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Research Article

ANTIFUNGAL ACTIVITY AND STAINING PROPERTIES OF SOME SYMMETRICALLY FUNCTIONALIZED METAL PHTHALOCYANINE DYES

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ABSTRACT

Effects of 1, 8, 15, 22-tetranitro Co(II) and Zn(II) phthalocyanines [3-NO_2CoPc, 3-NO_2ZnPc], 1,8,15,22-tetracyano Co(II) phthalocyanine [3-CNCoPc] and 2, 9, 16, 23-tetranitro iron(III)chlorophthalocyanine [4-NO_2FePc] on the growth of the fungus *Aspergillus niger* were studied. All the four complexes inhibited the radial spread of the fungus selected for the study to different extents. A considerable inhibitory effect on growth was seen even in l ppm concentration. The order of inhibitory effect, measured for a period of 5 days is 3-NO_2 ZnPc > 3-CNCoPc > 3-NO_2CoPc > 4-NO_2FePc. The presence of the peripherally substituted phthalocyanine complexes is found to alter the pigmentation property of the selected fungus. Safranin-phthalocyanine double staining technique using 3-NO_2CoPc, 3-NO_2ZnPc, 4-NO_2FePc and 2, 9, 16, 23-tetrahydroxy iron(III)chlorophthalocyanine [4-OHFePc] on the stem sections of *Hemidesmus indicus R Br* and *Polygonum punctatum Buch-Hum* have been studied.

Keywords: Phthalocyanines, *Aspergillus niger*, Staining, *Hemidesmus indicus*, *Polygonum punctatum*.

1. INTRODUCTION

Metal phthalocyanine complexes are very interesting class of bluish green coloured nontoxic materials in which the electronic environment around the metal is similar to that of biologically important molecules like haemin and chlorophyll [1]. The electronic structure and redox properties of the metal phthalocyanines made them applicable as industrial dyes and pigments [1-5], electrochemical catalysts [2, 6-7], materials for solar cells [8-10], thermally stable fuel cell electrodes [11-12], electrochromic display devices [13-16], photochromic display devices [17-18], data storage devices [19-21] and liquid crystal colour display devices [22-23]. Demonstration of the use of water soluble metal phthalocyanine derivatives as potential photosensitizers for photodynamic cancer therapy was considered as an important application of metal phthalocyanine complexes in biology and medicine [24-27]. Substituted metal phthalocyanine complexes and their gold nanoparticle and graphene conjugates have been known to exhibit antibacterial and photodynamic antibacterial activities. Representative precedents are mentioned here [28-32]. Though precedents related to staining of substrates like mucin, nuclear chromatin in insect gut, myelin sheaths, mitochondria, virulant germs and lipids, collagen and polycyclic mutagens using commercial unsubstituted phthalocyanine dyes such as Alcian blue, Monastral blue B,Luxol fast blue MBS, Heliogen blue SBL, Pontamone fast turquosie 8GL are reviewed [1, 33], reports on histological staining of sections of medicinal plants using simple substituted metal phthalocyanine have been scanty. In addition, literature survey reveals that the reports on the antifungal activity of the phthalocyanines on the fungus are meagre [34-35]. In this paper, we report the effects of 1, 8, 15, 22-tetranitro Co(II) and Zn(II) phthalocyanines [3-NO_2CoPc, 3-NO_2ZnPc], 1,8,15,22-tetracyano Co(II) phthalocyanine 3-CNCoPc) and 2, 9, 16, 23-tetranitro iron(III)chlorophthalocyanine [4-NO_2FePc] on the growth of the fungus *Aspergillus niger*, and the results of safranin-phthalocyanine double staining technique using 3-NO_2CoPc, 3-NO_2ZnPc, 4-NO_2FePc and 2, 9, 16, 23-tetrahydroxy iron(III)chloro-phthalocyanine [4-OHFePc] on *Hemidesmus indicus R Br* and *Polygonum punctatum Buch-Hum*. Both the plants are of

considerable medicinal importance in the southern interior Karnataka, India. Solubility of the complexes in dimethylsulphoxide (DMSO) and the associated high molar absorption coefficient in the blue-green region of the electromagnetic radiation are the criteria in selecting the above complexes for the studies.

