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EFFECT OF COPPER ON SPORE GERMINATION OF *Pteris vittata* Linn.

Subtitle: Gametophyte of *Pteris vittata*L can grow in Cu stress condition

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Abstract

Nowadays heavy metal contamination in environment is becoming a big problem. The aim of this research was to study the effect of heavy metal copper on spore germination of Pteris vittata Linn. The concentrations of copper sulphate were 0 ppm, 5 ppm and 50 ppm. The characteristics of germinated spores were observed on an interval of 10 days and the study was continued till 30th day from the day of spore sowing. The result showed that the spore germination percentage and rhizoid mean number both increased gradually day by day in case of control set and 5 ppm set, though in 5 ppm set spore germination was delayed but in 50 ppm set, spore germination started very late as compared to the former two sets and the germinated spores were arrested in single celled stage, no further development occurred till 30th day.

Key words: Pteris vittata Linn, copper sulphate, spore germination, protonema, spatulate, cordate.

I. INTRODUCTION

Heavy metals are significant environmental pollutants and their toxicity is an increasing problem. The term "heavy metals" refers to any metallic element that has a relatively high density. The major hazardous heavy metals of concern in terms of their environmental load and health effects are lead, mercury, chromium, cadmium, copper and aluminium. Many heavy metals are considered to be essential for plant growth. Some of these heavy metals like Cu and Zn either serve as cofactor or activators of enzyme reactions e.g. informing enzyme/substrate metal complex (Mildvan 1970). Copper is an essential heavy metal for higher plants and algae particularly for photosynthesis (Mahmood and Islam 2006; Chatterjee et al. 2006) but enhanced industrial and mining activities have contributed to the increasing occurrence of Cu in ecosystems. Cu is also added to soils from different human activities including mining and smelting of Cu-containing ores. Excess of Cu in soil plays a cytotoxic role, induces stress and causes injury to plants and animal. This leads to plant growth retardation and leaf chlorosis (Lewis et al. 2001). Exposure of plants to excess Cu generates oxidative stress and ROS (Stadtman and Oliver 1991). Oxidative stress causes disturbance of metabolic pathways and damage to macromolecules (Hegedus et al. 2001).

Physical approaches such as scavenging or burial of the contaminated surface soil or washing out of Cu from the contaminated field with or without electrical dialysis are effective but often too expensive for a large scale remediation. Phytoremediation, popularly known as "green clean" is a novel strategy for the removal of toxic contaminants from the environment by using plants. This concept is increasingly being adopted, as it is a cost effective and user friendly alternative. Many plants can remediate heavy metals from soil and water. The roots of Indian mustard are found to be effective in the removal of Cd, Cr, Cu, Ni, Pb and Zn and sunflower can remove Pb, U, Cs-137 and Sr-90 from hydroponic solutions (Zaranyika and Ndapwadza, 1995; Wang *et al.*, 2002; Prasad and Freitas, 2003). Water hyacinth accumulates trace elements such as Ag, Pb, Cd, etc. and is efficient for phytoremediation of wastewater polluted with Cd, Cr, Cu and Se (Zhu *et al*., 1999).

Fern spores are successfully used to screen the hyper accumulating ferns and also to test the toxicity of the metal contaminated samples. Chinese brake, *Pteris vitata* Linn., exhibits considerable

promise in the phytoremediation of arsenic-contaminated sites worldwide due to its unique ability of hyper accumulating arsenic. Since ferns have the characteristics of both primitive and land plants, an understanding of biological mechanism of hyper accumulation is necessary (Bondada and Qiying, 2003).

Pteris vittata Linn. is a roadside fern found in all places. They are present in all environmental conditions and are also show their sensitivity to heavy metal like arsenic. They are cytologically diversified (Manickam and Irudayaraj, 1988). The presence of cytological diversity which is the indication of genetical diversity provides a chance of having more of hyper accumulating potentiality. Before the application of a plant in phytoremediation program, it should be thoroughly checked up for its hyper accumulating capacity. Since ferns are with independent sporophytic and gametophytic generation, it is necessary to carry out such studies in both sporophytic and gametophytic generations. The present study was aimed to study the effect of heavy metal Copper (Cu) on spore germination of *Pteris vittata* Linn.

