



Annexure – IX

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002.

PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING
THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. NAME AND ADDRESS OF THE PRINCIPAL : Dr. N. B. Gaikwad
INVESTIGATOR
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2. NAME AND ADDRESS OF THE INSTITUTION: Shivaji University
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3. UGC APPROVAL NO. AND DATE: F.No.33-457/2007 (SR) dated 28/02/2008
4. DATE OF IMPLEMENTATION: 01-04-2008
5. TENURE OF THE PROJECT: April 2008- March 2010
6. TOTAL GRANT ALLOCATED: 1, 00,000/-
7. TOTAL GRANT RECEIVED: 50,000/-
8. FINAL EXPENDITURE: 99,272/-
9. TITLE OF THE PROJECT: "Studies on *in vitro* development of gametophyte and
cytology of *Ophioglossum* "
10. OBJECTIVES OF THE PROJECT:
 - Collection and maintenance of germplasm of *Ophioglossum* species in Botanical garden of Department of Botany, Shivaji University, Kolhapur.
 - *In vitro* gametophyte development.
 - Cytology and anatomical studies of selected population.

11. WHETHER OBJECTIVES WERE ACHIEVED: (GIVE DETAILS)

- Through extensive field visits, we have collected four species of *Ophioglossum* viz. *O. reticulatum*, *O. costatum*, *O. vulgatum*, *O. nudicaule*, *O. nudicaule* var *macrorrhizum*, *O. petiolatum*, *O. lusitanicum* and *O. gramineum* from Junnar, Malshej Ghat, Khopoli, Mulshi, Panchgani, Patgaon, Ajara, Radhanagari, Panhala, Appachiwadi, Nagpur, Bhandara, Gondia and Chandrapur and maintained in the Botanical garden.
- For *in vitro* gametophyte development spores of *O. costatum* and *O. reticulatum* spores were inoculated on Nitsch and Nitsch basal medium and incubated at 28°C with dark conditions, but *in vitro* development of gametophyte was found to be difficult even after several trials.
- Anatomical studies of root, petiole and spike stalk in above four *Ophioglossum* species of was carried out.
- Scanning electron microscopic study of spores was carried out in *O. costatum*, *O. reticulatum* and *O. vulgatum*.
- During conservation of *Ophioglossum* sp in garden, we have noticed that only *O. reticulatum* showed the incidence of leaf spot disease caused by *Alternaria alternate*.
Incidence of *Alternaria alternate* is reported for the first time in India on any *Ophioglossum* sp.

During the first year of the project the survey, collection and maintenance of *Ophioglossum* sp. was done from the following locations:

Locality	<i>Ophioglossum</i> sp. collected	GPS Location
Junnar	<i>O. reticulatum</i> , <i>O. vulgatum</i> & <i>O. costatum</i>	N 19 ⁰ .19.904' E 73 ⁰ .74.668'
Malshej	<i>O. reticulatum</i> , & <i>O. costatum</i>	N 19 ⁰ .20.126' E 71 ⁰ .47.377'
Khopoli	<i>O. reticulatum</i> , & <i>O. vulgatum</i>	N 19 ⁰ .00.268' E 73 ⁰ .17.780'
Panchgani	<i>O. reticulatum</i> , & <i>O. vulgatum</i>	N 17 ⁰ .23.734' E 73 ⁰ .39.833'
Patgaon	<i>O. reticulatum</i> , <i>O. vulgatum</i> & <i>O. costatum</i>	N 16 ⁰ .17.846' E 74 ⁰ .05.386'
Ajara	<i>O. reticulatum</i> , & <i>O. vulgatum</i>	N 16 ⁰ .08.001' E 74 ⁰ .08.884'
Radhanagari	<i>O. nudicaule</i> & <i>O. vulgatum</i>	N 16 ⁰ .22.910' E 73 ⁰ .51.442'
Panhala	<i>O. reticulatum</i> , <i>O. nudicaule</i> & <i>O. vulgatum</i>	N 16 ⁰ .49.110' E 74 ⁰ .04.525'

Ophioglossum species collected from different localities are maintained in botanical garden in second year of the project