2. MATERIAL AND METHODS

2.1. Material

All the chemicals used were of analytical grade and from either Merck or SD Fine chemicals. Distilled water was used wherever necessary.

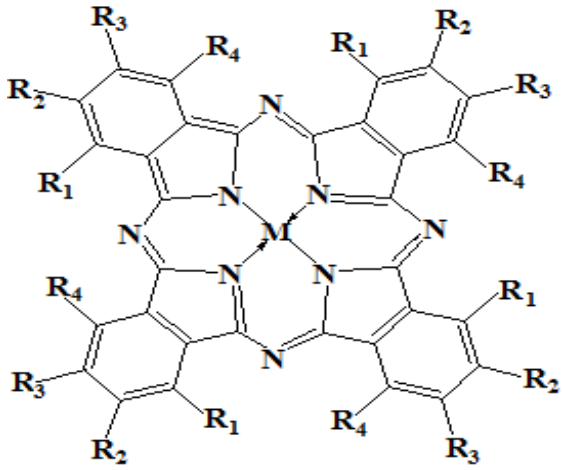
2.2. Methods

The metal phthalocyanine complexes 3-NO_2CoPc, 3-NO_2ZnPc, 4-NO_2FePc, 4-OHFePc and 3-CNCoPc were prepared as described elsewhere [36-38]. Pure cultures of *Aspergillus niger* were obtained from colonies grown by exposing the potato-dextrose-agar (PDA) medium to air. 3-4 cm stem pieces of *Hemidesmus indicus* and *Polygonum punctatum* plants were collected from the fields. The samples were fixed by placing in a solution of 5 mL formalin, 5mL glacial acetic acid and 90 mL 50% alcohol for 4 hours and dehydrated by carrying through the solutions of increased concentrations of tertiary butyl alcohol (TBA) in water and alcohol [39]. A rotatory microtone was used to take the thin sections of the paraffin infiltrated and embedded samples. The selected microtone sections were fixed to the slides and stained with 0.5 % safranin solution in absolute alcohol for 5 minutes. The sections were washed with water and rinsed with 95 % alcohol. The stained sections were counter stained with 0.05% solutions of 3-NO_2CoPc, 3-NO_2ZnPc, 4-NO_2FePc, and 4-OHFePc in DMSO for two minutes. The double stained sections were washed thoroughly with DMSO, water and with 95% alcohol, treated briefly with xylene and mounted using coverslip with DPX mountant. Sections on the slides were also stained individually with safranin or the selected substituted phthalocyanine complexes for comparison.

The potato dextrose agar (PDA) media was prepared using 300 g potato, 25 g dextrose, 25g agar-agar and required quantity of the test complex in 500 mL. The substituted metal phthalocyanine complexes required for the preparation of 1 ppm, 10 ppm, 100 ppm, 1000 ppm and 2000 ppm solutions in PDA media were weighed accurately, dissolved in 2ml of dimethyl sulphoxide separately and adulterated to the PDA media. The sterilized adultrated PDA media was poured into the sterilized petri dishes in an aseptic chamber. *Aspergillus niger* was inoculated as point at the centre of the each petri dish. The petri dishes were incubated at 23+1°C.

3. RESULTS AND DISCUSSION

The results and analyses of the synthesis and physico-chemical characterization of the title complexes were reported elsewhere [36-38]. The structure of the symmetrically functionalized metal phthalocyanine complexes used for this study is given in fig. 1.



R_1= NO_2; R_2 = R_3=R_4=H; M = Co(I) [3-NO_2CoPc]
R_1= NO_2; R_2= R_3= R_4=H; M= Zn(II) [3-NO_2ZnPc]
R_2= NO_2; R_1= R_3= R_4 = H; M = Fe(III) [4-NO_2FePc]
R_2 OH; R_1=R_3=R_4=H; M=Fe(III) [4-OHFePc]
R_1= CN; R_2= R_3=R_4= H; M= Co(II) [3-CNCoPC].

