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## **A STUDY ON IAA'S INFLUENCE ON FERN LIFE CYCLES: FOCUS ON CERATOPTERIS THALICTROIDES IN SITAMATA WILDLIFE SANCTUARY**

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**Abstract:** Pteridophytes, characterized by their affinity for shade and moisture, are a significant component of Rajasthan's vegetation, with 21 genera and 39 species documented in the region. The Sitamata forest, located in Chittorgarh and Pratapgarh districts, stands out as a biodiversity hotspot, renowned for its rich diversity of pteridophytes. Notably, *Ceratopteris thalictroides*, a prominent species within this botanical group, holds cultural and medicinal significance, being consumed as a leafy vegetable and valued for its antibacterial properties against *Streptococcus mutans* and *Shigella flexneri*, as reported by Behera et al. (2022). This paper explores the ecological importance and cultural relevance of pteridophytes in Rajasthan's landscape, drawing upon historical records and recent scientific studies. It traces the pioneering work of Bir and Goyal (1982), who provided the first comprehensive inventory of pteridophytic flora in the iconic Mt. Abu region, laying the groundwork for subsequent research endeavors. Through a synthesis of botanical surveys and ethnobotanical investigations, the paper sheds light on the diverse uses and ecological functions of pteridophytes within the region. By highlighting the medicinal and nutritional value of *Ceratopteris thalictroides* and other pteridophytic species, the paper underscores the potential contributions of these plants to human health and well-being. This study contributes to a deeper understanding of the ecological significance and cultural significance of pteridophytes in Rajasthan's landscape. It underscores the need for integrated approaches to biodiversity conservation that recognize the intrinsic value of these plants while addressing the socio-economic needs of local communities.

**Keywords:** Pteridophytes, Biodiversity, Ethnobotany, Medicinal plants, Conservation

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### INTRODUCTION

Pteridophytes, known as shade and moisture loving plant, form a sizeable component of the vegetation of Rajasthan. This group is represented by 21 genera and 39 species (Gena, 1998). Bir and Goyal (1982) were the first to provide comprehensive records of the pteridophytic flora of Mt. Abu, listing 22 species of ferns. Sitamata forest, located in Chittorgarh and Pratapgarh district in the southern part of Rajasthan, comprises 422.95 sq. km and is known to be one of the richest localities of pteridophytes in the state. Behera et al. (2022) reported that *Ceratopteris thalictroides* is consumed as a leafy vegetable and used medicinally, with an antibacterial activity against *Streptococcus mutans* and *Shigella flexneri*. However, the taxa in this area are likely facing extinction due to natural and manmade factors (Yadav, 2008). This necessitates the formulation of conservational strategies for this rapidly depleting group of plants. In light of this present work, the effect of different concentrations of Indole-3-Acetic Acid (IAA) from 1 to 10 Parts Per Million (ppm) in an even mode after 1 ppm treatment on spore germination and gametophytic development, starting from 2-dimensional (2-D) growth, spatulate growth, cordate gametophyte and gametophyte with germinal organs of *Cheilanthes thalictroides* has been studied. Previous studies have investigated the effect of growth hormones on spore germination and gametophyte development in ferns (Hurel-Py, 1943; Miller, 1961; Hickok and Kiriluk, 1984). An extensive review of the gametophytic generation and experimental studies has been provided by Bir (1987). Sharma and Vangani (1988) investigated the effect of Gibberellin (GA<sub>3</sub>) on spore germination, gametophyte development and sex expression in *Cheilanthes farinosa* Forssk. They reported that small linear gametophytes were protandrous and did not produce archegonia at any stage of development. Large bilobed gametophytes have distinct median ridge and were protogynous. According to them, there was a male to hermaphrodite sequence in prothallial development and the antheridial system was not evident. Gupta and Bhambi (1991) have investigated that cytokinins are better than other hormones for the induction and promotion of spore germination, leading to the faster rate of gametophyte and sporophyte development in