Locality	<i>Ophioglossum</i> sp. collected	GPS Location
Amgaon	<i>O. nudicaule</i> & <i>O. costatum</i>	N 21 ⁰ .22.006' E 80 ⁰ .22.050'
Tumsar	<i>O. lusitanicum</i> , <i>O. nudicaule</i> var <i>macrorrhizum</i> & <i>O. gramineum</i>	N 21 ⁰ .20.034' E 79 ⁰ .45.046'
Darekasa	<i>O. petiolatum</i> & <i>O. reticulatum</i>	N 21 ⁰ .16.020' E 80 ⁰ .35.017'
Allapali	<i>O. reticulatum</i> ,	N 19 ⁰ .25.007' E 80 ⁰ .03.058'
Todaba	<i>O. lusitanicum</i> & <i>O. costatum</i>	N 20 ⁰ .10.026' E 79 ⁰ .17.022'
Moharli		N 16 ⁰ .29.057'
Appachiwadi	<i>O. gramineum</i>	E 74 ⁰ .20.002'



Conservation of *Ophioglossum* species



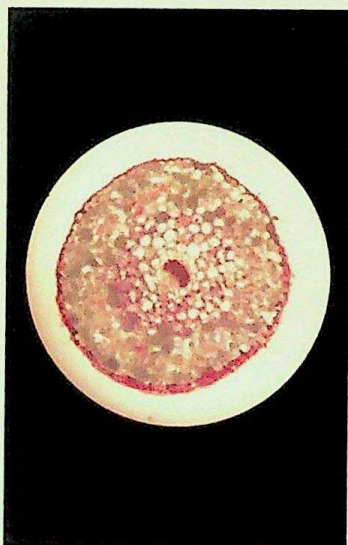
Conservation of *Ophioglossum* species

Anatomical studies: For anatomical studies fresh root, petiole and spike stalk was used from above collected *Ophioglossum* sp. and with the help of hand sections followed by staining with 0.1% safranin following results are observed.

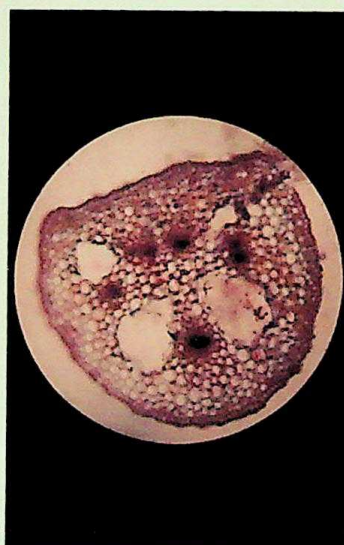
1. *Ophioglossum reticulatum*: T. S. of root shows presence of mycorrhizic fungus and starch grains with monarch stele, T. S. of petiole shows 5-8 vascular bundles which are arranged in circle while in case of T. S. of spike stalk 8-11 vascular bundles are observed in circular arrangement. These bundles are collateral and endarch.
2. *Ophioglossum costatum*: T. S. of root shows presence of mycorrhizic fungus and starch grains with diarch stele, T. S. of petiole shows 11-12 vascular bundles which are arranged in circle while in case of T. S. of spike stalk 4-6 vascular bundles are observed in semicircular arrangement. These bundles are collateral and endarch.
3. *Ophioglossum vulgatum*: T. S. of root shows presence of mycorrhizic fungus and starch grains with diarch stele, T. S. of petiole shows 2-4 vascular bundles which are arranged in semicircle while in case of T. S. of spike stalk 5-6 vascular bundles are observed in semicircular arrangement. These bundles are collateral and endarch.
4. *Ophioglossum nudicaule*: T. S. of root shows presence of mycorrhizic fungus and starch grains with monarch stele, T. S. of petiole shows 3-5 vascular bundles which are arranged in semicircle while in case of T. S. of spike stalk 3-4 vascular bundles are observed in semicircular arrangement. These bundles are collateral and endarch.