II. MATERIALS AND METHODS

Matured spores *of Pteris vittata* Linn. were collected from the natural habitats at Santiniketan,West Bengal. At first the fronds, bearing mature spores of *Pteris vittata* Linn. were kept in paper bags and placed in a dry and hot place for two days. After spores were released, they were sterilized with 0.1% mercuric chloride for 4-6 minutes and rinsed with sterile distilled water and used as explants.

The sterilized spores were sown onto half strengthed Murashige and Skoog (MS) medium solidified with 1% agar and supplemented with three concentrations of copper sulphate (0, 5 and 50 ppm) in glass petri plates. The inoculation operation was performed under the laminar air flow chamber. The cultures were kept in culture room. The temperature of the culture room was maintained at 22 ± 2˚C temperature and 3000 Lux light intensity. Light intensity was provided for 16 hours light photoperiod followed by 8 hours dark period. The observations were made on 10^{th}, 20^{th} and 30^{th} day from the spore sowing date. Spore germination percentage, rhizoid formation, protonema, spatulate and cordate structures were observed using research microscope.

III. RESULTS

In control set the spore germination started on seven to ten days from the day of spore sowing and the spore germination percentage reached 88.80 on 10^{th} day. The protonemal structures were mostly uniseriate (fig. 3.E). In 5 ppm set spore germination started on nine to ten days from the spore sowing date thus on 10^{th} day all germinated spores formed were either single celled or two celled protonema (fig. 3.D). On the other hand there was no germination in 50 ppm set till 10^{th} day. The mean No. of rhizoids was half in 5 ppm set of the control set, which is 0.80.

On 20^{th} day control set showed 90.00% spore germination, where it was 85.04% in of 5 ppm set and in case of 50 ppm set it was 5.36%. Both uniseriate and biseriate protonema, spatulate stages and few cordate structures were observed in control set. In 5 ppm set only protonemal stage was observed; among them biseriate stage (fig. 3.F) was fewer than uniseriate stage; spatulate structures were also observed. In 50 ppm set only single celled protonemal structures were seen. The mean No. of rhizoids was 4.40, 3.20 and 0 in control, 5 ppm and 50 ppm set respectively.

The spore germination percentages were 95.35%, 87.71% and 12.50% in control, 5 ppm and 50 ppm sets respectively on 30^{th} day. In 50 ppm set spore germination percentage had increased but the protonemal structures were still in single cell stage. In control set most of the germinated spores were in cordate stage and in 5 ppm set mostly were in spatulate stage. Mean No. of rhizoids were 12 in control and 11 in 5 ppm set but there was no rhizoid formation observed till 30^{th} day.

IV. CONCLUSIONS

From the above study we can conclude that *Pteris vittata* Linn. spores can germinate in copper contaminated environment but in that case spore germination is quite delayed and germination percentage and mean number of rhizoids are affected and morphological differences from control environment are absent. The gametophyte of *Pteris vittata* thus can withstand in Cu contaminated soil and may be used as a tool of phytoremadiation in polluted soil. Further study is required for determining maximum and minimum capacity of copper tolerance and hyper accumulation ability of gametophyte of *Pteris vittata* Linn.

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Table – 1: Characteristics of spore germination in Pteris vittata Linn. in 0, 5 and 50 ppm concentrations of copper sulphate on 10th day from spore sowing.

Concentrations of $CuSO_4$	Spore germination %	Mean No. of rhizoids	Stages			
			Protonema		Spatulate	Cordate
			Uniseriate	Biseriate		
0 ppm (control)	88.80	1.60	+++	++	---	---
5 ppm	46.97	0.80	Only one or two celled protonema were found			
50 ppm	0	0	No germination			

Table – 2: Characteristics of spore germination in Pteris vittata Linn. in 0, 5 and 50 ppm concentrations of copper sulphate on 20th day from spore sowing.

