

## Contribution to the Taxonomy of the genus *Helminthosporium* II

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While studying the mycoflora of this region, the authors have isolated four members of the genus *Helminthosporium* from various sources. When these isolates were compared among themselves and with other species previously recorded they were found to be new species.

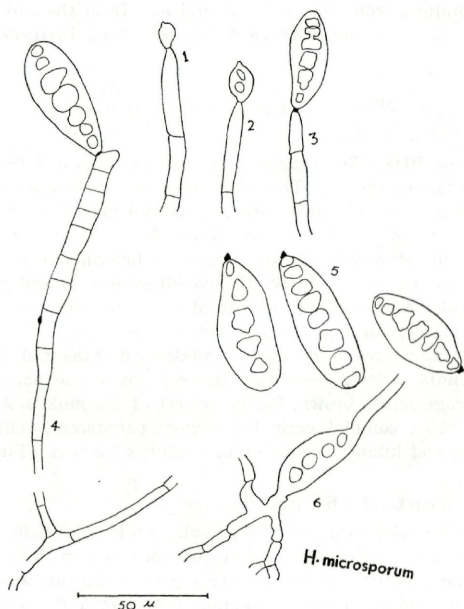


Fig. I. *H. microsporium*. 1, 2 and 3. Stages in the development of the conidium. — 4. Conidiophore with an acrogenous conidium. — 5. Mature conidia. — 6. A germinating conidium

Two of them have already been reported in an earlier communication from this laboratory (Deshpande and Deshpande 1966). The remaining two species have been described in this paper.

#### Material and methods

Dilution plate method was used to obtain isolate from soil. Soil samples (Black cotton soil) were plated on PDA at 1 : 1000 dilutions. The pH of the medium was adjusted to 4.5 to avoid bacterial contamination. Plates were incubated at  $26 \pm 3$  C. When the fungus appeared along with other fungi, it was transferred to other petriplates and after obtaining pure culture it was maintained on PDA slants.

In the case of grass isolate, portions of the leaf spots on leaves of *Cynodon dactylon* Pers. were surface sterilized with alcohol-dipped cotton swab and cut into small pieces by sterilized scalpel. PDA plates were inoculated with 4—5 infected pieces each and after 24 hours' growth the colonies were lifted and inoculated into PDA slants. Cultures of fungi obtained from diseased material and from the soil were called the primary isolates and were used for describing their morphological characters.

#### Morphology of the species

##### Isolate from soil.

Colony on PDA: The fungus grew well on PDA and reached a diameter of 90 mm on 6th day. The colony was round, olivaceous with slight bluish tinge and sporulated on 5th day. Aerial mycelium was abundant and the colony had a wooly texture. *Morphology*: The vegetative hyphae were profusely branched, septate, yellow to brown, darkened with age and measured 4.6—6.9  $\mu$  in width; conidiophores lateral or terminal, narrower, paler and distantly septate at base, gradually broadened into darker, closely septate tip; the tip produced percurrent, sterile, proliferations; conidiophores were 1—16 septate and measured  $234\text{--}468 \times 10.8$   $\mu$ ; conidia were porogenous, distoseptate, borne arogenously or rarely pleurogenously, brown, fusoid, widest at the middle, 2—7 septate,  $26\text{--}41 \times 22$   $\mu$ ; conidial germ tubes were percurrent, conidial scar a simple pore, and hilum a protuberent peglike structure (Fig. 1).

##### Development of the conidium

The first conidium arose as a globular protrusion through the pore at the tip of the conidiophore. This conidium initial maintained its contact with the conidiophore through this pore. A septum soon developed at about the middle of the developing conidium and successive septa were laid down. Simultaneously the point of contact darkened and the hilum in the form of peg like protrusion appeared. The conidial scar was seen as a simple pore after the abstriction of the conidium.

Already about six species have been described from soil. The present isolate was compared morphologically with them (Table 1). It is clear that the conidiophores in this isolate are never fasciculate and are the longest of all. In these two characters conidiophores differed from all species isolated from soil. Conidia also showed certain differences with those of other species in their small size and in their shape, which is fusoid with both ends roughly pointed or one bluntly hemispherical. This fungus possesses smallest conidium so far recorded for species of *Helminthosporium* from soil. In view of these differences this has been described here as a new species.

