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MYCOPARASITE - AMPELOMYCES IN ARTIFICIAL CULTURE I. MORPHOLOGY AND CULTURAL BEHAVIOUR

by

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ABSTRACT

This paper reports detailed studies and observations made on five isolates of the common mycoparasite on powdery mildews viz. *Ampelomyces quisqualis* CES. in artificial culture. The results revealed some significant variations among these isolates in respect of morphological characters of pycnidia and pycnidiospores and colony characters, which provide evidence on the existence of physiologic forms within this species of the hyperparasite.

INTRODUCTION

The conidial stages of the Erysiphaceae are often parasitized by a pycnidial fungus, *Ampelomyces* CES. (= *Cicinnobolus* EHRENB.) which forms pycnidia in the mycelium, conidiophores and conidia of the powdery mildews and is known to suppress the growth of the Oidial phase of the mildew. (KAMAT & PATWARDHAN, 1966). Although this interesting hyperparasite is widely distributed in the tropics, no reports are available on parasitism, cultural behaviour and other nutritional aspects of this mycoparasite except of a purely taxonomic nature. A detailed investigation was, therefore, undertaken by the writer on these lines. The results obtained on the cultural behaviour of five isolates of this hyperparasite are briefly reported in this paper.

HISTORICAL REVIEW

The earliest references to this fungus report its pycnidia being accessory spore-producing organs of its host – *Oidium* sp. [1]) or *Erysiphe* sp. Some workers utilized the presence of the hyperparasite as a diagnostic character for differentiating Oidial powdery mildews, until CESATI (1852) recognized the fungus as distinct from the powdery mildews and named it *Ampelomyces quisqualis.* DE

[1]) Now referred to as *Acrosporium* sp.

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BARY (1870) was the first to make a detailed study of this genus with special reference to nature of parasitism. He rejected the earlier names and named it as *Cicinnobolus cesatii* under which it had been known for a long time. EMMONS (1930) made some studies into the host-parasite relationship, the mode of pycnidial development and the cultural behaviour of this fungus isolated from *Erysiphe cichoracearum* affecting *Helianthus tuberosus*. ROGERS (1959) discussed the synonymy and pleaded for the validity of the name *Ampelomyces quisqualis* CES.

From India, SYDOW & BUTLER (1916) first recorded the occurrence of the hyperparasite on *Oidium* sp. parasitizing *Phaseolus mungo* var. *radiatus* from Pusa (Bihar). This hyperparasite was subsequently reported by VENKATRAYAN (1946), CHONA & MUNJAL (1956), RAO & SALAM (1959), PRASAD et al. (1960), PATWARDHAN (1964), BHATNAGAR et al. (1966), KAMAT & PATWARDHAN (1966) and KOTHARI & BHATNAGAR (1966) all referring their collections to the type species viz. *Ampelomyces quisqualis* CES. (= *Cicinnobolus cesatii* DE BARY) on the basis of morphological studies. The only report from India on the parasitism of this hyperparasite and its role in suppressing the host mildew is by KAMAT & PATWARDHAN (1966).

MATERIALS AND METHODS

The hyperparasite was collected from the conidial stages (*Acrosporum* sp. and *Oidiopsis taurica* (LEV.') SALM.) of the powdery mildews affecting five hosts belonging to diversified families viz. *Abelmoschus esculentus* W. & A. (FAM. MALVACEAE), *Cyamopsis tetragonoloba* (L.) TAUB. (Fam. Papilionaceae), *Impatients balsamina* L. (Fam. Balsaminaceae), *Pedilanthus tithymaloides* POIT. (Fam. Euphorbiaceae) and *Zinnia elegans* JAC*. (Fam. Compositae). Isolations were made on poured plate agar to obtain the hyperparasite in pure culture and these were designated as *Isolate A*, *Isolate, C*, *Isolate I*, *Isolate P* and *Isolate Z* according to the name of the plant host respectively. The isolates were grown in duplicate petridishes of uniform size containing uniform quantity of agar media (15 cc.) using standard formulae [1]) (AINSWORTH, 1961). Colour of of the colony was determined by comparison with C.M.I.'s Mycological Colour Chart prepared by RAYNER (1970).