*Adiantum capillus-veneris* L. Vidhya et al. (2000) tested 2, 4-D and GA<sub>3</sub> for their effects on spore germination and gametophyte development in three ferns viz., *Ceratopteris thalictroides* L., *Pityrogramma calomelanos* (L.) Link and *Pteris vittata* L. They observed that effect of 2, 4-D on gametophyte development in *Ceratopteris* and *Pityrogramma* were more pronounced than in *Pteris*. Higher concentration of 2, 4-D was found to be detrimental to the gametophyte development. Higher concentrations of GA<sub>3</sub> stimulated early germination of spores of *Pteris*; delay in *Pityrogramma* and without any significant effect in *Ceratopteris*. Low concentration of GA<sub>3</sub> stimulated early appearance of antheridia in *Ceratopteris* and *Pityrogramma* and reduction of number of archegonia were observed in *Ceratopteris*. Purohit and Bohra (2007) described gametophyte development and impact of various plant growth regulators (GA<sub>3</sub>, IAA and morphactin) on sex expression and sporophyte formation in *Equisetum* L., *Cheilanthes* Sw. and *Hypodematum* Kunze which were collected from different localities in the Aravalli hills, Rajasthan.

### MATERIALS AND METHODS

For germination experiments, spores of *C. thalictroides* found in marshy place along the road side punga pond (route to jhakham dam) of Sitamata sanctuary from Pratapgarh district were collected in the months of August-September. Spores were surface sterilized by 2% sodium hypochlorite solution and then kept on Knop's nutrient

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medium (Half strength) in petri-plates. For control set of experiments, one set of petri-plates containing 100 spores on nutrient medium (without growth regulators) was treated as control. Similarly, IAA treatment of vary concentration was given to another set of petri-plates containing 100 spores of selected taxa. Total seven sets of petri plates (including one set as a control) containing spores were taken for study. The culture medium used for these experiments consisted of Knop's major elements and Nitsch's trace elements (1 ppm). The composition of Knop's medium was as follows: KNO<sub>3</sub>: 100 mg; MgSO<sub>4</sub>: 100 mg; Ca(NO<sub>3</sub>)<sub>2</sub>: 400 mg; K<sub>2</sub>HPO<sub>4</sub>: 100 mg; Distilled water to make dilution of 1000 ml. Composition of Nitsch's medium was as follows: H<sub>2</sub>SO<sub>4</sub>: 0.5 ml; MnSO<sub>4</sub>: 3.0 ml; ZnSO<sub>4</sub>: 7H<sub>2</sub>O: 500.0 mg; H<sub>3</sub>BO<sub>3</sub>: 25.0 mg; CuSO<sub>4</sub>, 5H<sub>2</sub>O: 25.0 mg; NaMoO<sub>4</sub>, 2H<sub>2</sub>O: 25.0 mg; CoCl<sub>2</sub>: 25.0 mg; Distilled water: 1000.0 ml.

Illumination was provided by two 40 watt fluorescent tubes kept at a distance of 60 cm. Spores were allowed to germinate in a culture chamber maintained at 25 ± 2°C. For each treatment, spores were sown in two sets of petri-dishes (7.5 cm. diameter) thus total 14 petri dishes were used each pair is used for different concentration in ppm (1, 2, 4, 6, 8, and 10 ppm as well as control) of IAA. The data are based on counts of 100 spores from each petri-dish.

A control set was invariably included in all the experiments.

### Treatment of IAA

For treatment of IAA on spore germination after 48 h of dark inhibition were made to germinate on liquid Knop's medium (half strength) supplemented with Nitsche's trace elements in 7.5 cm petri-dishes. White light was obtained by two fluorescent tubes fixed 60 cm above the petri-dishes. Different sets of petri dishes were supplied with different concentrations of IAA. Stock solutions of required concentration of the plant hormone were prepared to investigate the effect of IAA, and the desired concentrations for the experiment were incorporated in the culture medium and kept in petri-dishes before sprinkling spores on the surface of the medium. Stock solution of IAA was prepared by dissolving the required quantity of the chemical in 2 ml. of ethanol, the required volume of distilled water being added afterwards. Further concentrations were made by diluting this stock solution. Entire experimental work was carried out under aseptic conditions including the use of sterilized glassware in a thermostatically controlled culture chamber in the laboratory. The temperature during the course of these experiments was maintained at 25± 2°C. Microscopic observations were made on an Olympus HSA microscope. Most of the photomicrographic work was done from the temporary preparations using photomicrographic attachment on Olympus trinocular microscope.

