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Studies in aquatic fungi of Varanasi

VI. Taxonomy and distribution of some peculiar isolated Species of *Allomyces*

THAKUR JI*

INTRODUCTION

The genus *Allomyces* with its type species *arbuscula*, one of the aquatic fungi belonging to *Blastocladiaceae*, was isolated from humid soil in this country (Butlér 1911). Since its discovery a number of species have been added to the genus by many investigators from various districts in several countries. Now, the genus *Allomyces* includes 8 species with varieties, all of which are known to be saprophytic or terrestrial. These species exist in tropical or temperate zones all over the world (Emerson 1941; Wolf 1941; Cejp 1947; Remy 1948; Shen and Siang 1948; Sparrow 1952; Kobayashi and Ookubo 1952, 1954; Sörgel 1952; Gaertner 1954; Harder and Gallwitz-Uebelmesser 1959; Kato 1963; Jeffrey and Willoughby 1964).

In India, only one contribution has been published on the classification and morphology of the genus by Butler (1911), and therefore the present author noted them with the above objects. The present paper deals with a complete description and distribution of *Allomyces* species hitherto found in India, with the possibility to locate a distribution pattern based upon a more local scale.

MATERIALS AND METHODS

The procedure used by the author in the isolation of species of *Allomyces* from the soil was essentially similar to the techniques employed by Butler (1907) and Harvey (1925). In order to obtain material free from outside contamination, sterile collecting bottles of 50 ml or 100 ml size were used. Soils were secured from various locations at usually 2 inches depth from fresh excavations made with a borer. The bottles were labelled and brought to the laboratory where the soils were properly treated with sterile pond water after which it was baited with halves of boiled hempseed. The seeds were removed to fresh sterile

* Assistant Mycologist, Gort. Iute Research Station, Bahvaich, U. P.

water after 3—4 days incubation at laboratory temperature. Sample baits were examined when the mycelia of any kind relative to aquatic fungi present had grown clear of adhering soil samples.

Collection data recorded for the water samples used in the present investigation indicates that members of the genus *Allomyces* have appeared less frequently in fresh waters. Therefore soil sample collections were made twice a week. Nearly 125 samples were investigated and these were classified into four categories: (a) *Allomyces* only, (b) *Allomyces* plus *Pythiaceae* and/or *Saprolegniaceae*, (c) *Pythiaceae* or *Saprolegniaceae* only (d) no filamentous water moulds of any kind. Regarding category (b) the sample was air dried and re-baited, although competitive fungi were still present to a reduced degree, *Allomyces* were then recorded.

RECORDED SPECIES

Allomyces arbuscula Butler, Ann. Bot. 25: 1027, 1911.

This fungus was described by Butler in 1911 from Pusa in India. Since then it was recorded from various districts of the world by many investigators (Barrett 1912; Coker and Braxton 1926; Emerson 1941; Kato 1963). It seems a typical cosmopolitan.

Allomyces javanicus Kniep, Berichte Deutsch. Bot. Ges. 47: 211, 1929.

This fungus was originally isolated by Kniep (loc. cit.) from Java, and studied the anisogamous planogametic reproduction of the genus for the first time. Emerson (1938) collected the fungus in North America, Middle America, Africa, India, Burma, Fiji Islands etc., but the frequency of the appearance is rather low. In Varanasi, it is found also very rarely.

Since it has been merely listed by Galloway (Chaudhuri et al. 1947) from Pusa, Darbhanga, without any description and diagrams. The following information has been included in the present paper.

Isolated on hempseed from pond water near Tikari village, Varanasi, U. P., India. Leg. Thakur Ji, 16.II.1967. Culture RD 3a (Figs. 1—6).

Allomyces moniliformis Coker and Braxton, Jour. Elisha Mitch. Sci. Soc. 42: 139, 1926; emend. Emerson, Mycologia 30: 127, 1938.

This fungus was isolated by Coker and Braxton (loc. cit.) from North Carolina for the first time and later Wolf (1939) and Emerson (1941) collected it from Mexico. This species is similar to *A. cystogenus* in the zoospore discharge from the chlamydocysts, developing into minute gametophytes, but differs from it in having rhombic chlamydocyst. In Japan, it was isolated only in two localities in Tôkài and Tôsan areas (Kato 1963). It has not been described or recorded from this country so far.

