e-ISSN:2582-7219

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY RESEARCH IN SCIENCE, ENGINEERING AND TECHNOLOGY

Volume 6, Issue 9, September 2023

INTERNATIONAL STANDARD SERIAL NUMBER INDIA

Impact Factor: 7.54

| ISSN: 2582-7219 | www.ijmrset.com | Impact Factor: 7.54| Monthly Peer Reviewed & Referred Journal |

| Volume 6, Issue 9, September 2023 |

| DOI:10.15680/IJMRSET.2023.0609015 |

Diversity of Water Borne Conidial Fungi in Shipra River of Ujjain, Madhya Pradesh

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ABSTRACT: Microorganisms are ubiquitous and cosmopolitan in distribution. They colonize, grow, survive and multiply on diversified habitats. The physic-chemical parameters of habitat, seasonal variation and meteorological conditions influence their occurrence, distribution and activity. The microbes have been found associated with this planet since the origin of life and without these life is not feasible. It is estimated that there exist about 5 million living organisms and the number of undescribed microbial species seems to be 1.9 million. Microorganisms play an important role in agriculture, pharmaceutical/medicine, health, cycling of elements, food chain cycle, biodegradation, forestry and industry.

Fungi are non-chlorophyllous living organisms. The absence of chlorophyll has enforced them to live as saprophytes or parasites. Out of estimated 1.5 million fungi over 97, 861 species are reported from different parts of the world and over 29, 000 fungal species are recorded from India indicating that S, global fungal diversity exists in India. These fungi are known to colonize diversified habitats including water. Aquatic fungi live and multiply in water and are involved in the cycle of life in nature.

Zoosporic fungi are known to be present in water either as saprophytes or parasites. The aquatic fungi can be isolated from water mainly by means of baiting techniques. Solid agar media such as mineral agar, cornmeal agar, oatmeal agar, hempseed agar, potato dextrose agar and others have been used to grow them artificially. Water culture, silica gel, lyophilisation etc. are some of the methods used for conservation.

Water-borne conidial fungi are the asexual phases of Ascomycotina and Basidiomycotina. The fungal conidia get trapped in foam, germinate and multiply on submerged dark brown leaves. Their perfect stages may exist on submerged wood, litter etc.

Fungi are essential part of the living world and their activities have a direct bearing on human welfare. Detailed knowledge of fungi involved in biodegradation and substrates attacked under different ecological conditions make it possible to increase the efficiency of degradation of cellulose and lignocellulosic materials, which are resistant to attack.

KEYWORDS-diversity,water,borne,conidial,fungi,Shipra,river,Ujjain,Madhya,Pradesh

I. INTRODUCTION

A conidium sometimes termed an asexual chlamydospore or chlamydoconidium (PL: chlamydoconidia),[1] is an asexual,[2] non-motile spore of a fungus. The word *conidium* comes from the Ancient Greek word for dust, κόνις (*kónis*).[3] They are also called mitospores due to the way they are generated through the cellular process of mitosis. They are produced exogenously. The two new haploid cells are genetically identical to the haploid parent, and can develop into new organisms if conditions are favorable, and serve in biological dispersal.

Asexual reproduction in ascomycetes (the phylum Ascomycota) is by the formation of conidia, which are borne on specialized stalks called conidiophores. The morphology of these specialized conidiophores is often distinctive between

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species and, before the development of molecular techniques at the end of the 20th century, was widely used for identification of (*e.g. Metarhizium*) species.

The terms microconidia and macroconidia are sometimes used. There are two main types of conidium development:[5]

- *Blastic* conidiogenesis, where the spore is already evident before it separates from the conidiogenic hypha which is giving rise to it, and
- *Thallic* conidiogenesis, where first a cross-wall appears and thus the created cell develops into a spore.

Conidia germination

A conidium may form germ tubes (germination tubes) and/or conidial anastomosis tubes (CATs) in specific conditions. These two are some of the specialized hyphae that are formed by fungal conidia. The germ tubes will grow to form the hyphae and fungal mycelia. The conidial anastomosis tubes are morphologically and physiologically distinct from germ tubes. After conidia are induced to form conidial anastomosis tubes, they grow homing toward each other, and they fuse. Once fusion happens, the nuclei can pass through fused CATs. These are events of fungal vegetative growth and not sexual reproduction. Fusion between these cells seems to be important for some fungi during early stages of colony establishment. The production of these cells has been suggested to occur in 73 different species of fungi, in Shipra river of Ujjain, Madhya Pradesh.[1,2]

Structures for release of conidia

Conidiogenesis is an important mechanism of spread of plant pathogens. In some cases, specialized macroscopic fruiting structures perhaps 1 mm or so in diameter containing masses of conidia are formed under the skin of the host plant and then erupt through the surface, allowing the spores to be distributed by wind and rain. One of these structures is called a conidioma (plural: conidiomata).[8][9]

Two important types of conidiomata, distinguished by their form, are:

- pycnidia (singular: pycnidium), which are flask-shaped, and
- acervuli (singular: acervulus), which have a simpler cushion-like form.