Fig. 1: Structure of symmetrically tetrasubstituted metal phthalocyanines

The UV-visible spectra of these complexes are characteristic of two bands. A band at ~340 nm (B band) and the other in the range of 650-680 nm (Q band). The molar absorption coefficient for both the bands was found to be 10^4-10^5.

3.1. Effect on fungal growth

In the present investigation, Poison-Food-Technique was employed wherein 3-NO_2CoPc, 3-NO_2ZnPc, 4-NO_2FePc and 3-CNCoPc in the concentration ranges of 1-2000 ppm were added to the media in which *Aspergillus niger* fungi were cultured. DMSO was used to dissolve the complexes before dispersing it in the media. Growth of the fungus was tested in the fixed volume of the unamended and amended media with 2 mL of DMSO. Since no difference in the fungal growth between these two systems was observed, media with 2 mL of DMSO

was considered as control. The observed radial growth for a period of 2 days and 5 days with respect to various concentrations in ppm are summarized in figure 2.

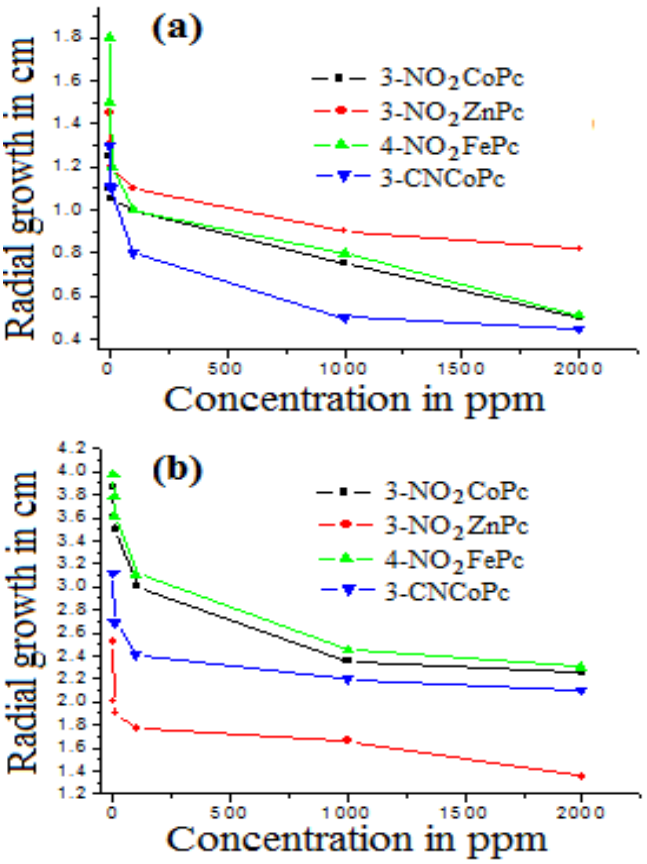


Fig. 2: Effects of symmetrically tetrasubstituted metal phthalocyanines, 3-NO_2CoPc, 3-NO_2ZnPc, 4-NO_2FePc and 3-CNCoPc on the radial growth of the *Aspergillus niger* after 2 days (a) and after 5 days (b) of incubation.

Fungi, a class of chlorophyllous plants are nucleated thallophytes, which are unable to manufacture their own food in nature from carbon dioxide and water. Fungi depend on external source for their food, unlike green plants. Hundreds of species of Aspergillus, which are known to cause deterioration of stored food grains, have been identified. The representative species of this genus, *Aspergillus niger* was selected for the study to test the effect of the selected phthalocyanine complexes on their growth.