### RESULTS

To determine the effect of IAA, spores of *C. thalictroides* were treated with different concentrations of IAA ranging from 1 to 10 ppm. The data presented in Table 1 indicate that spores of selected taxa require different period for spore germination. The spores started to germinate after 9 days under control where only 55% of spores were able to germinate while rest undergone dormant and later disintegrate. 10 ppm concentration of IAA has been recorded to induce earliest (within 7 days) with minimum spore germination (25%) as compared to other treating concentrations of IAA and control treatment (Table 1). An increase in percent spore germination was observed under 1ppm (60.00%) to 6ppm (66%) concentration as compared to control (55%) treatment. Amongst different concentrations of IAA, maximum spore germination (66.6%) was observed in 2-6 ppm. However, a decrease in

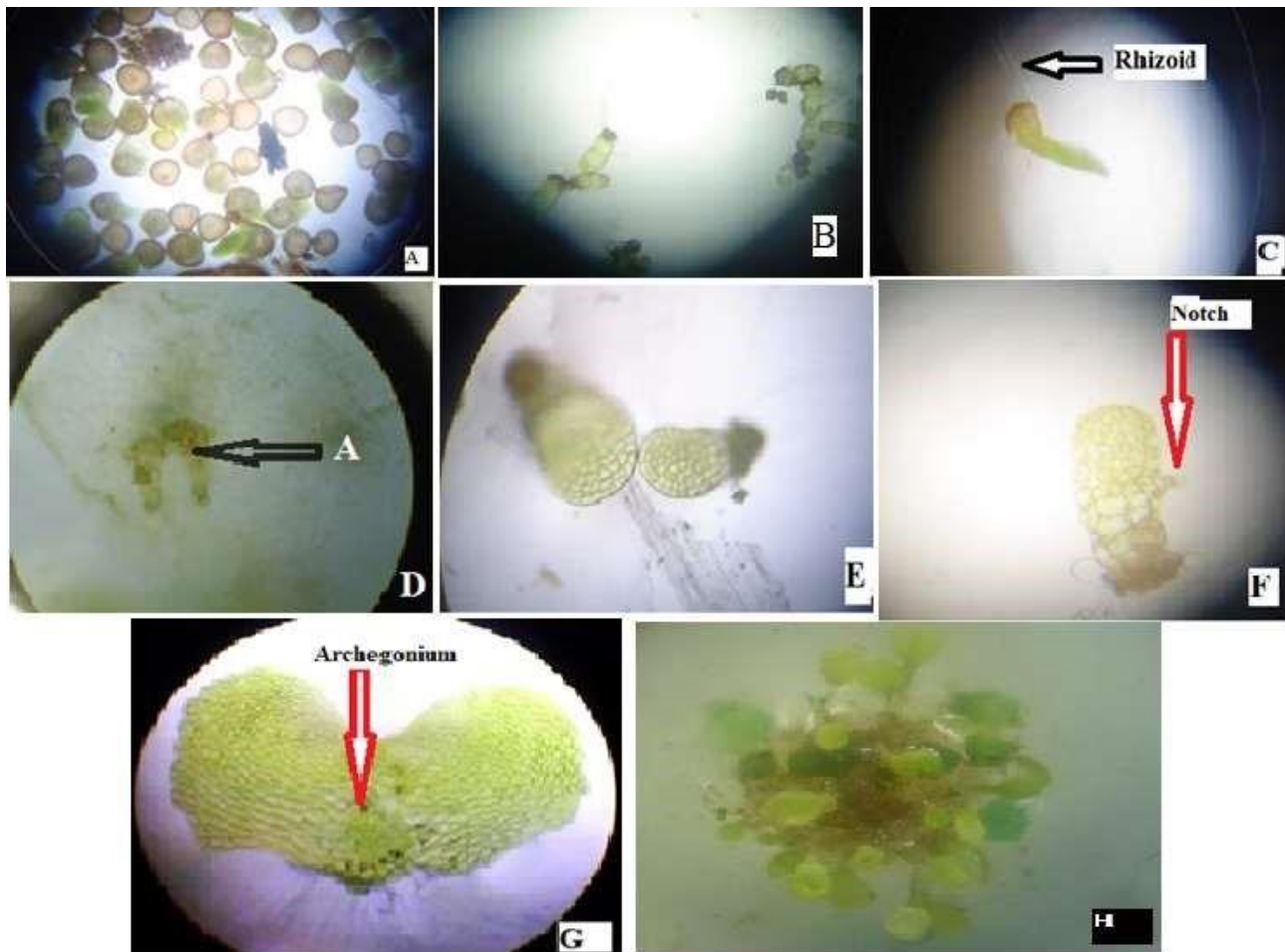
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percentage spore germination after 6 ppm IAA concentration has been observed. Data represented in Table 2 indicates that number of cells in protonemal filament is found to be varying in different concentrations of IAA at different time periods. 2-D gametophyte percentage was found maximum (80%) after 12 days of sowing in 1ppm of IAA while minimum (23%) after 19 others were lies in between these two. Similarly, the days of sowing in 10 ppm and percentage growth of highest percentage growth of spatulate gametophyte (67%) and cordate gametophyte (71%) are observed in 1 ppm of IAA treatment after 13 and 17 days of sowing respectively (Figure 1, D and E). Sex organs were developed after a month of spore inoculation. However, the further growth of these gametophytic portions underwent decline from 6 to 10 ppm (Table 2). The gametophytes were found protandrous under control and hormonal treatment. Antheridial formation took place in spatulate gametophyte (Figure 1D). Sex organs begin to develop after 20 days of sowing. Antheridial formation takes place earlier then archegonia and it is found that treatment of IAA usually has not promontory effect for the development of antheridia in compare to control (66%) except 1 ppm (55%). However, 1 ppm of IAA causes earlier promoter effect for the development of archegonia (61%) in compare to control (45%) and other concentrations of IAA. Similarly, Yadav and Uniyal (2021) studied in vitro spore germination and gametophyte development of *Drynaria mollis* Beddome, and *Pteris aspericaulis* wall and noticed early development of antheridia after 90 days of sowing and later archegonial formation began after 100 days of sowing in both the treated species. Delayed formation of sex organs and the least number of antheridia and archegonia are recorded in 10 ppm. Sporophytes were observed after 45 days in control while in treated plates they appeared earlier between 35-38 days of sowing. Maximum percentage of sporophytes (80%) was calculated in 4 ppm and 6 ppm hormonal treatment (Figure 1). The least percentage observed in 10 ppm after 2 months of sowing