Concentrations of $CuSO_4$	Spore germination %	Mean No. of rhizoids	Stages			
			Protonema		Spatulate	Cordate
			Uniseriate	Biseriate		
0 ppm (control)	90.00	4.40	++	++	++	+
5 ppm	85.04	3.20	+++	++	+	---
50 ppm	5.36	0	Only single celled protonema were found			

Table – 3: Characteristics of spore germination in Pteris vittata Linn. in 0, 5 and 50 ppm concentrations of copper sulphate on 30th day from spore sowing.

Concentrations of $CuSO_4$	Spore germination %	Mean No. of rhizoids	Stages			
			Protonema		Spatulate	Cordate
			Uniseriate	Biseriate		
0 ppm (control)	95.35	12.00	---	+	++	+++
5 ppm	87.71	11.00	+	+	+++	+
50 ppm	12.50	0	Only single celled protonema were found			

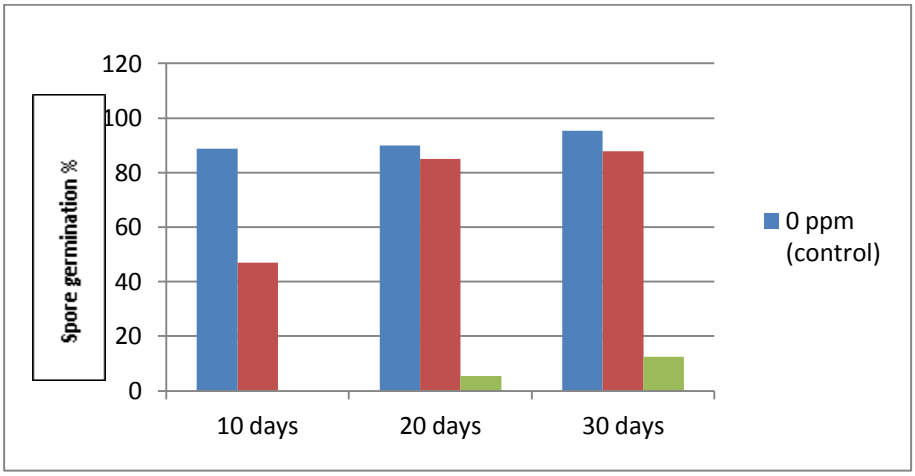