All the four examined complexes when poisoned with PDA media inhibit the radial spread of the fungus selected for the study, to different extents and hence the selected complexes are found to be antifungal. The results are contrasting in nature when compared to those with respect to water soluble 2, 9, 16, 23-tetrasulpho and tetracarboxy copper phthalocyanines [34], where the complexes promotes the growth of the fungus. After two days of incubation, the fungi exhibited minimal growth. Inhibiting effect observed with respect to control increased with increase in concentration of the complexes. A considerable inhibitory effect on growth was seen even in the small concentration of l ppm. When measured after 2 days, 3-NO_2ZnPc inhibited less and 3-CNCoPc inhibited more while 3-NO_2CoPc and 4-NO_2FePc inhibit to the same extent. However, when the observation was extended to a period of 5 days, 3-NO_2ZnPc exhibited a maximum inhibitory effect. This indicates the sustainability of its antifungal nature. The order of inhibitory effect, measured for a period of 5 days on the radial growth of the fungus for various complexes is 3-NO_2ZnPc > 3-CNCoPc > 3-NO_2CoPc > 4-NO_2FePc. An interesting observation made during the experiments was the change in the colour of the fungus. Though *Aspergillus niger* is known for its black colour, in the presence of even a very low concentration (1 ppm), in the presence of the selected phthalocyanine complexes, matured colonies of the fungus were pale brown and the new colonies were pale green (fig. 3).

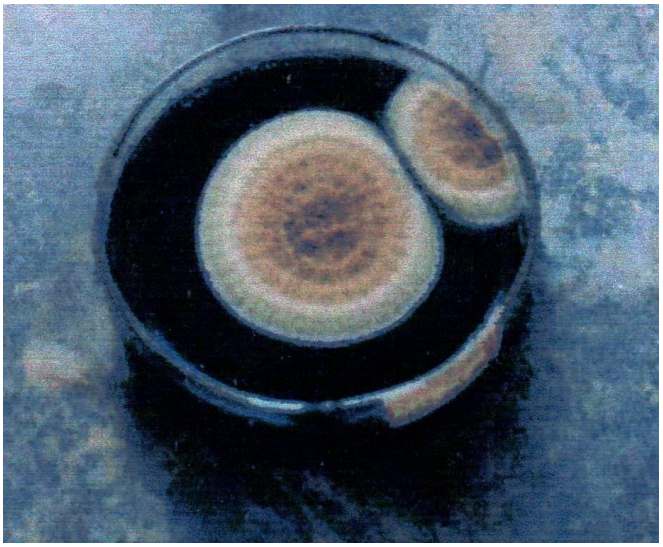


Fig. 3: *Aspergillus niger* grown on PDA media suplimented with 5 ppm 3-NO_2CoPc, observed after five days

Prolonged culture of this fungus in the PDA media amended with the test complexes has not resulted in the black colour of the fungus. It was confirmed by the parallel experiment with and without the addition of 2 mL of DMSO in the media that the change in colour of

the fungus was not due to the presence of DMSO in the medium. The change in colour of the fungus is attributed to the effect of complexes on the pigmentation properties in the growing fungus.

A qualitative and comparative study of the sporulation characteristics of the fungus using ocular-stage microscope and stereo microscope in the control and in the presence of complexes revealed that, the size of the conidial heads appear diminished when cultured in the presence of complexes in the concentration range of l000-2000 ppm when compared to that for the fungus grown in control. Also, watery exudation over the conidial head was observed in the fungus grown on media supplemented with higher concentrations of 3- NO_2CoPc and 4-NO_2FePc.

3.2. Staining properties

The stem sections [40] of *Polygonum punctatum* and *Hemidesmus indicus* were stained clearly with 3-NO_2ZnPc. Parenchymatous cells and vascular bundles of the sections of both the species did not take up the stain of this complex (fig. 4(a) and 4(b)). Pericycle region of the sections took the stain but the staining was not superior to the standard safranin (fig. 4(c) and 4(d)). 3-NO_2ZnPc complex effectively stained the pith or stellar region of the *Hemidesmus indicus*. Conjunctive tissue of the *Polygonum punctatum* was found selectively stained on double staining with safranin and 3-NO_2ZnPc (fig. 5).