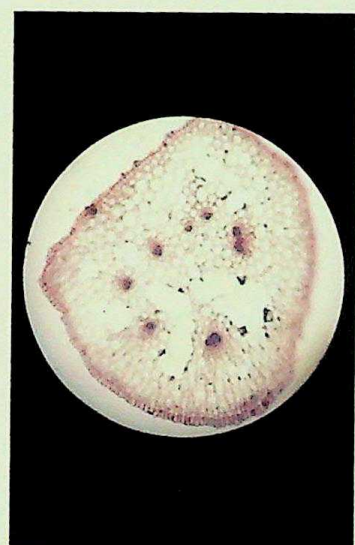
Ophioglossum reticulatum habit



T. S. of Root



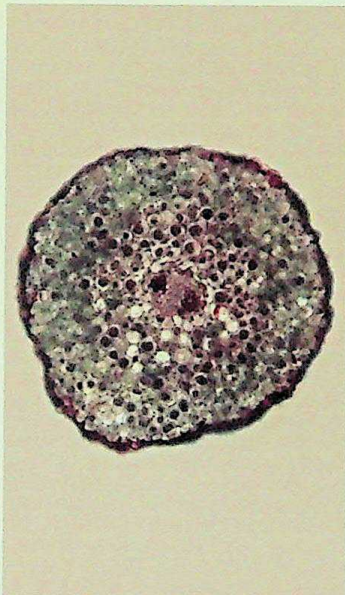
T. S. of Petiole



T. S. of Spike stalk



Ophioglossum costatum habit



T. S. of Root



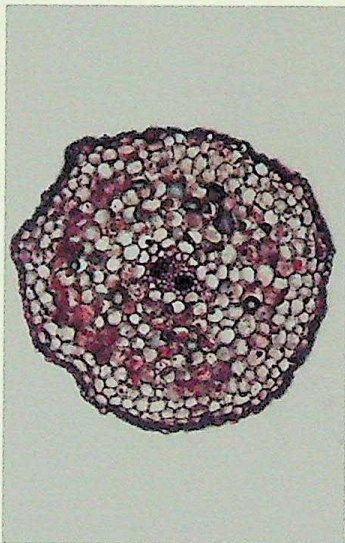
T. S. of Petiole



T. S. of Spike stalk



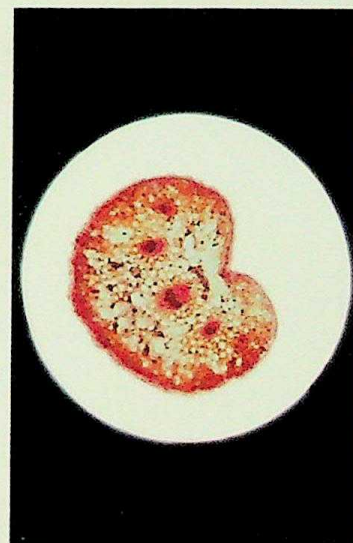
Ophioglossum vulgatum habit



T. S. of Root



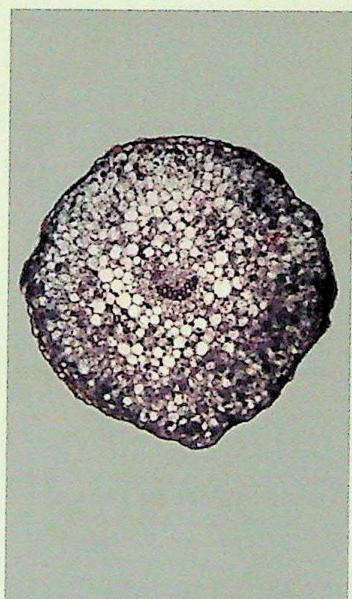
T. S. of Petiole



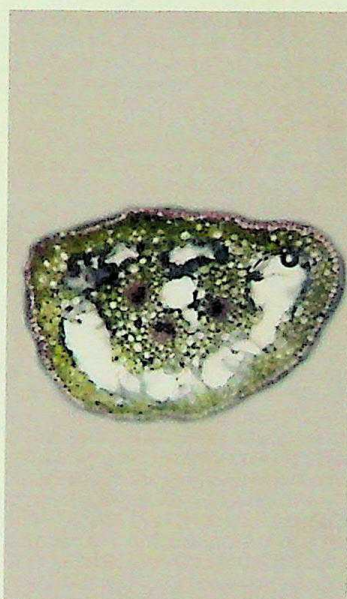
T. S. of Spike stalk



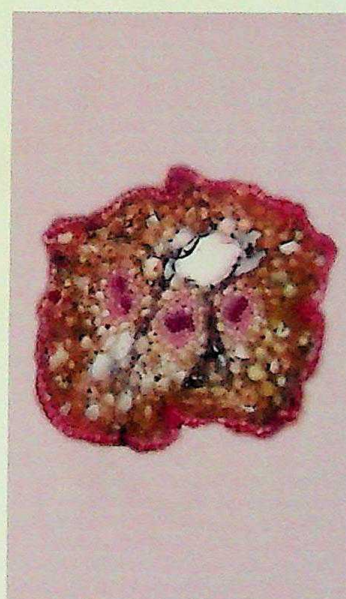
Ophioglossum nudicauli habit



T. S. of Root



T. S. of Petiole



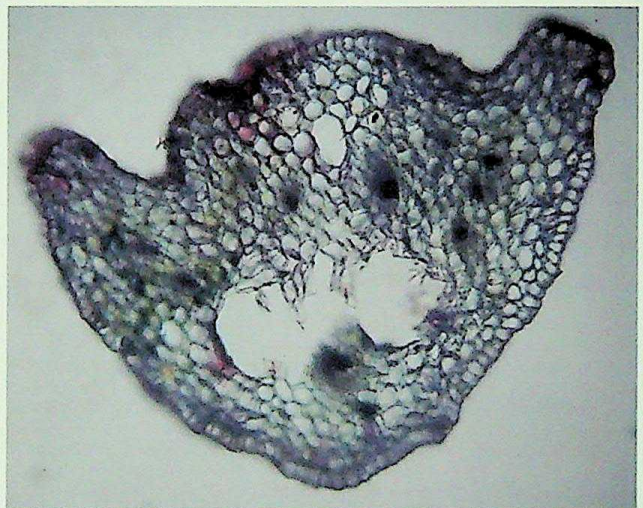
T. S. of Spike stalk



Ophioglossum gramineum Habit