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[1]) Composition:

M_2Agar NaCl: 10 gm., yeast extract 5 gm., Glucose : 10 gm., $KH_2POï$: 0.1 gm., $MgSO_4$: 0.05 gm., Agar: 20 gm., Glycerine: 6 ml., Distilled water: 1 L.

BROWN's Agar: L-Aspargine: 2 gm., D-glucose: 2 gm., K_3PO_4: 1.23 gm., $MgSO_4$: 0.75 gm., Agar: 20 gm., D.water: 1 L., pH: 5.5—6.

ASTHANA & HAWKER's Agar: Glucose: 5 gm., KNO_3: 3.5 gm., KH_2PO_4: 1.75 gm., $MgSO_4$: 0.75 gm., D.water: 1 L. pH: 5.5—6.

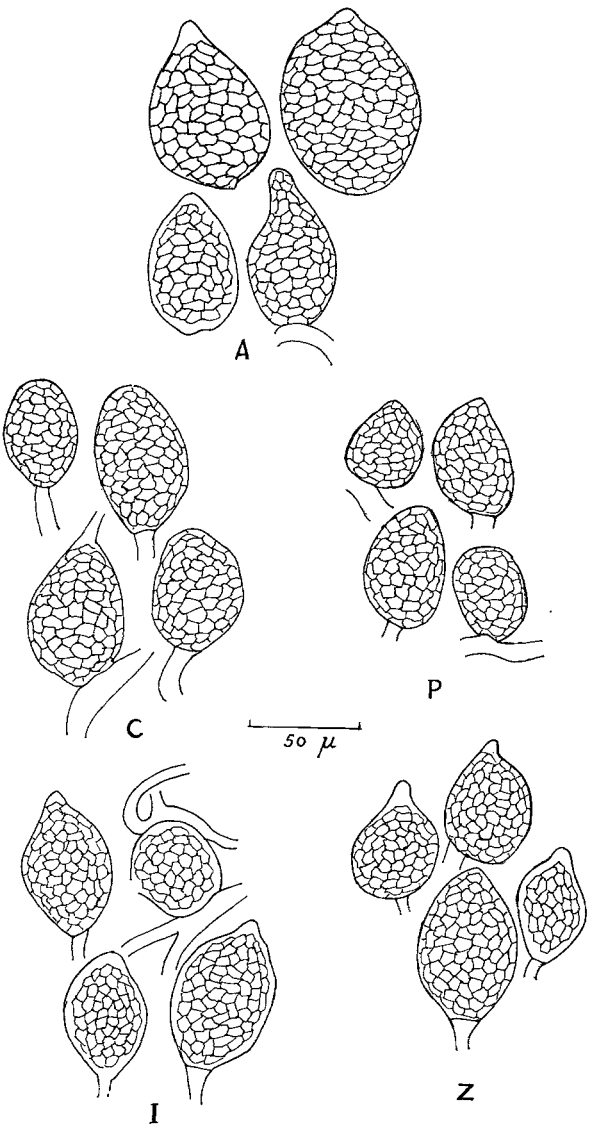

Plate I. Comparison between pycnidia of *Ampelomyces quisqualis* CES. from hosts.
Fig. A: Isolate A (On *Acrosporium* sp. affecting *Abelmoschus esculentus*).
Fig. C: Isolate C (On *Oidiopsis taurica* on *Cyamopsis tetragonoloba*).
Fig. I: Isolate I (Om *A.* sp. parasitizing *Impatiens balsamina*).
Fig. P: Isolate P (On *A.* sp. on *Pedilanthus tithymaloides*).
Fig. Z: Isolate Z (On *A.* sp. parasitizing *Zinnia elegans*).

