Research on association of VAM with *Azolla pinnata* and other aquatic plants and its seasonal distribution of *Azolla* in various location of Jabalpur city.

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Abstract

An experiment was conducted at J.N.K.V.V Jabalpur to study the association of VAM (Vesicular arbuscular mycorrhiza) with *Azolla pînata* rhizoids and other aquatic plants. As it may be an example of unique obligate symbiosis with *Anabaena azollae*, present in the dorsal cavity of *Azolla*. This has been promoted to find out association of VAM with *Azolla pinnata* because of earlier finding of Firdans e-bareen (1990) was quite interesting enough to establish whether this tripartite symbiosis does exit or feasible in aquatic or littoral environment. After investigations, apart from there major sites of Jabalpur city seven more lactations were surveyed for not only *Azolla pinnata* but *Lemma minor*, *Echhnaria crasipes*, *Trapa bispinosa* and *Iponea aquatica* also. The rhizodis of the samples were examined for finding association of VAM. Our finding has gone contrary to the existence of VAM association with *Azolla pinnata*. However, ecophysiological studies was also done for occurrence of *Azolla* in Jabalpur region.

Keywords: Associations of VAM (Vesicular arbuscular mycorrhiza) rhizoids, obligate symbiosis.

1. Introduction

*Azolla* is a small aquatic fern which floats on the water surface. It is also a unique example of obligate symbiosis due to presence of Cyanobacterium, *Anabaena azollae* in the dorsal cavity of *Azolla*. The Nitrogen fixing capacity of *Anabaena azollae* enables *Azolla* to thrive on nitrogen free waters. It lives in swamps, ditches, and even in lakes and rivers where the water is not turbulent (Lumpkin and Plucknett,1982). *Azolla* leaf consists of two lobes, an aerial dorsal lobe, which is chlorophyllous, and a partially submerged ventral lobe. Each dorsal lobe contains a leaf cavity, which houses the symbiotic *Anabaena azollae*. *Azolla* is historically cultured in the rice farming system in China and southeast Asian countries. The fern is doubled in 3-5 days and N input of 110-330 kg /N/ hm² per annum has been obtained under optimal conditions. This importance of *Azolla* was increased due to report of its associations with VAM (Firdus-e-bareen 1990) as its quite interesting to establish whether this tripartite symbiosis exist or not. However, the importance of *Azolla* for low land rice production has been evaluated in numerous investigation (C.C. Liu and Zheng W.W. 1989), (X.Liu 2008)

2. Materials and methods

Sampling of littoral mud carrying the fern *Azolla* was collected during May – June period from a pond of Deotal Jabalpur. *Azolla* frond was found growing in green condition on littoral mud .Whereas it was growing completely red inside its water body Fig 1(a)1(b). At this stage of eutrophication of the site *Azolla* frond alongon littoral mud was ripped off through a bladé
of knife so as to keep its rhizoids in fully intact and unfurled conditions. Several sample were placed inside a polythene bag, brought to the laboratory and stored in a refrigerator till examined for occurrence of vesicular arbuscular mycorrhiza (VAM).

3. **Microscopic examination of Azolla rhizoids for associations (VAM)**

Rhizoids from *Azolla* growing on littoral mud in 10 localities of different sites were cut into 1cm pieces after subsequent washing with water and making free of soil particles. The segments of Rhizoids were heated in 10 % KOH for 30 minute at 90ºC rinsed with water and acidified with dilute HCL. These were stained in 0.05% trypan blue in lactophenol for 5 minutes. The segments so stained were thus mounted on slides and viewed under Stereo Microscope (wild M3Z) at 40x magnification for the presence of vesicules.

4. **Results and discussion**

After examination in more than three times, we found that *A. pinnata* occurred in 80% followed by *Lemna minor* (70%), *E. crassipes* (20%), *T. bispinosa* (50%) and *I. aquatica* (20%) Rhizoids of *Azolla Pinnata* and other aquatic plants were stained for vesicular arbuscular mycorrhiza by simmering in trypan blue (0.05% W/V)in lacto phenol for 5 minutes. When examined under Microscope, no trace of the mycorrhizal strands was visible there, though all care had been taken to detach the rhizoids, to cut into segments and to reduce the time of boiling in 10% KOH at 90º C. These plants did not bear any incidence of the VAM in their rhizods. The location and distributions pattern of the floating plants are given in table1.