***Figure – 1. Effect of different copper concentrations in spore germination percentage of Pteris vittata* L.**

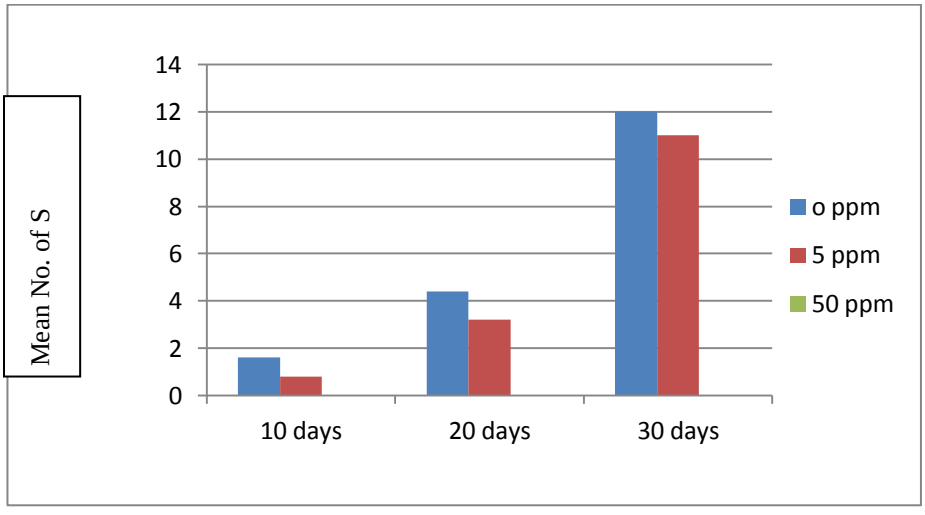

***Figure – 2. Effect of different copper concentrations in mean No. of rhizoids of Pteris vittata* L.**

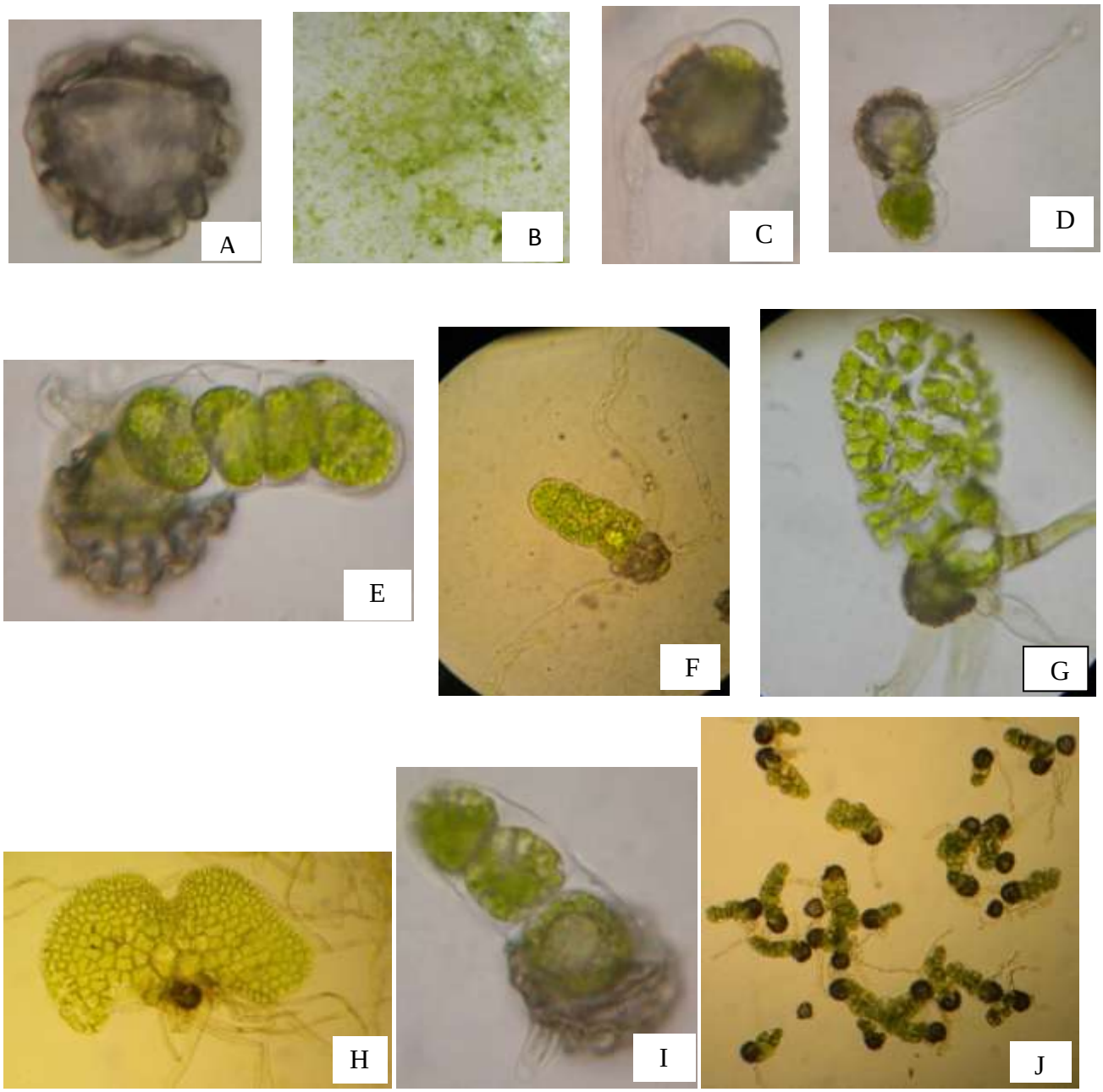

Figure -3. Pteris vittata Linn. spore germination. A. spore of P. Vittata. B. Germinated spores of control set. C. Single celled germinated spore of control set. D. 2-celled germinated spore of 5 ppm set. E. Uniseriate protonema of control set. F. Biseriate protonema of 5 ppm set. G. Spatulate stage of control set. H. Cordate stage of 5 ppm set. I. Rhizoid formation. J. Germinated spores in 10x.