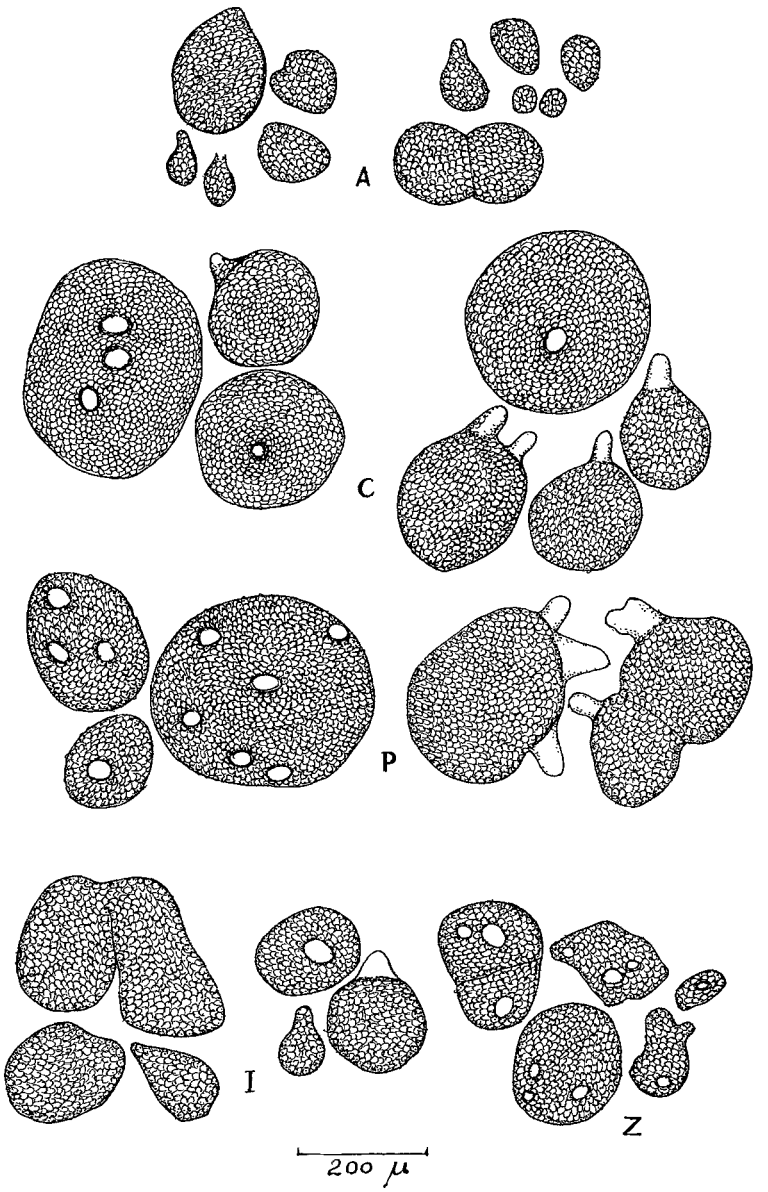

Plate II.: Comparison between pycnidia of *Ampelomyces quisqualis* CES. in artificial culture.
Fig. A: Isolate A; Fig. C: Isolate C; Fig. I: Isolate I;
Fig. P: Isolate P; Fig. Z: Isolate Z.

Observations and Results

Morphological Characters (On natural hosts):