The ecophysiological studies were also done in the condition of Jabalpur city as it is located 23.10 North latitude and 79.59 East longitude and is 411.78m above MSL. The area experiences a tropical, monsoonal climate. During the summer, whether is dictated by the SW monsoon bringing wet, hot, and humid summer. It has a maximum rainfall in August – September. In the winter months the SE monsoon is weakly active and thus there is sporadic rainfall with slight drizzle during March and April. For a typical rainfall, and maximum and minimum temperature data for the area is exhibited in fig 2. Being monsoonal, rainfall is seasonal, mainly falling in June to September, and this pattern is possessed of a 12 year cycle.

The fig also depicts that a seasonal variation in growth of *Azolla pinnata* in ponds of various sizes, treanches and ditches. It is usually preceded by Lemna sp, and its young plantlets begin to appear in August resulting in bloom in January and February. It is then succeeded by cyanophycean flora in total accompaniment with dying Azolla. However, this cyanophycean flora is in no way the same as *Anabaena azollae*, the microsymbiont of *Azolla pinnata* (Verma and Dube 1984)
Fig 2: Showing ecophysiological study of *Azolla pinnata* at Jabalpur Region

**Table 1**: Incidence of VAM on aquatic plants in various locations of Jabalpur city

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Location</th>
<th><em>Azolla pinnata</em> (80%)</th>
<th><em>Lemna minor</em> (20%)</th>
<th><em>Eichhornia crassipes</em> (20%)</th>
<th><em>Trapa bispinosa</em> (50%)</th>
<th><em>Ipomea aquatica</em> (20%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Barhatal (Rani Durgavati Road)</td>
<td>+(-ve)</td>
<td>+(-ve)</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Thonat Tal (Jhansighat Road)</td>
<td>-</td>
<td>+(-ve)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Deotal (NH7)</td>
<td>+(-ve)</td>
<td>(-ve)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Kisrod (NH12)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Panagar (NH7)</td>
<td>+(-ve)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Adhartal (NH7)</td>
<td>+(-ve)</td>
<td>+(-ve)</td>
<td>-</td>
<td>+(−ve)</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Kachhpura (Bombay Rail track)</td>
<td>+(-ve)</td>
<td>+(-ve)</td>
<td>+(−ve)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Sukha (Patan Road)</td>
<td>+(-ve)</td>
<td>+(-ve)</td>
<td>+(−ve)</td>
<td>+(−ve)</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Rausra (Patan Road)</td>
<td>+(-ve)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Patan (Patan Road)</td>
<td>+(-ve)</td>
<td>+(-ve)</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+(-ve) – No VAM; a-not sampled for observation of VAM; Bracketed numerals indicate of the aquatic plants in all locations.
5. Conclusion

As reported earlier by Firdus e-bareen (1990) that there is a association of vesicles of VAM with rhizoids of A.pinnata in littoral mud condition, it becomes unique example of tripartite symbiosis in the field of biological Science but our findings had gone contrary to the existence of VAM association with \textit{Azolla pinnata} and other aquatic plants. This did not preclude existence of VAM in limbic environment or in the littoral mud possibly drying \textit{Azolla} on higher level of transplanted region might associate itself with VA mycorrhiza. \textit{Azolla} has many uses, it can be utilized as a biofertilizer on rice and many other crops and water purifier. \textit{Azolla} can accumulate more than 10 kg N/ha./day, and thus has the potential of supplying the entire nitrogen requirement for highyielding many crops within a few weeks due to its rapid multiplication and N2-fixation. \textit{Azolla} as organic matter can also improve physical and chemical properties of the soil. The ecophysiological study enables the farming community of Jabalpur region to utilize the \textit{Azolla} fern in the farmers field.

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