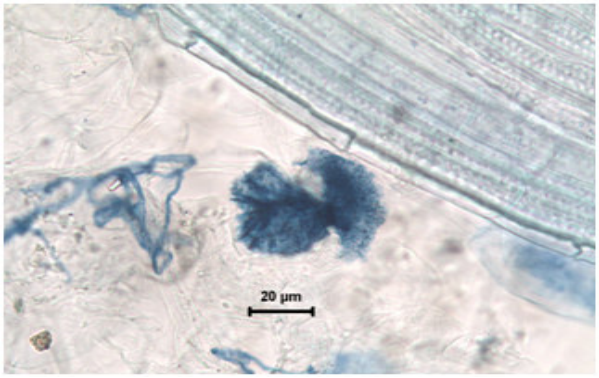
Medicinal Herbs	**Local Name** (Bengali)	**VAM colonization Status (% root length infected)**	**Spore count (Extraradicle spores/100 gm of soil)**
Andrographis paniculata (Burm.f.) Wall.ex Nees	Kalmegh	21 ± 1.20	310 ± 9.43
Barleria lupulina Lindley	Bisalokaroni	49.5 ± 1.36	280 ± 11.26
Hygrophila auriculata (Schumacher) Heine	Kulakhara	27 ± 0.93	320 ± 11.06
Justicia adhatoda L.	Basak	14 ± 1.22	390 ± 15.35

Andrographis paniculata (Burm.f.) Wall.ex Nees

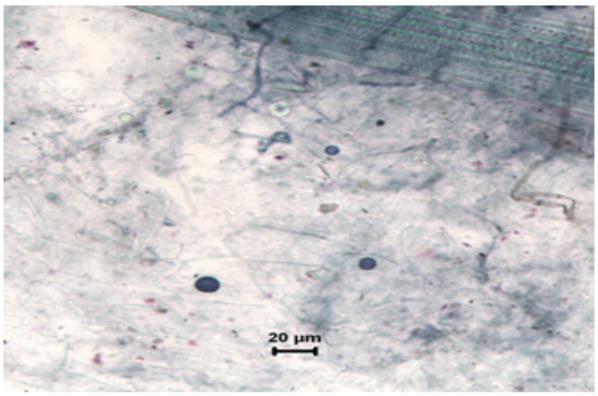
Barleria lupulina Lindley

Hygrophila auriculata (Schumacher) Heine

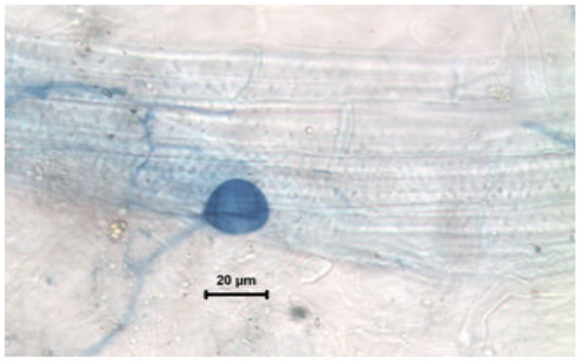
Justicia adhatoda L.



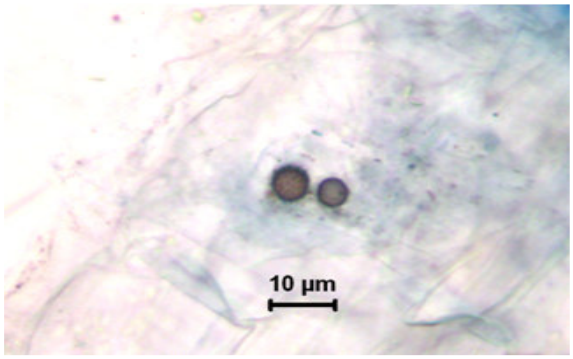
Arbuscles of Andrographis paniculata (Burm.f.) Wall.ex Nees



Spores of Barleria lupulina Lindley



Vesicles of Hygrophila auriculata (Schumacher) Heine



Spores of Justicia adhatoda L.

RESULTS AND DISCUSSION

It was apparent 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 *Barleria lupulina* Lindley (49.5%), followed by *Hygrophila auriculata* (Schumacher) Heine (27%), *Andrographis paniculata* (Burm.f.) Wall.ex Nees (21%), and lowest in *Justicia adhatoda* L.(14%). The VAM/AM fungi obtained from this investigation were identified using standard manual and the synoptic key.[24,25,26] The VAM/AM fungi recorded in the present study mostly belong to the genus *Glomus*. The genus includes both sporocarpic and non-sporocarpic species.

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 Acanthaceae 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 large number of medicinal and aerometric plants are present in Darjeeling Himalaya region, extensive research works are required to establish a database of mycorrhizal colonization in these plants and to determine their efficiency towards improving quality and quantity of bioactive medicinal compounds present in these plants. However, it should be mentioned here that though earlier reports about VAM association in members of Acanthaceae was inadequate but the present observation may certainly be a significant finding.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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