**Table 1.** Effect of different concentrations of IAA on spore germination of *Ceratopteris thalictroides* from Sitamata wild life sanctuary, Rajasthan.

| Serial number | Name of plant <i>Ceratopteris thalictroides</i> |             |                     |
|---------------|---|-------------|---------------------|
|               | Concentration of IAA (ppm)                      | Days sowing | after % germination |
| 1.            | Control   | 9           | 55                  |
| 2.            | 1   | 8           | 60                  |
| 3.            | 2   | 8           | 66                  |
| 4.            | 4   | 9           | 66                  |
| 5.            | 6   | 9           | 66                  |
| 6.            | 8   | 10          | 40                  |
| 7.            | 10  | 7           | 25                  |

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**Figure 1.** Spore germination and different stages of gametophyte development in *Ceretopteris thalictroides*, Sitamata forest (AH). A. Initiation of Spore germination in 4 ppm concentration of IAA (after 9 days of sowing); B-C. Protonema formation with rhizoids in 8 and 2 ppm of IAA respectively; D. Spatulate gametophyte with “Antheridia (A)” in control (after 14 days of sowing); E. Spatulate gametophyte in 6 ppm of IAA (after 17 days of sowing); F. Cordate gametophyte showing notch in 6 ppm IAA (after 21 days of sowing); G. Mature prothallus showing Archegonia in 2 ppm IAA (after 32 days of sowing); H. Clusters of Sporophyte in 8 ppm concentration of IAA (after 38 days of sowing).