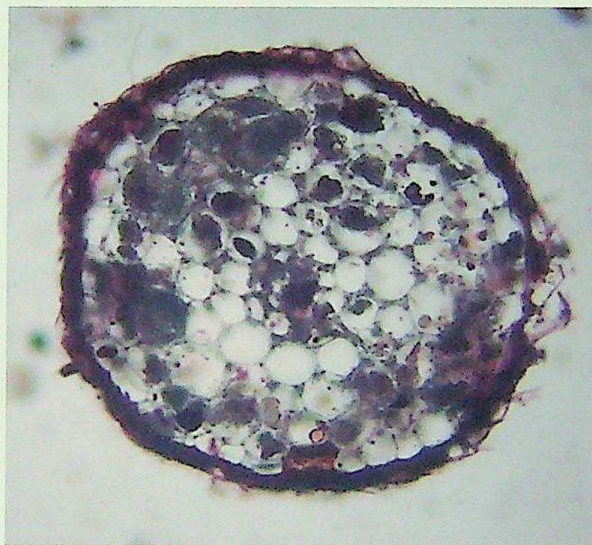
T. S. of root



T. S. of Petiole



Ophioglossum lucitanicum Habit



T.S. of root

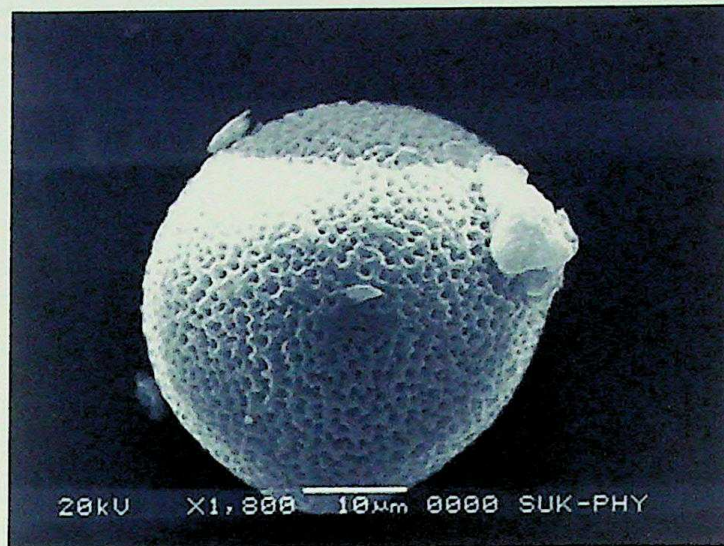
5. ***Ophioglossum gramineum***: T. S. of root shows presence of mycorrhizic fungus and starch grains with monarch stele and T. S. of petiole shows 4-11 vascular bundles which are arranged in circular arrangement.
6. ***Ophioglossum lusitanicum***: T. S. of root shows presence of mycorrhizic fungus and starch grains with monarch stele.

***In vitro* gametophyte development:** For *in vitro* gametophyte development spores of *O. costatum* and *O. reticulatum* spores were inoculated on Nitsch and Nitsch basal medium and incubated at 28⁰C with dark conditions, but *in vitro* development of gametophyte was found to be difficult even after several trials.