#### ***Helminthosporium microsporum* Deshpande and Deshpande sp. nov.**

Colony on PDA round, olivaceous with slight bluish tinge, wooly textured; aerial mycelium abundant; hyphae profusely branched, septate, yellow to brown, 4.6—6.9  $\mu$ ; conidiophores lateral or terminal, narrower, pale brown and distantly septate at base, gradually broaden towards the dark, close septate tip; sometimes producing sterile proliferations through the tip, 10—16 septate, 234—468  $\times$  10.8  $\mu$ ; conidia porogenous, distoseptate, borne acrogenously or rarely pleurogenously, brown, fusoid, widest at the middle, 2—7 septate, 26—41  $\times$  22  $\mu$ ; conidial germ tubes percurrent; conidial scar a simple pore and hilum a protuberent peg like structure.

Caespites in PDA orbiculares, olivacei, lenissime coerulescentes, lanuginosi; mycelium aereum bene evolutum; hyphae profuse ramosae, septatae, luteae vel brunneae, 4.6—6.9  $\mu$ ; conidiophora lateralia vel terminalia, pallide brunnea et ad basim remote septata, sursum gradatim latiora, in apice obscura, densiuscule septata, septis 10—16 praedita, 234—468  $\times$  10.8  $\mu$ ; conidia acrogena, raro pleurogena, fusoidae, 2—7-septata, 26—41  $\times$  22  $\mu$ .

#### **Isolate from grass leaves**

This isolate was obtained from the leaves of the grass, *Cynodon dactylon* Pers. The leaves were collected in the vicinity of Millind College, Aurangabad. The spots were ashgray green with brown margin which in turn was surrounded by yellow halo. Dark brown specks with yellow halo were also present on leaves from old plants.

Colony on PDA: The fungus grew fast on PDA and attained a diameter of 85 mm on 6th day. The colony was spreading, round, olivaceous, with inconspicuous advancing white margin, and sporulated on 3rd day. Aerial growth was abundant and the colony had a wooly texture.

#### **M o r p h o l o g y (Fig. 2 and 3)**

The vegetative hyphae were profusely branched, septate, pale olive, darkened with age and measured 2.3—4.6  $\mu$  in width; conidio-

phores arose as lateral branches, usually unbranched, they were of two types; determinate conidiophores were uniform, olivaceous, 1—3 septate, mostly bearing a single terminal conidium, represented about 5% of the total number of conidiophores per microscopic field and measured  $182 \times 5.2 \mu$ ; Indeterminate conidiophores were 5—20 septate, narrower, paler and distantly septate at base, gradually broadened into a darker close septate tip and measured  $208—520 \times 7.8 \mu$ ; the conidiophores proliferated between successive conidia; the conidia were porogenous distoseptate, mostly pseudopleurogenous, at time acrogenous or pleurogenous, olivaceous, darkened at maturity, cylindrical to clavate and measured  $20.8—152 \times 7.8 \mu$ ; conidial germ tubes percurrent, conidial scar a simple pore and hilum a cylindrical protuberance. The conidia did not show any definite pattern of arrangement; when acrogenous, tip bore single conidium or a cluster of two or three conidia; when pseudopleurogenous, conidia were uniparous, or alternate.

#### Development of the conidium

The first conidium arose as a globular protoplasmic protrusion through a pore at the tip of the conidiophore; it enlarged and a septum developed at about the middle of the conidium; successive septa were soon laid down. The tip of the conidiophore elongated pushing aside the first formed conidium and other conidia were formed in a similar manner giving an appearance of pleurogenous arrangement. The point of contact at the base of the conidium darkened and a cylindrical short protuberance (hilum) developed. The conidial scar was seen as a flat ringed pore after the abstriction of the conidium.