1. *Isolate A*: On *Acrosporium* sp. parasitizing *Abelmoschus esculentus* Pycnidia broadly ovate to lemmon-shaped, pedicellate, papellate, dark brown, 32—90 × 24—38 μ; pycnidiospores hyaline, oblong, 1-celled, 3.5—8 × 3.5 μ. (Pl. I, Fig. A).
2. *Isolate C*: On *Oidiopsis taurica* on *Cyamopsis tetragonoloba* Pycnidia sub-globose, with short pedicel, indistinctly papillate, dark brown, 40—60 × 24—40 μ; pycnidiospores hyaline, oblong, 1-celled, 4—6 × 3 μ. (Pl. I, Fig. C).
3. *Isolate I*: On *Acrosporium* sp. parasitizing *Impatiens balsamina* Pycnidia globular, short-pedicellate, papillate, dark brown, 32—72 × 32—52 μ; pycnidiospores hyaline, sub-globose to oblong, 1-celled, 3—8 × 2—3 μ. (Pl. I, Fig. I).
4. *Isolate P*: On *Acrosporium* sp. on *Pedilanthus tithymaloides* Pycnidia sub-globose, short-pedicellate, indistinctly papillate, dark brown, 30—60 × 24—40 μ; pycnidiospores hyaline, oval to oblong, 1-celled, 4—8 × 2—4 μ. (Pl. I, Fig. P).
5. *Isolate Z*: On *Acrosporium* sp. affecting *Zinnia elegans* Pycnidia globose, short-pedicellate, papillate, dark brown, 44—72 × 30—45 μ; pycnidiospores hyaline, sub-globose, to oblong, 1-celled, 3—6 × 2—3 μ. (Pl. I, Fig. Z).

Morphological Characters (On Agar Media):

1. *Isolate A*: Pycnidia globose to flask-shaped, non-ostiolate, dark brown, 48—176 × 48—144 μ; pycnidiospores hyaline, globose to oblong, 1-celled, 5.5—7 × 2—3.5 μ. (Pl. II, Fig. A).
2. *Isolate C*: Pycnidia globose to flask-shaped, ostiolate, distinctly papillate or with prominent beak (on OMA, M_2A, RA*),), often producing large globose compound pycnidia, dark brown, 80—300 (—352) × 48—304 μ; pycnidiospores hyaline globose to oblong, 1-celled, 5.5—9 × 2—3.5 μ. (Pl. II, Fig. C).
3. *Isolate I*: Pycnidia flask-shaped, often coalescing to form compound pycnidia of irregular shape, rarely ostiolate (on A & H A), dark brown to black or olivaceous, 64—272 × 64—224 (—272) μ; pycnidiospores sub-hyaline to olivaceous, globose to oblong, 1-celled, 5—11 × 3.5—7 μ. (Pl. II, Fig. I).
4. *Isolate P*: Pycnidia globose to flask-shaped, multi-ostiolate, (4—6 in number on RA), often forming large globose to oval compound pycnidia measuring 80—320 (—432) μ in diam. with prominent beaks (48—80 μ long on A & H A); pycnidiospores hyaline, oblong, 1-celled, 3.5—7 × 2—3.5 μ. (Pl. II, Fig. P).
5. *Isolate Z*: Pycnidia globose, distinctly smaller, sometimes coalescing to form compound pycnidia of irregular shape (on OMA), separating wall of the two coalescing pycnidia was clearly observed on M_2A, multi-ostiolate (1—3 in number), dark brown, 32—192 (—256) × 32—118 (—160) μ; pycnidiospores hyaline, globose to oblong, 1-celled, 5.5—9 × 2—3.5 μ. (Pl. II, Fig. Z).

The results of the comparative study on the behaviour of the five isolates of *Ampelomyces* in artificial culture are assembled in Table I.

Discussion

It is evident from the above results that the five isolates of *Ampelomyces quisqualis* obtained from different hosts showed some interesting variations in respect of morphological characters including dimensions of fruiting bodies as well as their cultural behaviour on various vegetable and synthetic agar media which are briefly indicated below:

On Hosts: Isolate A produced significantly bigger pycnidia

*) Throughout this paper: PDA = Potato-Dextrose-Agar; OMA = Oatmeal Agar; M_2A = M_2 Agar; NA = Nutrient Agar; A & H A = Asthana & Hawker's Agar; CzA = Czapek (Dox) Agar; BA = Browns' Agar and RA = Richard's Agar.

TABLE I

Comparative Statement Showing Behaviour Of The Five Isolates Of *Ampelomyces* In Artificial Culture.