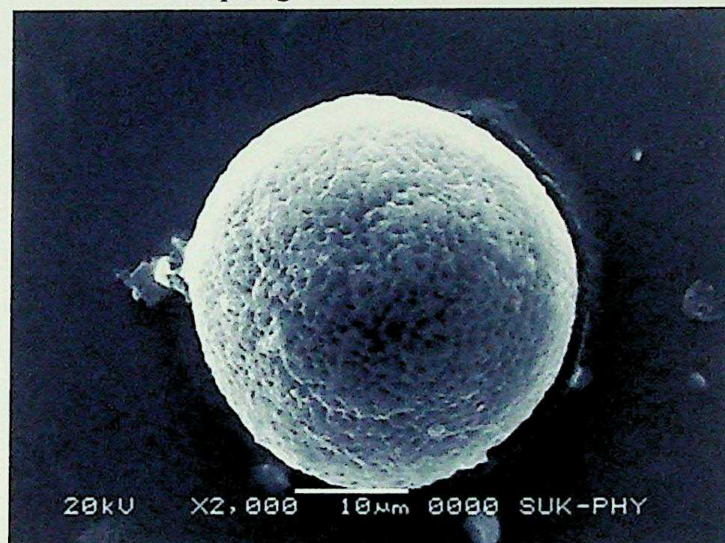
Scanning electron microscopic (SEM) study of spores: According to Erdtmans (1971) acetolysis method scanning electron microscopic study of spores was carried out in *O. reticulatum*, *O. costatum*, and *O. vulgatum*. with Joel JSM-6360 Scanning Electron Microscope. Results of SEM study are as follows.

1. ***Ophioglossum reticulatum***: Spores prolate spheroidal, non spinulose, 40X 40 µm, exine reticulate.
2. ***Ophioglossum costatum***: Spores prolate spheroidal, non spinulose, 35.62 X 35 µm, exine reticulate.
3. ***Ophioglossum vulgatum***: Spores oblate spheroidal, non spinulose, 44.34 X 47.82 µm, exine reticulate