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**Table 2.** Effect of different concentrations of IAA on gametophyte development in *Ceratopteris thalictroides*.

| Serial number | IAA Conc. (ppm) | Protonemal filament |                    | Initiation of 2-D growth |                     | Spatulate gametophyte |                            | Cordate gametophytes |                          | Development of sex organs |  |                   |
|---------------|-----------------|---------------------|--------------------|--------------------------|---------------------|-----------------------|----------------------------|----------------------|--------------------------|---------------------------|--|-------------------|
|               |                 | Days after sowing   | No. of cell sowing | Days after sowing        | % of 2D gametophyte | Days after sowing     | % of spatulate gametophyte | Days after sowing    | % of cordate gametophyte | Days after sowing         | Gametophyte with Antheridia <sup>o</sup> | Days after sowing |
| 1             | Control         | 10                  | 3                  | 12                       | 40                  | 14                    | 64                         | 18                   | 63                       | 27                        | 66                                       | 35                |
| 2             | 1 ppm           | 10                  | 7                  | 12                       | 80                  | 13                    | 67                         | 17                   | 71                       | 27                        | 52                                       | 30                |
| 3             | 2 ppm           | 10                  | 7                  | 11                       | 45                  | 13                    | 56                         | 18                   | 54                       | 26                        | 35                                       | 32                |
| 4             | 4 ppm           | 11                  | 3                  | 12                       | 44                  | 15                    | 60                         | 19                   | 50                       | 26                        | 43                                       | 32                |
| 5             | 6 ppm           | 10                  | 5                  | 12                       | 64                  | 17                    | 52                         | 21                   | 53                       | 25                        | 42                                       | 31                |
| 6             | 8 ppm           | 12                  | 6                  | 13                       | 36                  | 18                    | 36                         | 20                   | 20                       | 23                        | 37                                       | 28                |
| 7             | 10 ppm          | 9                   | 6                  | 19                       | 21                  | 18                    | 23                         | 17                   | 16                       | 53                        | 28                                       | 46                |

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### . DISCUSSION

The effect of growth regulators on spore germination under different concentration of IAA indicate that the spores of *C. thalictroides* start to germinate earlier in different concentrations of treated hormone except 8 ppm where it causes little delayed to germinate. In control the germination was recorded on 9th days of sowing. Raghavan (1971b) reported that the longer period of exposure of white light for the induction of spore germination may be due to longer hydration period. Under 10 ppm of IAA treatment the spores of *C. thalictroides* germinate earlier while percent spore germination decreases gradually from the concentration of 1 to 8 ppm. Thus, the spore germination is not much affected under PGRs in the selected fern taxa of Sitamata forest as reported by Sharma et al. (1996) in some common ferns of Rajasthan. Roshni and Hegde (2020) suggested that the spore germination of *Pityrogramma calomelanos* L. needed knops medium for in vitro culture and follow vittaria-type of spore germination pattern. Prothallus development is of Ceretopteris-type. In general, germination and protonemal development take one to two weeks under optimum conditions and mature gametophytes are formed after 6-8 weeks

(Table 2). Similar observations were made by

Praptosuwiryo (2017) who reported that the germination of spores in *Platyserium wande* Racib takes place between 7-14 days on natural media. However, in *C. thalictroides*, fertilization can take place only in two weeks after germination (Stein, 1971). The whole life cycle can be completed in vitro in 3-4 months in *C. thalictroides* and

*C. pteridiodes* (Loyal and Chopra, 1977). The gametophytic development of *Christella hispidula* (Deene) Holttum has been described and illustrated by Bejoy et al. (1994).

Sex organs were developed after a month of spore inoculation. The gametophytes were found protandrous under control and hormonal treatment. Maximum percentage of gametophytes with sporophytes in *C. thalictroides* (Table 1) was observed in 4 and 6 ppm concentration of IAA in comparison to the control. A decrease in percentage development of sporophytes in selected taxa is observed under higher concentration of IAA. 1ppm concentration of IAA has been found effective in antheridial development however; Ohishi et al. (2021) suggested that GA<sub>4</sub> stimulates both protonemal elongation and antheridium formation in *Lygodium japonicum* Thunb. On the other hand, IAA promoted protonema elongation but causes reduced antheridium formation. Overall it may be concluded that formation of spatulate gametophytes and antheridial development in this taxa are not favoured by hormone, especially at higher concentration of IAA, while archeogonial development is enhanced by the minimum concentration of treated hormone as compare to control in the selected fern taxa.

### CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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