Media	Colony Characters	Isolate A	Isolate C	Isolate I	Isolate P	Isolate Z
PDA	Diameter in mm	9	65	69	68	64
	Shape	Circular	Circular	Circular	Circular	Circular
	Nature	Submerged to subaerial	Subaerial	Aerial	Submerged to subaerial	Submerged to subaerial
	Colour	Ochreous to dirty white	Olivaceous buff to citrine	Dark olivaceous green	Citrine	Dark olivaceous to pale olivaceous.
	Pycnidial development	+++, G.	++++, C.	++++, C.	++++, C.	++++, S.
OMA	Diameter in mm.	8	59	58	58	57
	Shape	Circular	Circular	Circular	Circular	Ciruclar
	Nature	Aerial	Aerial	Aerial	Subaerial	Aerial
	Colour	Dirty white	Olivaceous to ochreous	Olivaceous black	Rosy buff	Glaucous grey to greenish greyish sepia
	Pycnidial development	++, S.	++++, C.	++++, C.	++++, C.	++++, S.
M_2A	Diameter in mm.	8	63	68	57	69
	Shape	Dome-shaped circular	Circular wavy margin	Circular	Circular	Circular serrate margin
	Nature	Subaerial	Subaerial	Aerial	Subaerial	Subaerial
	Colour	Olivaceous	Greenish glaucous to citrine	Smokey grey	Greenish glaucous to citrine	Rosy buff
	Pycnidial development	++, S.	++++, C.	++++, C.	++++, C.	++++, S.
NA	Diameter in mm.	10	45	40	46	44
	Shape	Circular	Circular	Circular	Circular	Circular
	Nature	Subaerial	Subaerial	Subaerial	Subaerial	Submerged
	Colour	Dirty white	Dirty white	Dirty white	Dirty white to umber	Flesh to umber
	Pycnidial development	+++, S.	++++, S.	++++, S.	++++, S.	++++, S.
A & H A	Diameter in mm.	5	38	39	35	40
	Shape	Circular	Irregular estoid margin	Circular serrate margin.	Irregular estoid margin.	Circular
	Nuture	Subaerial	Subaerial	Submerged	Subaerial	Subaerial
	Colour	Ochreous to dirty white	Olivaceous buff to pale olivaceous	Honey to Isabelline	Dirty white with apricot coloured pycnidia	Olivaceous buff
	Pycnidial development	++, G.	+++, C.	++++, S.	++++, C.	++++, S.
CzA	Diameter in mm.	—	45	49	59	48
	Shape	—	Circular	Circular	Circular	Circular
	Nature	—	Subaerial	Submerged	Submerged	Submerged
	Colour	—	Salmon to flesh	Salmon to ochreous	Hyaline to dirty white	Olivaceous buff
	Pycnidial development	—	+++, S.	++++, S.	++++, C.	++++, S.
RA	Diameter in mm.	—	43	53	40	51
	Shape	—	Circular wavy margin	Circular serrate margin	Circular wavy margin	Circular smooth margin

TABLE I (cont.)

Media	Colony Characters	Isolate A	Isolate C	Isolate I	Isolate P	Isolate Z
	Nature	—	Subaerial	Submerged	Subaerial	Submerged
	Colour	—	Dirty white	Umber to olivaceous	Dirty white	Greenshi glaucous to olivaceous grey
	Pycnidial development	—	+++, C.	++++, S.	+++, C.	++++, S.
BA	Diameter in mm.	—	**45**	**36**	**34**	**40**
	Shape	—	Circular wavy margin	Irregular serrate	Circular estoid margin	Circular smooth margin
	Nature	—	Submerged	Subaerial	Submerged	Submerged
	Colour	—	Olivaceous buff	Greyish sepia to dark brick	Dirty white	Hyaline to dirty white
	Pycnidial development	—	++, S.	+++, S.	+++, C.	++++, S.