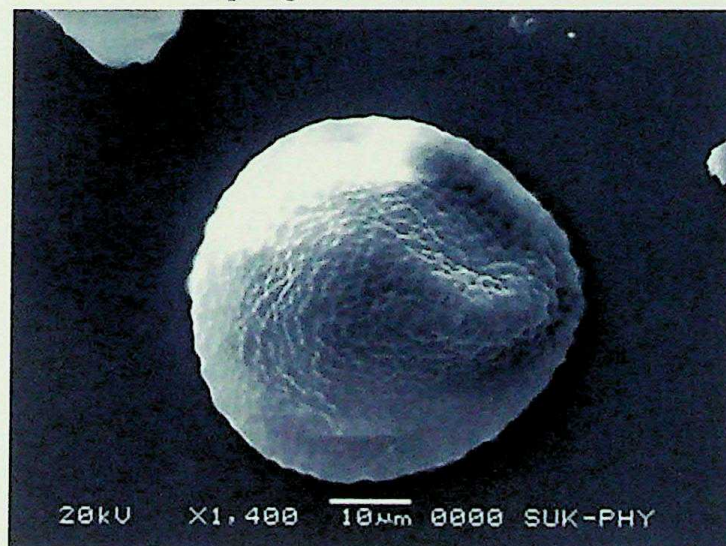
SEM photographs of spores



Ophioglossum reticulatum



Ophioglossum costatum



Ophioglossum vulgatum

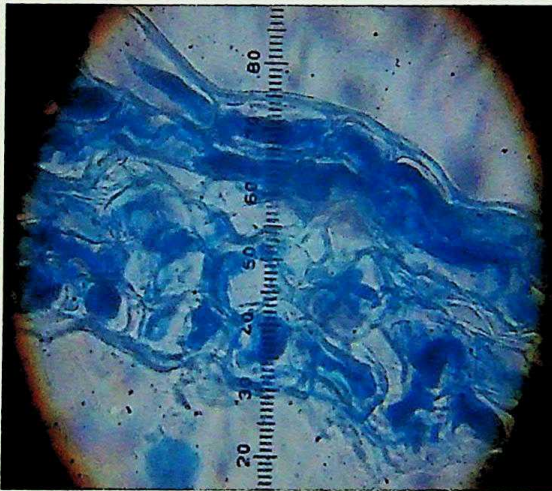
Leaf Spot of *Ophioglossum*:

During the present study of conservation of *Ophioglossum* sp in garden, we have noticed the incidence of leaf spot disease on *Ophioglossum reticulatum* only caused by *Alternaria alternata*. Details of the same are given below.

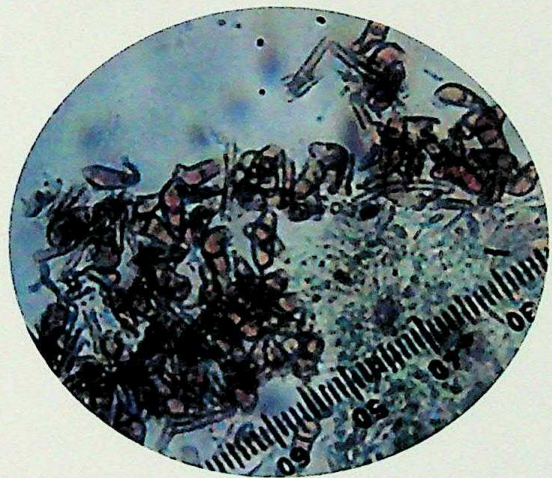
- **Symptoms** :- Small, round, elliptical dry spots appears on the margine of leaves. These spots rapidly increase in size centrifugal growth and became oval, circular or irregular shape. Progressive growth of spot leads to destruction of whole leaf lamina under favorable condition. Internally leaf tissue of infected part is completely invaded by fungal hyphae and haustoria.
- **Colonies** olive black without aerial mycelium growing rapidly on P.D.A (Potato Dextrose Agar). reaching 5-6cm diameter on MEA (Malt Extract Agar) in 7-8 days at 25-30°C,
- **Conidiophores** short, simple straight, 3- septate medium/pale golden brown $50 \pm 2.5\mu$ long $\times$ $5 \pm 2\mu$ wide.
- **Conidia** (from culture) often in long chains & 2-10 in numbers. , muriform with 3-5 transverse septa in conidial walls rough in lower part with longitudinal or slightly oblique septa, ovoid, ellipsoidal or obclavate often with a short or cylindrical beak, smooth or verruculose medium golden brown, pale. $45 \pm 15 \times 10 \pm 2\mu$; beak pale and up to one third ($1/3$) the length of conidium.



Leaf spot of *Ophioglossum reticulatum*



T.S. passing through infected part of leaf



Alternaria alternata (Fr.) Keissil. conidia

12. ACHIEVEMENTS FROM THE PROJECT:

The distribution pattern of *Ophioglossum* species from Maharashtra was studied. Similarly anatomical and palynological aspects were studied in different *Ophioglossum* species. During conservation studies leaf spot disease on *Ophioglossum reticulatum* was first time reported in India.