Pycnidial conidiomata or *pycnidia* form in the fungal tissue itself, and are shaped like a bulging vase. The conidia are released through a small opening at the apex, the ostiole.

Acervular conidiomata, or *acervuli*, are cushion-like structures that form within the tissues of a host organism:

- *subcuticular*, lying under the outer layer of the plant (the cuticle),
- *intraepidermal*, inside the outer cell layer (the epidermis),
- *subepidermal*, under the epidermis, or deeper inside the host.

Mostly they develop a flat layer of relatively short conidiophores which then produce masses of spores. The increasing pressure leads to the splitting of the epidermis and cuticle and allows release of the conidia from the tissue, in Shipra river of Ujjain, Madhya Pradesh

Conidia are always present in the air and water, but levels fluctuate from day to day and with the seasons. An average person inhales at least 40 conidia per hour.[10] Exposure to conidia from certain species, such as those of *Cryptostroma corticale*, is known to cause hypersensitivity pneumonitis, an occupational hazard for forest workers and paper mill employees.

Conidia are often the method by which some normally harmless but heat-tolerating (thermotolerant), common fungi establish infection in certain types of severely immunocompromised patients (usually acute leukemia patients on induction chemotherapy, AIDS patients with superimposed B-cell lymphoma, bone marrow transplantation patients (taking immunosuppressants), or major organ transplant patients with graft versus host disease). Their immune system is not strong enough to fight off the fungus, and it may, for example, colonise the lung, resulting in a pulmonary infection isolated from Shipra river of Ujjain, Madhya Pradesh.[3,5]

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Asexual reproduction occurs via vegetative spores (conidia) or through mycelial fragmentation are transferred to water. These are waterborne conidia. Mycelial fragmentation occurs when a fungal mycelium separates into pieces, and each component grows into a separate mycelium. Mycelial fragmentation and vegetative spores maintain clonal populations adapted to a specific niche, and allow more rapid dispersal than sexual reproduction.[86] The "Fungi imperfecti" (fungi lacking the perfect or sexual stage) or Deuteromycota comprise all the species that lack an observable sexual cycle.[87] Deuteromycota (alternatively known as Deuteromycetes, conidial fungi, or mitosporic fungi) is not an accepted taxonomic clade and is now taken to mean simply fungi that lack a known sexual stage isolated from Shipra river of Ujjain, Madhya Pradesh.

II. DISCUSSION

Conidia arise as blow outs from one side of the tip of the conidiophore, and after the first conidium has been put out before it fully matures, the next conidium is blown out from the opposite side. The conidia thus are pinched out one after the other and remain attached to each other on the shoulder to form zig-zag chains giving rise to a characteristic head. The conidia are ovoid or pear shaped, two celled, with the apical cell being larger and globose than the basal cell which is curved and conical. The conidia are hyaline or lightly coloured pink or pale, appear hyaline under a microscope, but pink in masses in culture or on the host. The conidia are attached to the conidiophore at the pointed end of their basal cell. The size of conidia is 12–18 µm long × 8–10 µm broad. Conidiophores and conidia of *T. polybrochum*, *T. cystosporium*, *T. pravicovi* are morphologically different from *T. roseum*, isolated from Ujjain, Madhya Pradesh.

The stress resistance of conidia is, in general, higher than that of vegetative fungal cells. Waterborne conidia exhibit a number of cellular properties that are more similar to fungal vegetative cells than to dormant airborne conidia. For instance, airborne conidia show a low staining of ergosterol in the plasma membrane when compared to waterborne spores and have a higher cytoplasmic viscosity (Van Leeuwen et al., 2010). Moreover, waterborne conidia are nonpigmented and show higher sensitivity for antibiotics than airborne conidia.