Thirtytwo species of *Helminthosporium* have been known so far parasitizing different types of grasses. The present isolate differs from all these species in having two types of conidiophores. Species approaching the dimensions of either conidiophores or conidia of the grass isolate were compared with it (Table 2). It is clear that species with longer conidiophores (*H. siccanis*, *H. triseptatum* and *H. monoceros*) differ from grass isolate in septation, size or mode of germination of conidia, whereas species attaining dimensions of conidia differ either in size of conidiophores, arrangement of conidia or in mode of germination. Thus the comparison justifies its description as a new species and hence owing to the lack of definite pattern of conidial arrangement, it has been designated as *H. appatarnae*.

#### *H. appatarnae* Deshpande and Deshpande sp. nov.

Colony on PDA round, ovilaveous, spreading with white advancing margin, woolly textured; aerial mycelium abundant; hyphae profusely branched, septate, pale olive,  $2.3—4.6 \mu$ ; conidiophores lateral, usually unbranched, of two types; determinate conidiophores uniform, oliva-

ceous, 1—3 septate, mostly bearing single apical conidium,  $182 \times 5.2 \mu$ ; indeterminate conidiophores narrower, paler and distantly septate at base, gradually broadened into a darker, close septate, tip,  $208-520 \times 7.8 \mu$ ; the conidiophores proliferated between successive conidia; conidia porogenous, distoseptate, conidial arrangement pseudopleurogenous, acrogenous or pleurogenous,  $20.8-152.0 \times 7.8 \mu$ , 6—18 septate; conidial germ tubes percurrent, conidial scar a simple pore and hilum a cylindrical protuberence.

Caespites in PDA olivacei, albido-marginati, lanuginosi; mycelium aereum bene evolutum; hyphae profuse ramoe, septatae, pallide olivaceae,  $2.3-4.6 \mu$ ; conidiophora lateralia, plerumque simplicia, biformia:

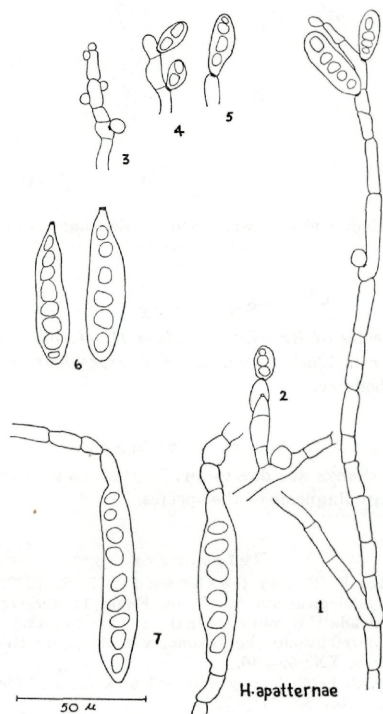


Fig. II. *H. apatternae*. 1. Indeterminate conidiophore with conidia. — 2. A determinate conidiophore with conidium. — 3, 4 and 5. Stages in the development of conidia. — 6. Mature conidia. — 7. Germinating conidia

conidiophora determinata uniformia, olivacea, 1—3-septata, conidium unicum terminale gignentia,  $182 \times 5.2 \mu$ ; conidiophora indeterminata pallidiora, ad basim remonte septata, sursum paulatim latiora, in apice obscuriora, densiuscule septata,  $208-520 \times 7.8 \mu$ ; conidia pseudopleurogena, acrogena vel pleurogena, 6—18-septata,  $20.8-152 \times 7-8 \mu$ .

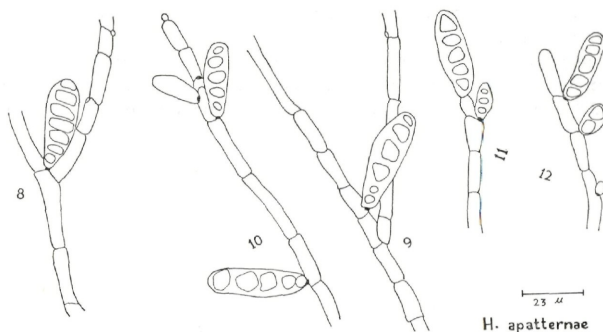


Fig. III. 8—12. Conidiophores with conidia showing different patterns of arrangement

### Summary

Two new species of *Helminthosporium*, *H. microsporium* and *H. apatternae* isolated from black cotton soil and grass leaf spots respectively have been described here.