3-NO_2CoPc was found to be suitable for staining *Hemidesmus indicus* (fig. 6(a)) and collenchyma of the *Polygonum punctatum* (fig. 6(b)). This complex could be used as a double stain with safranin to selectively visualize the collenchyma and conjunctive tissues of *Polygonum punctatum* (fig. 6(c)). Endodermis, pericycle and cambium of the *Polygonum punctatum* were selectively stained with safranin during the double staining of tissues with 3-NO_2CoPc and safranin.

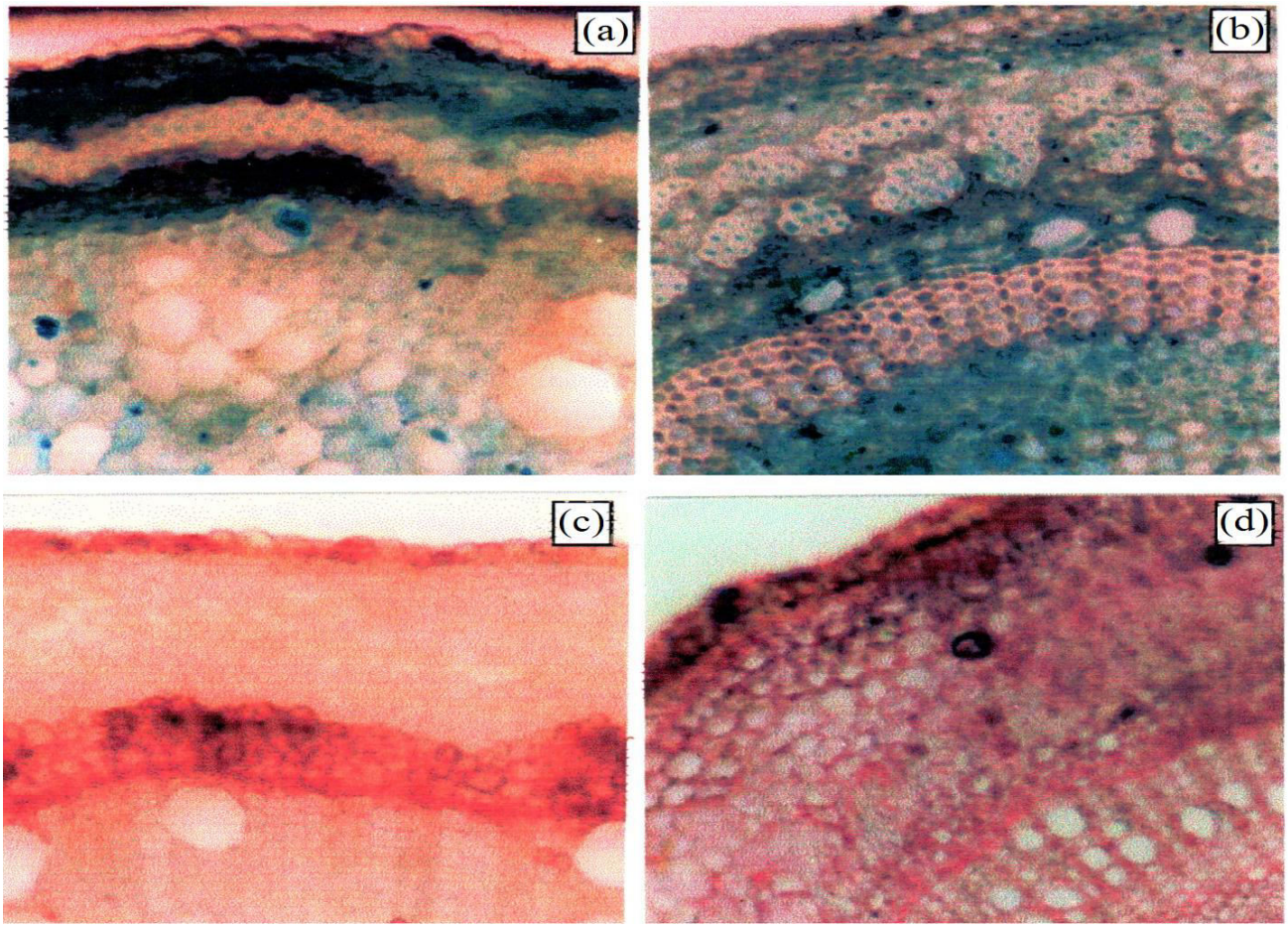


Fig. 4: Stem sections of *Polygonum punctatum R.Br.*, stained with 3-NO_2ZnPc (a),of *Hemidesnmus indicus* stained with 3-NO_2ZnPe (b), of *Polygonum punctatum R. Br.*, stained witlh safranin (c) and of *Hemidesmus indicus* stained with safranin (d)