1. —: No development; ++: Moderate; +++: Good; ++++: Excellent.
2. S: Scattered; C: in concentric rings; G: Gregarious.

(32—90 × 24—38 μ) as compared to the rest of the four. Isolate P produced smallest pycnidia (30—60 × 24—40 μ) whereas Isolates C, I and Z produced approximately the same type of pycnidia. All the five isolates produced broadly ovate to lemmon-shaped pycnidia provided with papillae but no ostioles. In all the isolates the pycnidiospores were uniformly hyaline and showed no significant differences in size and shape.

In Culture: Isolates C & P showed distinct protruded necks (beaks) while other lacked this character. Distinct ostioles (up to 6 in number) were also noted in pycnidia of these isolates. Pycnidia of Isolates I & Z however, showed less distinct and fewer (up to 2) number of ostioles. Pycnidiospores in all the five isolates showed striking uniformity in respect of their dimensions, being 3.5—7 (—11) × 2—3.5 (—7) μ. However, Isolate I was characterized by sub-hyaline to olivaceous pycnidiospores as compared to those of other four isolates which had hyaline pycnidiospores.

As regards developmental pattern of pycnidia and colony characters these isolates showed some interesting but distinctive behaviour. The types of growth, colony characters and developmental pattern of pycnidia were similar in case of Isolates C & P; whereas, Isolates I & Z showed fewer striking resemblances in colony, shape and growth pattern. Isolate A on the other hand was found to be strikingly slow growing with non-spreading type of colony. It produced no growth on CzA, RA and BA, thereby indicating its remarkable distinctiveness from the other four isolates. Besides, this isolate was characterized by lack of development of compound pycnidia of irregular shape as in the other four isolates. Isolates C & P were more inclined to develop large globose to oval pycnidia