### Acknowledgements

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Table 1

Comparison between the species of *Helminthosporium* isolated from soil

Name of the species	Conidiophores	Conidia
<i>H. sativum</i> PK and B.	Fasciculate, 150—180 × 6—10 μ, dark reddish brown	Straight or curved, ends rounded, olivaceous, 90—130 × 15—20 μ, 7—14 celled
<i>H. anomalum</i>	bent, long, brown, 105—400 μ	Elongate, straight, rounded at both ends, 5—12 septate, 40—90 × 10—15 μ
<i>H. microsorum</i>	—	Cylindrical
<i>H. victoriae</i> Meeham and Murphy	Erect, single or in clusters, olive, 6—8 septate, 60—280 × 6.8—10 μ	Dark olive, thin walled, slightly curved, 40—130 × 11—25 μ
<i>H. microsorum</i>	Single, Brown, proliferating through the tip. 234—468 × 10.8 μ 10—16 septate	Olivaceous to brown, tapering at both ends, widest at the middle, 26—41 × 22 μ

Table 2

Comparison between the species of *Helminthosporium* isolated from different grasses

Sr. Name of the No. species	Host.	Conidiophores	Conidia
1. <i>H. sicans</i> Drechs.	<i>Lolium multiflorum</i>	Olivaceous, single or in pairs, 50—300 × 7—9 μ	Yellow to brown, 35—130 × 14—20 μ, germination bipolar or lateral

Sr. Name of the No. species	Host.	Conidiophores	Conidia
2. <i>H. triseptatum</i> Drechs.	<i>Holcus lanatus</i>	Dark olive, single or in pairs, 200—400 × 9—12 μ	Dark olive, ellipsoidal or cylindrical, 2—3 septate, 30—50 × 15—21 μ, Germination bipolar or lateral
3. <i>H. monoceros</i> Drechs.	<i>Echinochloa Crus-alli</i>	Dark brown or olivaceous, 120—325 × 6—9 μ	Yellowish to dark olive, 40—150 × 15—22 μ, Germination bipolar
4. <i>H. Panici</i> Von Overeem	<i>Panicum maximum</i>	Grey brown, Single, 250 × 5—7 μ	Pale brown, acrogenous, 70—140 × 13—16 μ, Germination bipolar
5. <i>H. sachhari</i>	<i>Sachharum Officinarium</i>	Deep gray brown, 70—200 × 5.5—7.5 μ	32—120 × 11—17 μ, 3—10 septate
6. <i>H. Stenacrum</i>	<i>Agrostis stolonifera</i>	Dark olive, 80—250 × 7—10 μ	53—135 × 15—23 μ, Germination from any segment
7. <i>H. vagans</i> Drechs.	<i>Poa pratensis</i>	Dark olive, branched, 50—280 × 8—10 μ	Dark olive
8. <i>H. rostratum</i> Drechs.	<i>Eragrostis major</i>	Dark olive, Single or in groups of 3—5, 40—180 × 6—8 μ	Dark olive, rostrate, 32—184 × 14—22 μ
9. <i>H. bromi</i> Diedicke	<i>Bromus inermis</i>	Single or clustered 100—150 × 7—10 μ	108—150 × 13—20 μ, hilum inconspicuous, Germination unipolar or lateral
10. <i>H. dictyoides</i> Drechs.	<i>Festuca elatior</i>	Dark brown or olive, single or in groups, 70—150 × 6—8 μ	Subhyaline to yellow, 23—115 × 14—17 μ, Germination bipolar
11. <i>H. turcicum</i> Passerini	<i>Eleusine indica</i>	In clusters of 2—6, 150—264 μ	45—132 × 15—25 μ
12. <i>H. cynodontis</i> Marignoni	<i>Cynodon dactylon</i> Pers.	80—150 × 6—7 μ	Olivaceous, 27—80 × 11—14 μ, Germination bipolar
13. <i>H. apatternae</i>	— do —	Olivaceous, single; Determinate: 182 × 5.2; indeterminate: 208—520 × 7.8 μ	Olivaceous, cylindrical to clavate, 20.8—152 × 10.4 μ

\* only those species approaching dimension of either conidiophores or conidia of *H. apatternae* are compared.



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