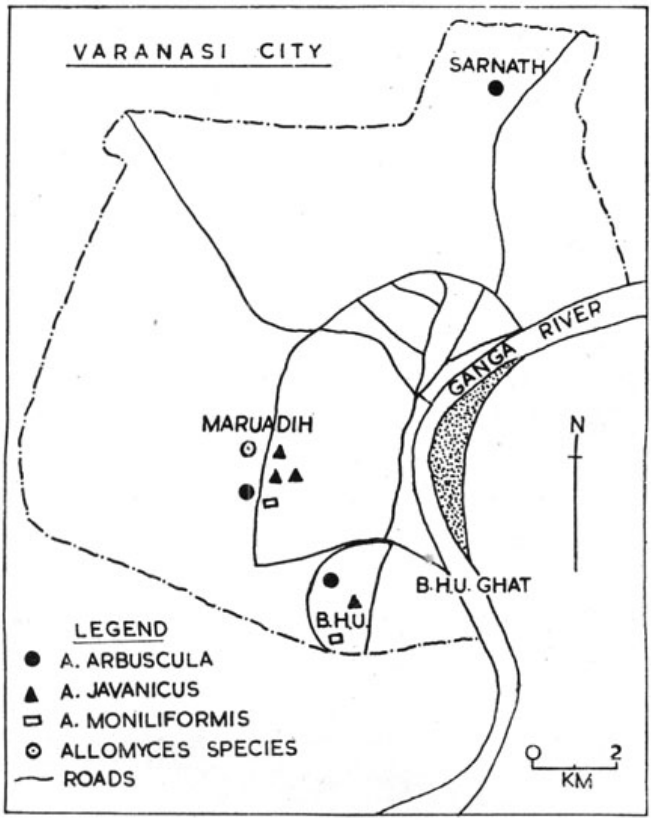
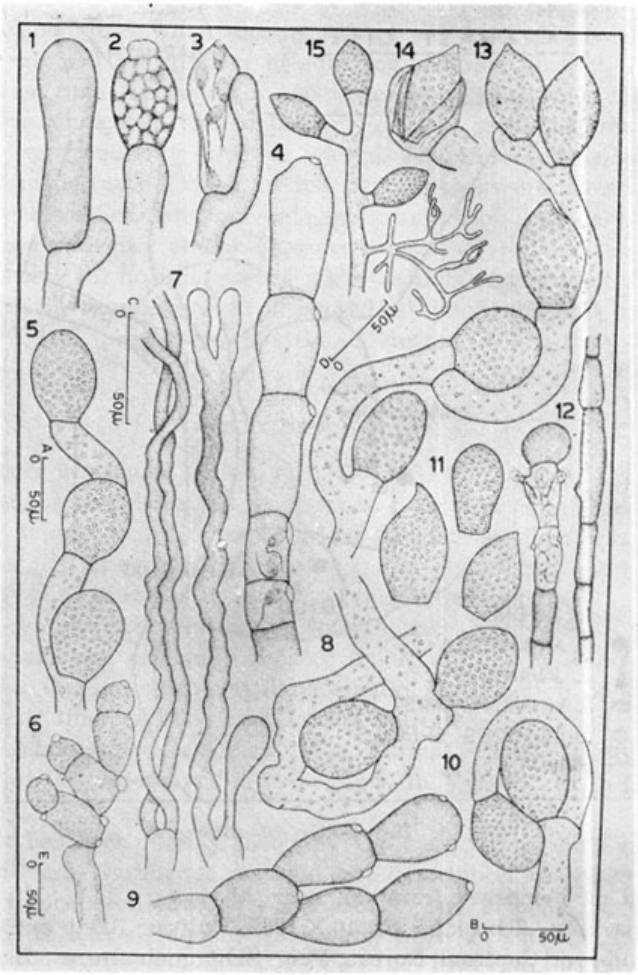


Fig. 1. Distribution of *Allomyces*

Isolated on hempseed from soil near Manduadih, Varanasi, U. P., India. Leg. Thakur Ji, 10.X.1965. Culture RD 1 and duplicate also deposited at Centraal Bureau Voor Schimmelcultures, Baarn, Holland (CBS 104. 67) (Figs. 11—15).

The general characters of the present isolate are in close resemblance to the description of *A. moniliformis* except in the size of zoosporangia, resistant sporangia, presence of rounded resistant sporangia (approximately 15%) and long finger like branching from the hypha (Fig. 15).