13. SUMMARY OF THE FINDINGS:

From the above study it was observed that *Ophioglossum* species are commonly observed in different districts of Vidharbha and Western Ghats of Maharashtra. These species are growing well in humus rich, clayey soils, sandy beds of streams, banks of rivers, ponds and other water sources.

O. reticulatum, *O. vulgatum*, *O. costatum*, *O. gramineum* and *O. nudicaule* are the commonly occurring species in all studied area and which are showing great variations in morphology (plant height, leaf size, number of stolons) whereas *O. lusitanicum*, and *O. nudicaule* var *macrorrhizum* are only observed in Vidharbha area.

Anatomical study of root, petiole and spike stalk revealed that presence of mycorrhizic fungus and starch grains with monarch to diarch steel in root sections. In case of petiole it was observed that presence 2-11 vascular bundles in circular arrangement, whereas 3-11 vascular bundles were observed in spike stalk of all *Ophioglossum* sp studied. These bundles are collateral and endarch in nature.

Scanning electron microscopic study of spores of three *Ophioglossum* species was carried out. This study revealed the presence of prolate spheroidal to oblate spheroidal shape with reticulate exine. Spores are none spinulose in nature. Spines observed on the spore wall are with uniform size.

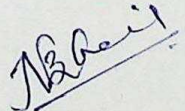
During the present study of conservation of *Ophioglossum* sp in garden, we have noticed the incidence of leaf spot disease on only *Ophioglossum reticulatum* caused by *Alternaria alternata*. It was the first report of leaf spot disease on any *Ophioglossum* sp. in India.

However, gametophyte development under *in vitro* condition was found to be difficult in present study.

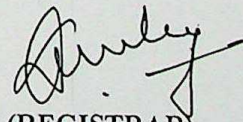
14. CONTRIBUTION TO THE SOCIETY: Leaf spot disease on *Ophioglossum reticulatum* caused by *Alternaria alternata* was reported for the first time from India.

15. WHETHER ANY Ph.D. ENROLLED/PRODUCED: No
OUT OF THE PROJECT

16. NO. OF PUBLICATIONS OUT OF THE PROJECT: One paper has been accepted for Bionano-Frontier Journal. Print of the same is attached.


(PRINCIPAL INVESTIGATOR)

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(REGISTRAR)

Registrar,
Shivaji University, Kolhapur.

Leaf Spot of *Ophioglossum* in India- a new report

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Abstract:

Ophioglossum reticulatum a pteridophytic plant having highest chromosome number $2n=1260$ is commonly growing in forest areas below bushy shrubs. During the present study leaf spots were observed on *Ophioglossum reticulatum* plants caused by *Alternaria alternata*. The spots are observed on mature and young leaves and showed fast spreading of necrotic areas which cover the leaf lamina. The causal organism *Alternaria alternata* was isolated from the infected leaves and it was first time reported on *Ophioglossum reticulatum*.

Key words: *Ophioglossum reticulatum*, leaf spot, *Alternaria alternata*

Leaf spot on *Ophioglossum reticulatum* was observed during 2009-10 till 2010-11 rainy seasons at Department Botanical garden of Shivaji University Kolhapur. Small, round, elliptical dry spots appears on the margin of leaves. These spots rapidly increase in size centrifugal growth and became oval, circular or irregular shape. Progressive growth of spot leads to destruction of whole leaf lamina under favorable condition. Internally leaf tissue of infected part is completely invaded by fungal hyphae and haustoria. On microscopic examination the leaf spot has been identified and described as *Alternaria*

alternata (Fr.) Keissil. The specimen has been deposited in Departmental herbarium SUK of Shivaji University Kolhapur.

For the morphological characteristics of fungus it was cultured on Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) at $25 \pm 2^{\circ}\text{C}$, for 7-8 days. Colony shows olive black without aerial mycelium growing rapidly having short, simple straight conidiophores, 3-septate medium/pale golden brown $50 \pm 2.5\mu$ long $\times$ $5 \pm 2\mu$ wide.