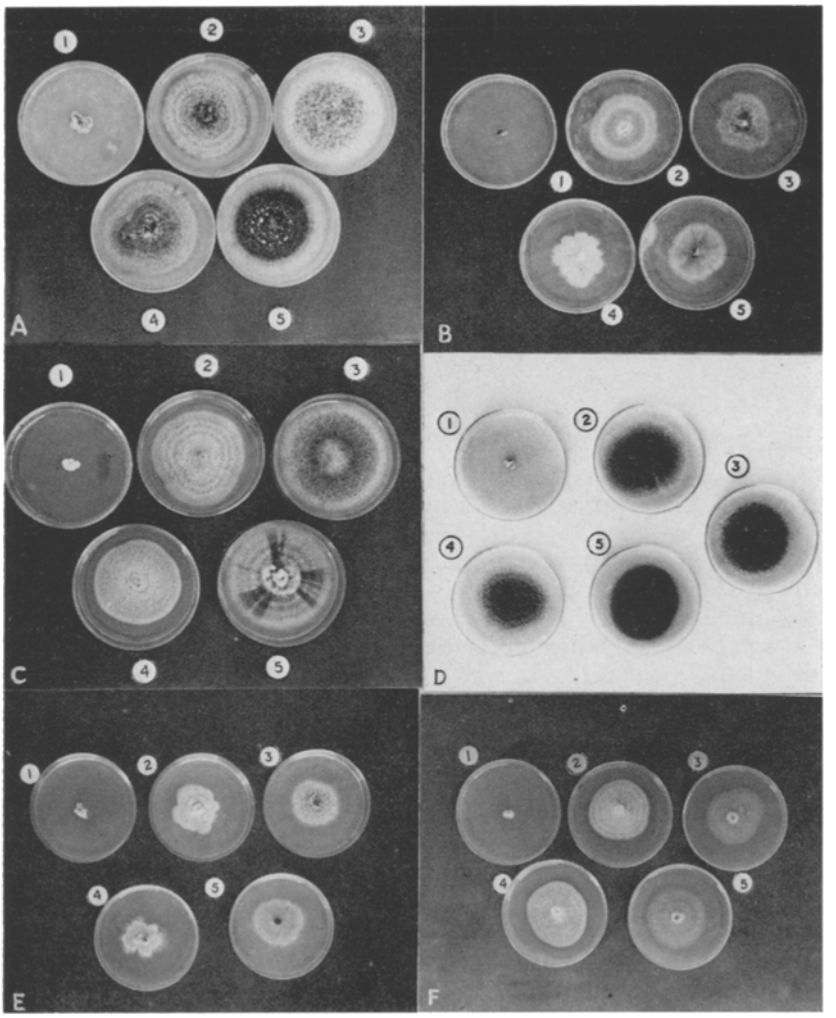

Plate III: Colony characters of five isolates of *Ampelomyces* on different cultural media.
A: Potato-Dextrose-Agar; B: BROWNS' Agar; C: M_2 Agar;
D: Oat-Meal Agar; E: ASTHANA & HAWKER's Agar; F: Nutrient Agar.
1.Isolate A; 2. Isolate C; 3. Isolate I; 4. Isolate P; 5. Isolate Z.

with 1—6 ostioles often provided with prominent beaks (Pl. II, Figs. C & P). Compound pycnidia of irregular shape were rarely observed in these two isolates. Isolate I on the other hand, exhibited a distinct tendency to produce compound pycnidia of irregular shape with distinct aerial mycelium as compared to the rest of the four isolates. (Pl. II, Fig. I). Distinct differences were also noted

in respect of colour of the pycnidiospores, which were sub-hyaline to olivaceous in the case of Isolate I and typically hyaline in the case of the rest of the isolates. Isolate Z was found to have marked tendency to extrude pycnidiospores in flesh-coloured gelatinous mass of ooze (Pl. III, Fig. A-5). This oozing nature was less frequently observed in the isolates C, I and P. Similarly, Isolate Z was found to produce distinctly smaller pycnidia in culture as compared to the other four, often showing a tendency to coalesce to form compound pycnidia of irregular shape. This tendency was best exhibited on OMA. On M_2 Agar seperation walls of the coalescing pycnidia were clearly noted. (Pl. II, Fig. Z).

Thus, in general, of the five isolates, Isolate A appeared to be distinct in its cultural behaviour and also in morphology of pycnidia. The other four isolates may be separated into two broad groups viz. Group I, (Isolates C & P) and Group II (Isolates I & Z). It is significant to note that the pycnidia developed distinct beaks and ostioles in culture, but not on hosts where the pycnidia were uniformly lemmon-shaped, papillate but entirely non-ostiolate. The development of lysignetic ostioles by pycnidia of this hyperparasite in culture was also reported by EMMONS (1930) in two strains of *Ampelomyces* isolated from *Erysiphe cichoracearum* affecting *Helianthus tuberosus* collected from Wisconsin and New Jersey. As regards the size of pycnidia, all isolates under study except Z produced much bigger pycnidia (64—320 × 64—276 μ) as against 100——170 × 50—80 μ) of the two American strains. Isolates C & P resembled the New Jersey strain of EMMONS (1930) in having pronounced beaks for the pycnidia produced by them. Significant differences were also noted in respect of spore dimensions which were 3.5—7 (—11) × 2—3.5 μ in all the Indian isolates as against 6—9 × 2.5—3 μ in Wisconsin and New Jersey strains.

This is the first detailed report from India on the behaviour of this hyperparasite in artificial culture. The previous reports available from India and elsewhere only refer to taxonomic and comparative studies in respect of pycnidial dimensions of various isolates of this fungus obtained from hosts on the basis of which these were all referred to the type species. – *Ampelomyces quisqualis* CES. The present study of the five isolates in artificial culture has revealed fairly good evidence of the existence of physiologic forms within the hyperparasite based on cultural behaviour, colony characters, rate of growth, pycnidial characters and dimensions and colour of pycnidiospores on the basis of which this fungus could be segregated into three distinct forms viz. Form I consisting of Isolate A, Form II consisting of Isolates C & P and Form III with Isolates I & Z.

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