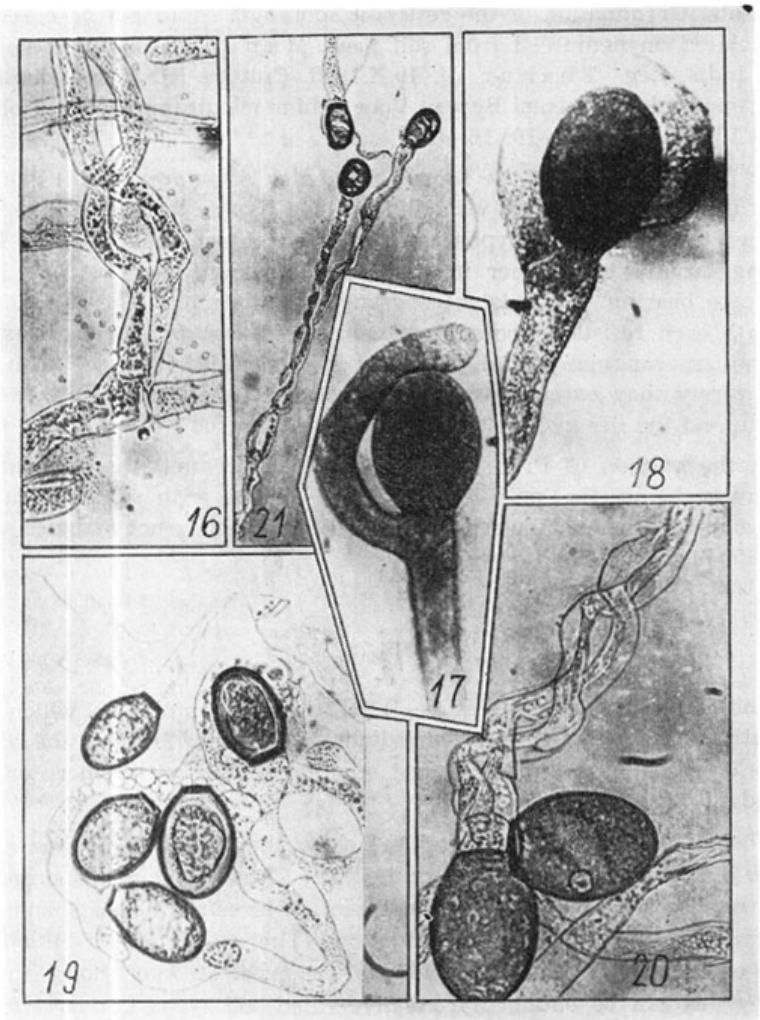
Recently, a peculiar representative of this genus was isolated and described from a students aquarium of tropical fish by Sparrow (1964), which exhibits finger-like vegetative structures of unknown fungus. The author agrees with Prof. Emerson (pers. comm.) that much emphasis should not be paid to such a mycelial character to give a new epithet.



Figs. 1—6. *Allomyces javanicus:* 1 — Immature zoosporangium, 2 — Mature zoosporangium with prominent papilla, 3 — Discharging zoosporangium, 4 — Tip of hypha bearing discharged zoosporangia; in the two below the zoospores are about to escape, 5 — Portion of hypha bearing resistant sporangia, 6 — Tip of hypha of gametangial plant bearing a terminal male gametangium and subterminal female one. Figs. 7—10. *Allomyces* sp.: 7, 8 and 10 — Coiling of hyphae, 9 — Basipetal immature zoosporangia. Figs. 11—15. *Allomyces moniliformis:* 11 — Mature resistant sporangia, two with beak type; 12 — Basipetal zoosporangia with some discharging spores; 13 — Hypha of a mature plant bearing resistant sporangia; 14 — Mature resistant sporangium slipping out from the container; 15 — Portion of hypha bearing immature resistant sporangia with finger-like vegetative outgrowth. Scale line *A* for figs. 1—5; *B* for figs. 8—10; *C* for fig. 7; *D* for figs. 11—15 and *E* for fig. 6

Allomyces sp.

Thallus consists of a differentiated basal cell; 51—323 × 14—34 μ; giving rise to cylindrical, dichotomously, subdichotomously branched, blunt — tipped hyphae of indefinite extent; bearing the reproductive organs. Zoosporangia born in basipetal succession, thin walled, 50—79 × 36—61 μ; discharge their fully formed zoospore through



Figs. 16—21. *Allomyces* sp.: 16, 17 — Origin of hyphal coiling 200×; 125× 18—20 — Hypha encircling the resistant sporangia 250×; 21 — Helical coiling of the vegetative hyphae 300 ×.

one or more pores produced upon the deliquescence of prominent papillae. Zoospore escaping in a vesicle that soon burst scattering in all directions, while some of the zoospores still remain inside the zoosporangium where they escape singly; 10—17 μ in diameter with anteriorly single flagellum; exhibiting amoeboid movement prior to encystment. Resistant sporangia abundant; ovoid with rounded apex and truncate base, 36—61 × 29—54 μ; exospore wall thick; reddish brown; minutely punctate. Germination of the resistant sporangia could not be observed.