Conidia (from culture) often in long chains & 2-10 in numbers, muriform with 3-5 transverse septa in conidial walls rough in lower part with longitudinal or slightly oblique septa, ovoid, ellipsoidal or obclavate often with a short or cylindrical beak, smooth or verruculose medium golden brown, pale. $45 \pm 15 \times 10 \pm 2\mu$; beak pale and up to one third ($1/3$) the length of conidium.

Leaf Spot of *Ophioglossum*

Symptoms :- Small, round, elliptical dry spots appears on the margine of leaves. These spots rapidly increase in size centrifugal growth and became oval, circular or irregular shape. Progressive growth of spot leads to destruction of whole leaf lamina under favorable condition. Internally leaf tissue of infected part is completely invaded by fungal hyphae and haustoria.

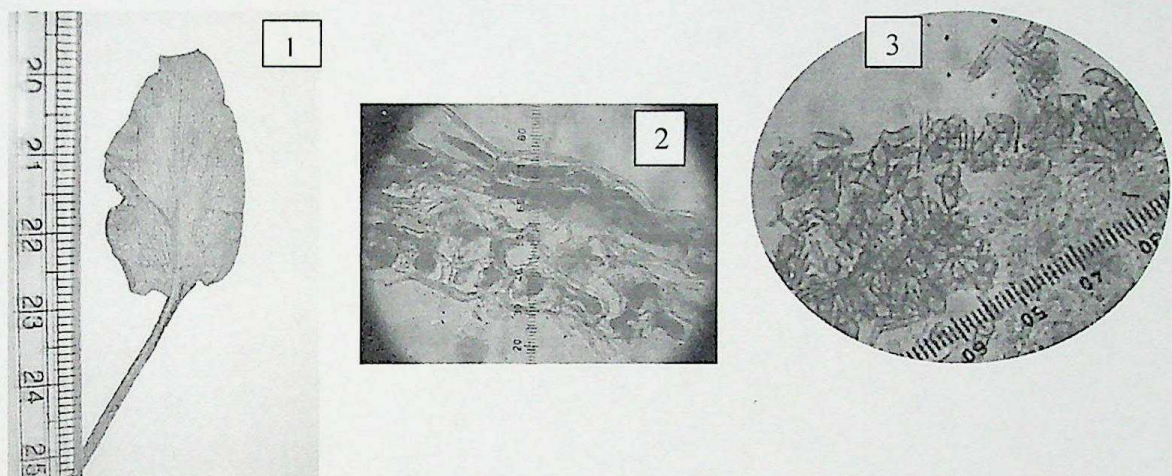


Fig1:1 Leaf Spot of *Ophioglossum reticulatum* caused by *Alternaria alternata* (Fr.) Keissil;
2. T.S. passing through infected part of leaf and 3. *Alternaria alternata* (Fr.) Keissil. conidia
Alternaria alternata (Fr.) Keissil.

Colonies olive black without aerial mycelium growing rapidly on P.D.A (Potato Dextrose Agar). reaching 5-6cm diameter on MEA (Malt Extract Agar) in 7-8 days at 25-30°C,

Conidiophores short, simple straight, 3- septate medium/pale golden brown $50 \pm 2.5\mu$ long $\times$ $5 \pm 2\mu$ wide.

Conidia (from culture) often in long chains & 2-10 in numbers. , muriform with 3-5 transverse septa in conidial walls rough in lower part with longitudinal or slightly oblique septa, ovoid, ellipsoidal or obclavate often with a short or cylindrical beak, smooth or verruculose medium golden brown, pale. $45 \pm 15 \times 10 \pm 2 \mu$; beak pale and up to one third ($1/3$) the length of conidium.

Acknowledgements:

The authors (NBG and RVG) are thankful to University Grants Commission, New Delhi for providing financial assistance. Thanks are also due to the Head, Department of Botany, Shivaji University, Kolhapur for facilities and encouragement.

References:

Nagmani, Kunwar and Manoharachary C.(2006) Handbook of Soil Fungi. I. K. International Publishing House Pvt. Ltd., New Delhi- 112-114 pp.