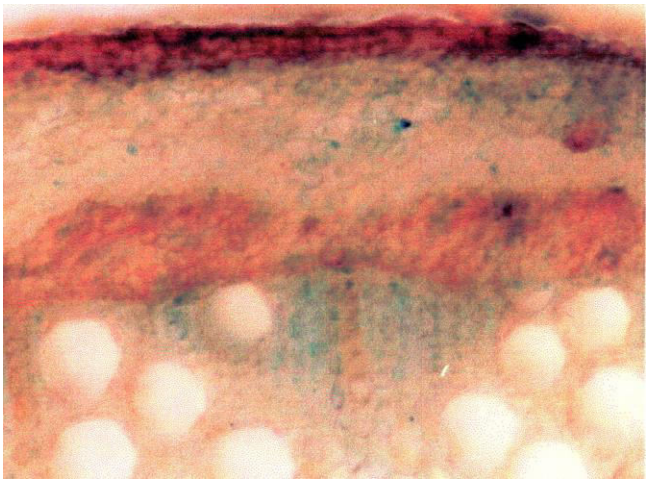


Fig. 5: Stem section of *Polygonum punctatum R.Br.*, double stained with safranin-3-NO_2ZnPc

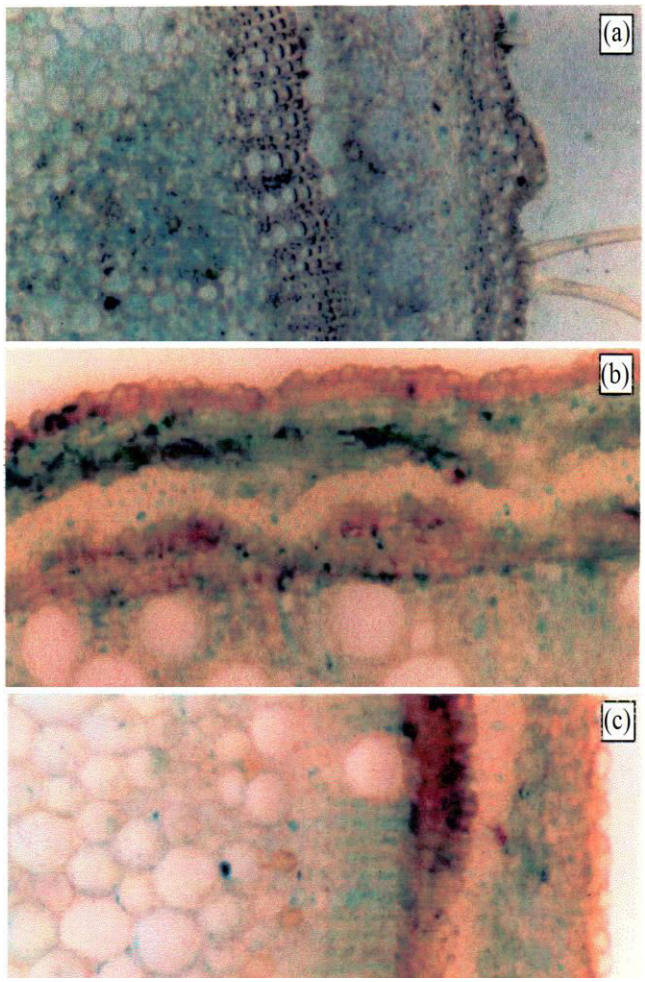


Fig. 6: Stem sections of *Hemidesmus indicus* stained with 3-NO_2CoPc (a), of *Polygonum punctatum R.Br.*, stained with 3-NO_2CoPc (b) and of *Polygonum punctatum R.Br.*, double stained with safranin-3-NO_2COPc (d).