Isolated on hempseed from soil near Manduadih, Varanasi, UP., India. Leg. Thakur Ji, 10.X.1965. Culture RD 2 and duplicate also deposited at Centraal Bureau Voor Schimmelcultures, Baarn, Holland (CBS 105.67) (Figs. 7—10; 16—21).

In its general aspect the fungus resembles *Allomyces*, but it exhibits some interesting features, viz., some of the hyphae originate in helical manner and the two hyphae come into contact, but do not fuse, coiling around each other up to a certain length, where they later bifurcate bearing the resistant sporangia at their top. The lateral bud beneath each resistant sporangium continues to grow and encircles the resistant sporangium partially or fully in a manner to protect them and very rarely they extend further. The resistant sporangia failed to germinate and the life cycle of the fungus could not be worked out.

In the opinion of Prof. Emerson (pers. comm.) the organism is unique in being the only species of the genus with such a constant vegetative character. However the specific epithest is not assigned until the life history is not known.

RESULTS

The collection data (tables 1—4) for the soil samples on which this investigation is based throuw some light on our knowledge of the occurrence of *Allomyces* species. Almost any soil sample yields more or less abundant flora of *Allomyces*.

The *Allomyces* records in table I show that the soil pH was never below 5.7. Where they were determined, the *Chytridiales* yields suggested that the majority of the samples represented terrestrial habitats, and only in few cases marginal aquatic situations. The association of *Allomyces* with certain members of *Chytridiales* was most striking. Some species of chytrids can be obtained from diversified soil types but have never been on record from purely aquatic situations e.g., *Rhizophlyctis rosea* Willoughby 1961; 1962). Further the restriction of *Phlyctorhiza variabilis* to soil pH 5.75 has been observed by Jeffrey and Willoughby

Table 1

Allomyces records obtained from Varanasi

Sample site	Soil group where known	pH	*Allomyces* determination	*Pythiaceae* and/or *Saprolegniaceae*	*Chytridiales* or other members
Sarnath pond 1	Grey soil of heavy texture	6.2	*A. arbuscula*	+	+
Sarnath pond 2	Grey soil of heavy texture	7.2	*A. arbuscula* *	—	+
Sarnath pond 3	Black soil	8.3	*A. javanicus*	+	—
Sarnath outlet channel		5.7	*A. arbuscula*	+	not sampled
University ditch	Black soil	7.8	*A. javanicus* *	—	not sampled
Univ. press pond	Calcareous yellow earth	6.5	*A. moniliformis* *	+	+
Technology pond	Calcareous yellow earth	5.9	*A. arbuscula*	—	not sampled
Engineering pond	Grey soil of heavy texture	8.8	*A. moniliformis* *	—	not sampled
Nizam colony pond	Black earth	8.3	*A. arbuscula*	+	—
Agri. farm ditch	Grey soil of heavy texture	6.2	*A. javanicus*	+	—
Manduadih pond 1	Yellow soil	7.4	*Allomyces* sp. *	+	+
Manduadih pond 2	Yellow soil	7.2	*A. moniliformis*	+	+
Manduadih pond 3	Black soil	8.3	*Allomyces* sp.	—	—
Manduadih pond 4	Grey soil of heavy texture	8.7	*A. arbuscula*	+	+
Road side ditch 1	Light brown soil	8.4	*A. javanicus* *	+	+
Road side ditch 2	Light brown soil	8.4	*A. moniliformis*	+	—
Road side ditch 3	Grey soil of heavy texture	6.2	*A. arbuscula*	+	+

* Denotes isolate was obtained only after air drying sample.

(1964) in Australia. These two conclusions have reinforced the author's impression that *Allomyces* must be regarded as a valid entity of soil microflora. It is also clear that *Allomyces* occurs only in a limited range of soil types. On the Ganga river bank near university, for example, samplings of sandy soils (pH 5.0) have not yielded the genus. Sandy soil samples from other parts of the site have also proved negative for *Allomyces* (pH 4.5—5.4).