A number of examples of resistance of hydrated conidia to heat or oxidative stress are summarized in Table below. Temperature inactivation of waterborne conidia of *Botrytis cinerea* (hyaline) and *Stagonospora nodorum* (from pycnidia, septate, hyaline) occurs within minutes at temperatures above 45 °C (Doehlemann, Berndt, & Hahn, 2006; Solomon et al., 2005). The median lethal temperature (LT_{50}) of *S. nodorum* conidia at 40 °C is 25 min (Solomon et al., 2005). *B. cinerea* conidia showed similar sensitivity with a LT_{50} of 8 min at 45 °C (Doehlemann et al., 2006). [7,8]The conidia of the insect pathogenic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* are more resistant to heat (Wang, Lu, & Feng, 2012; Ying & Feng, 2004). More than 50% of *B. bassiana* conidia survive up to 2 h at 48 °C, while *P. fumosoroseus* conidia survive up to 15 min at 48 °C (Ying & Feng, 2004). More heat resistant are conidia of *Aspergillus* (*A. nidulans*, *A. niger*, and *A. oryzae*). They survive 50 °C for minutes to even hours (Fillinger et al., 2001; Ruijter et al., 2003; Sakamoto et al., 2009). The LT_{50} values of conidia of *A. nidulans* and *A. oryzae* are 10 and 42 min, respectively, at 50 °C (Fillinger et al., 2001; Sakamoto et al., 2008, 2009), while 100% of the conidia of *A. niger* still germinate after 2 h at this temperature (Ruijter et al., 2003). Baggerman and Samson (1988) reported a D_{59} of 3.3 min for conidia of *A. niger*. This means that 10% of the conidia still survive a period of 3.3 min of heating at 59 °C. The resistance toward oxidative stress also shows variation among fungal species. Fifty percent of *A. nidulans* conidia survive 100 mM H_2O_2, while 60% of *A. oryzae* conidia survive 30 min at 400 mM of this oxidative agent. *A. niger* conidia are not inactivated after 1 h incubation in 1 mM NaOCl. Half of the conidia of *B. bassiana* survived 5 mM H_2O_2 for 24 h (Wang et al., 2012), while *S. nodorum* is sensitive to oxidative stress as no survivors were measured after a 15-min exposure to 1 mM NaOCl (Solomon et al., 2005).

Table . Stress resistance of fungal conidia

Species	Stress	Time	Survival
Aspergillus nidulans	50 °C	> 30 min	0%
	100 mM H_2O_2	> 60 min	0%
Aspergillus niger	50 °C	2 h	100%

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Species	Stress	Time	Survival
	Freeze–thaw	1 ×	76%
	Lyophilization		64%
	1 mM NaOCl	1 h	100%
Aspergillus oryzae	50 °C	1 h	30%
	400 mM H_2O_2	30 min	60%
	UV 254 nm	0.03 J cm^{-2}	50%
Beauveria bassiana	45 °C	50 min	> 50%
	0–10 mM H_2O_2	24 h	
	UV-B 312 nm	0.42 J cm^{-2}	50%
	48 °C	> 25–120 min	0%
Botrytis cinerea	45 °C	15 min	0%
	45 °C	10 min	20%
Paecilomyces fumosoroseus	48 °C	> 6–15 min	0%
Stagonospora nodorum	50 °C	15 min	0%
	40 °C	15 min	65%
	40 °C	> 60 min	0%
	1 mM NaOCl	15 min	0%

Airborne dormant conidia of *Aspergillus* and, most probably, also of *Penicillium* and *Paecilomyces* are moderate stress-resistant cells that survive drought, relatively high temperatures (1 h at 50 °C; several min at 60 °C, Ruijter et al., 2003; Samson et al., 2004) and UV radiation due to a melanized outer cell wall (Jørgensen et al., 2011; Tiedt, 1993). The dormancy of conidia ensures that these cells survive time intervals as in case of *A. nidulans* for 6 weeks in liquid (Fillinger et al., 2001), but much longer in a dried state or when cooled.

Hydrated conidia of *P. chrysogenum*, *P. italicum*, or *P. digitatum* are inactivated (a log 2.5 to log 6 decrease) after a 4-day treatment at 30 °C in either 5% ethanol vapor or 10% ethanol solution (Dantigny, Tchobanov, Bensoussan, & Zwietering, 2005; Dao, Bensoussan, Gervais, & Dantigny, 2008). *P. chrysogenum* showed marked less inactivation compared to the other species. This makes sense as this species is more stress resistant and grow at lower water activities. It is not clear whether conidia are still in their dormant state after such a long period of treatment or have started some stage of germination. Dry-harvested conidia of all three species are more resistant to ethanol vapors than hydrated conidia (Dao & Dantigny, 2009). Yet, the dry-harvested spores are inactivated at higher vapor pressures (10%; Dao, Dejardin, Bensoussan, & Dantigny, 2010). Mathematical models for the inactivation of fungal spores are reviewed (Dijksterhuis, Rodriquez de Massaguer, Da Silva, & Dantigny, 2012) and can be used for different sporicidal conditions including heat, drying, or vapor treatments (Dao & Dantigny, 2009; Dao et al., 2010), isolated from Shipra river of Ujjain, Madhya Pradesh.[9,10]