Endodermis region of the *Polygonum punctatum* was found to take up selectivelydark brown coloration on staining with 4-OHFePc eventhough the original colour of thecomplex is dark green (fig. 7(a)). All the regions of the section of *Hemidesmus indicus* were found to be stained by this complex (fig. 7(b)). However, its double staining with safranin highlights endodermis and pith regions (fig. 7(c)).

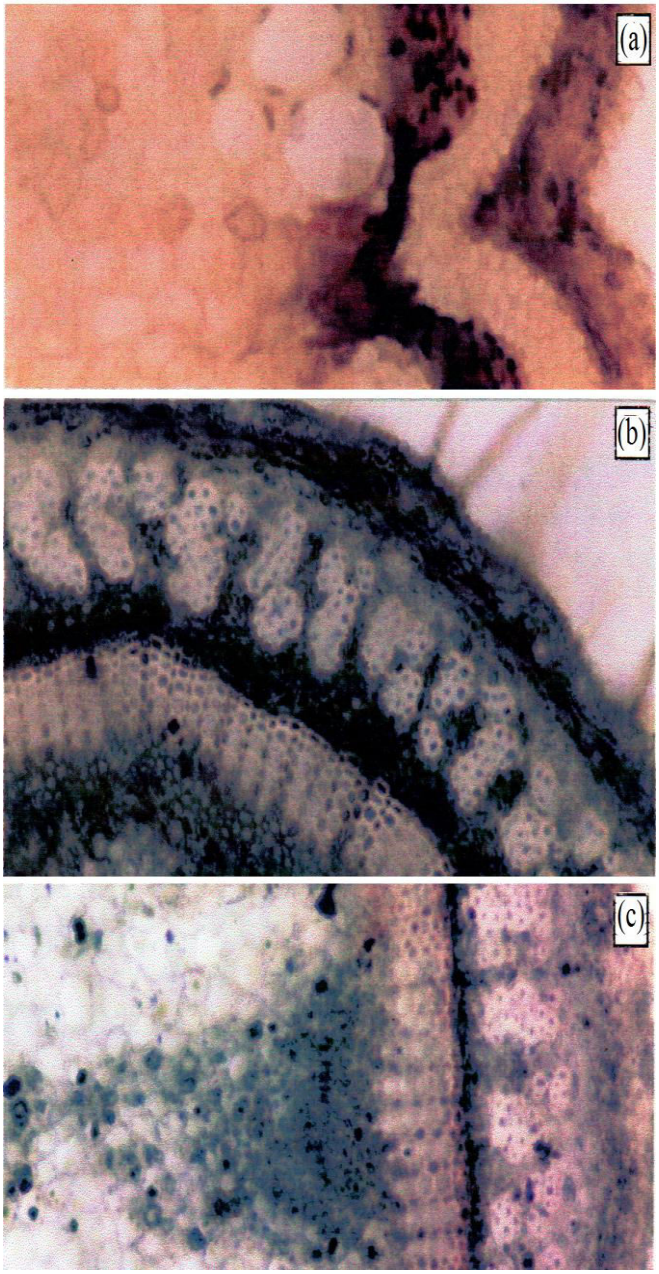


Fig. 7: Stem sections of *Polvgonum punctatum R.Br.*, stained with 4-OHFePc (a), of *Hemidesmus indicus* stained with 4-OHFePc (b) and of *Hemidesmus indicus* double stained with safranin-4-OHFePc(c)