Table 2

Detection of each taxon of *Allomyces* and their dominance expressed as percentage in each zone

Zone	Taxon				
	A. arbuscula	*A. javanicus*	*A. moniliformis*	*Allomyces* sp.	
Sarnath	15 (57.6)	7 (26.9)	4 (15.3)	— —	26
University campus	3 (49.9)	2 (33.3)	1 (16.7)	—	6
Manduadih	16 (69.5)	3 (13.4)	1 (4.3)	3 (13.4)	23
Total	34 (61.8)	12 (21.8)	6 (10.9)	3 (5.4)	55

Table 3

Frequency of detection of *Allomyces* in samples collected from different zones in Varanasi

Zone	Region	Number of collected soil samples	Number of soil samples from which *Allomyces* detected	Frequency of detection of *Allomyces* (%)
Sarnath	Pond	68	12	17.6
	Channel	76	8	10.5
University campus	Pond	125	13	10.4
	Ditch	125	6	4.8
Manduadih	Pond	85	2	2.4
	Road side ditches	67	—	—

Table 4

Climatic factors at the localities from where *Allomyces* were detected

Zone	Mean atmospheric temp. °C.		Annual temp. °C.	Rainfall (cm) annual
	Minimum monthly	Maximum monthly		
Sarnath	19.3	33.5	29.6	80.07
University campus	16.0	31.5	30.5	97.25
Manduadih	18.9	40.0	36.5	102.18

DISCUSSION

In Varanasi, the author found the following 4 taxa of *Allomyces: A. arbuscula, A. javanicus, A. moniliformis,* and one unidentified *Allomyces* species. The map shows roughly the distribution of each taxon in Varanasi with different zones dealt in the present investigation, and table I indicates the *Allomyces* records in relation to pH and soil types. Rolf Emerson (1941) in his monographic study of the genus considered this fungus as a tru aquatic genus. However, his conclusion was based on the frequency with which he isolated it from ditches and drains and margins of ponds. Samples from such situations have been well represented in the present collections, but have only rarely yielded *Allomyces.* In any case it is not advisable to regard its presence in a marginal situation as decisive evidence that the fungus is aquatic (Willoughby 1961). The author is of the opinion to consider *Allomyces* as a valid soil genus, occurring most frequently in naturally fertile soil over a wide pH range with different soil type.

The data presented in table II indicate the occurrence and dominance of *Allomyces* in various zones. The observation further reveals that *A. arbuscula* appears most abundant and is distributed widely in various zones and *A. javanicus, A. moniliformis* and *Allomyces* sp. follow it. It is interesting that the other 3 taxa are rare and exhibit different patterns of geographical distribution. *Allomyces* sp., an endemic one, was isolated only from the warm temperate industrial region near Manduadih while the other 3 species were distributed in the cold temperate zones in the inland region of the northen part of Varanasi. Table 3 indicates the frequency of detection of *Allomyces* at various regions. From the table, it is known that the northern parts of Varanasi have higher frequencies while the western parts have a lower frequency. It is a noticeable fact that the frequency of detection in Manduadih region, *Allomyces* shows the lowest value (2.4%) and no *Allomyces* have been observed from road side soils in spite of examination of many samples.

Among the environmental factors affecting the distribution of *Allomyces,* temperature seems to be most important. The habitat of *Allomyces* in Varanasi, is generally soil, where the environmental conditions excluding temperature, are almost identical in every region of the district investigated. As shown in table IV, the annual mean atmospheric temperature of the regions from where *Allomyces* was isolated, is lower than 36.5°C at Manduadih where rare *Allomyces* distribution was noted. It seems that the author's observation of no record of *Allomyces* above 36.5°C supports the above mentioned distribution. It canot be accepted that the rainfall effects on the distribution of the genus (cf. table IV).

SUMMARY

Four species of *Allomyces* hitherto found in Varanasi viz., *A. arbuscula, A. javanicus, A. moniliformis,* and one with unidentified specific epithet are described with illustrations. Some interesting observations previously unnoticed have been included in their description. The distribution in Varanasi has been investigated. The most abundant and widely distributed species is *A. arbuscula* and a commonly found species is *A. javanicus. Allomyces* species are found abundantly in the northern part of Varanasi. The density of the distribution decreases gradually towards the west. The annual mean atmospheric temperature of the regions, where *Allomyces* species are found, is lower than 36.5°C.

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Department of Plant Pathology
Faculty of Agriculture
Banaras Hindu University
Varanasi — 5, U. P., India

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Studia nad grzybami wodnymi Varanasi

VI. Taksonomia i rozprzestrzenienie niektórych gatunków *Allomyces*

Streszczenie

Wyizolowano 4 gatunki *Allomyces*, z których najpospolitszymi są *A. arbuscula* i *A. javanicus*. Gatunkami rzadkimi są *A. mobiliformis* oraz *Allomyces* sp.

Uzupełniono diagnozy i przedstawiono rozmieszczenie znalezionych grzybów w okolicy Varanasi; więcej stanowisk leży na zachód od miasta. Średnia temperatura roku w tej okolicy jest niższa od 36,5°C.