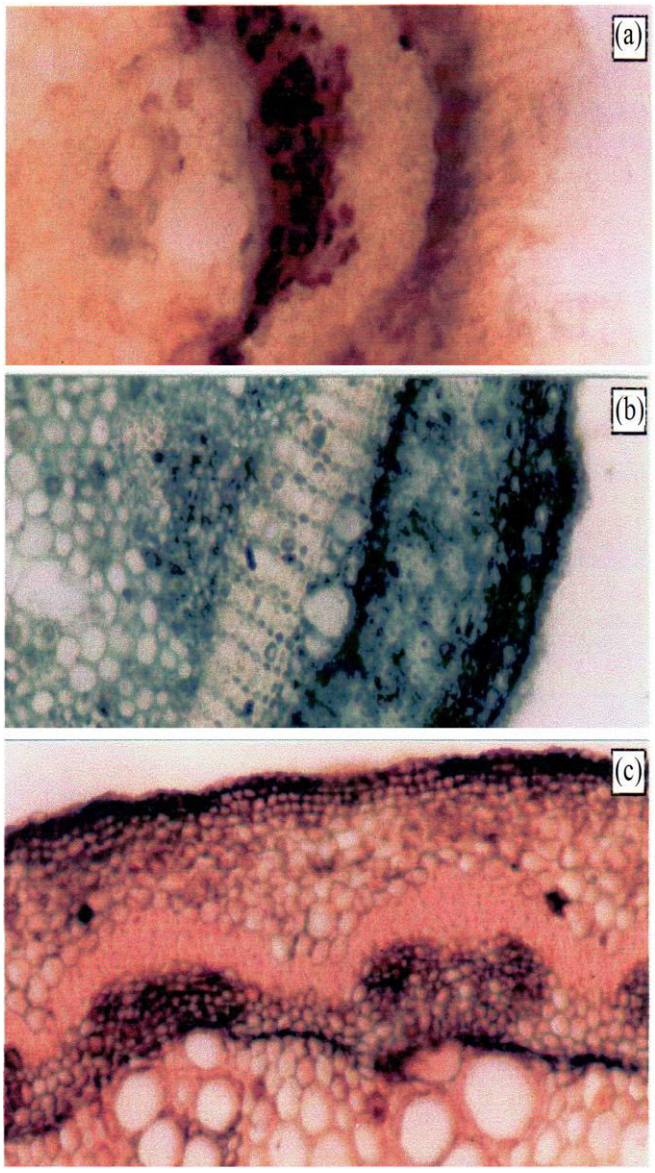


Fig. 8: Stem sections of *Polygonum punctatum R.Br.*, stained with 4-NO_2FePc (a), of *Hemidesmus indicus* stained with 4-NO_2FePc (b) and of *Polygonum punctatum R.Br.* double stained with safranin-4-NO_2FePc (c).

Important observations were made when 3-NO_2FePc was applied as pure compound and as double stain with safranin on *Polygonum punctatumn*. Pericycle of the section of this species was observed to be selectively stained with black colour by 3-NO_2FePc (fig. 8(a) and 8(c)), whereas on double staining with safranin, thin layers of epidermis and cambium were seen dark. Sections of *Hemidesmus indicus* were found to be stained totally with 3-NO_2FePc (fig. 8(b). No significant changes were observed on double staining of this complex with safranin on *Hemidesmus indicus*.

4. CONCLUSION

All the four symmetrically tetrasubstituted phthalocyanine complexes, 1, 8, 15, 22-tetranitro Co(II) and Zn(II) phthalocyanines [3-NO_2CoPc, 3-NO_2ZnPc], 1,8,15,22-tetracyano Co(II) phthalocyanine [3-CNCoPc] and 2, 9, 16, 23-tetranitro iron(III) chlorophthalocyanine [4-NO_2FePc] inhibit the radial growth of the fungus *Aspergillus niger* when adultrated with PDA media. The pigmentation property of *Aspergillus niger* found altered when cultured in the presence of selected complexes as the matured colonies appear pale brown and new colonies appearing pale green. The deep blue to bluish green coloured 3-NO_2CoPc, 3-NO_2ZnPc, 4-NO_2FePc and 2, 9, 16, 23-tetrahydroxy iron(III)chloro-phthalocyanine [4-OHFePc] complexes could be used for visualizing the tissues of *Polygonum punctatum* and *Hemidesmus indicus* by staining and double staining techniques with safranine.

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Conflict of interest

Authors declare that they have no conflict of interest.

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