III. RESULTS

During the present study, 18 species of water-borne conidial fungi belonging to 14 genera were isolated and identified from submerged decomposed leaf-litters isolated from Shipra river of Ujjain, Madhya Pradesh.Comparison of species composition along altitudinal gradients in relation to temperature and pH Alatospora acuminata ,Conidia isolated from Shipra river of Ujjain, Madhya Pradesh,pH 7 and 8, sporulation temperature ranging from 17-30 °C, were hyaline, tetra

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radiate, with curved main axis 21- 40 µm long, and two curved appendages 25-50 µm long having 3-4 septations. Anguillospora crassa: Conidia isolated from Shipra river of Ujjain, Madhya Pradesh,pH 6 and 8, sporulation temperature ranging from 30-32 °C, were hyaline, vermiform, 90-200 µm long, 10-20 µm wide, 5-10 septate, tapering towards ends. Anguillospora longissima , Conidia isolated from Shipra river of Ujjain, Madhya Pradesh,pH 8, sporulation temperature ranging from 30-36 °C, were hyaline, unbranched, filiform, curved with main axis 150-350 µm long, 4-6 µm wide at middle having 5-12 septations, tapering at both the ends. Beltrania rhombica , Conidia isolated from Shipra river of Ujjain, Madhya Pradesh,pH 6, sporulation temperature ranging from 30-32 °C were light-brown to pale-olive, consisting of a bi-conic, symmetrical, main axis, 16-30 µm long, 6-9 µm wide, with a distinct, hyaline, transverse band and an apical, hyaline 10-15 µm long appendage with a basal septum. Campylospora chaetocladia , Conidia isolated from Shipra river of Ujjain, Madhya Pradesh,pH 6, sporulation temperature ranging from 20-30 °C were tetra radiate, main axis alantoid, composed of a smaller part 10-15 µm in length and 8-11 µm in width, while arms are 27- 38 µm long. Apical cells of axis conoid to bulbous, each end in one of the slender appendages.[11,12]

Clavariopsis aquatica ,Conidia isolated from Shipra river of Ujjain, Madhya Pradesh,sporulation temperature ranging from 17-30 °C were hyaline, 2-3 celled obconical main axis, 25- 60 µm long, 10-16 µm wide, lateral arms arising from the broad end of the 1st arm are 20- 100 µm long. Usually, appendages or arms are longer than the axis. Clavatospora tentacula , Conidia isolated from Shipra river of Ujjain, Madhya Pradesh, sporulation temperature ranging from 15-17 °C were tetraradiate, main axis 30-75 µm long and 1.5-2.5 µm wide at the base, 4-7 µm wide at apex, with 3-equidistant, divergent 30-55 µm long and 1-2 µm wide appendages arising from the apex and constricted at the base. Cylindrocarpon aquaticum , Conidia isolatedfrom Shipra river of Ujjain, Madhya Pradesh,temperature ranging from 27-30 °C were brown, cylindrical, small, septate and measured up-to 15-18 µm in length and 3-4 µm in width. Helicomyces roseus Conidia isolated from Shipra river of Ujjain, Madhya Pradesh,sporulation temperature ranging from 30-32 °C were helical, coiled up to 2.5-3 times with rounded ends, 20-30 µm in diameter with 10-15 septa, tapering to an enlarge, obliquely flattened basal cell. Lemonniera pseudofloscula ,Conidia isolated from Shipra river of Ujjain, Madhya Pradesh,sporulation temperature ranging from 17-20 °C were hyaline, tetra-radiate, main axil cell spherical 3-6 µm in diameter, 1-6 septations, consisting of 3-4 appendages, 13-72 µm long and 3-4 µm wide. Lemonniera terrestris , Conidia isolated from Shipra river of Ujjain, Madhya Pradesh,sporulation temperature ranging from 17-20 °C were hyaline, tetra-radiate main axil cell spherical 4-7 µm in diameter, appendages 9-13 µm long and 3-6 µm wide. Lunulospora curvula , Conidia isolated from Shipra river of Ujjain, Madhya Pradesh,sporulation temperature ranging from 17-28 °C were hyaline, lunate, unbranched, elongated, aseptate, dematiaceous conidia 40-50 µm long and 4-6 µm wide. Lunulospora cymbiformis , Conidia isolated from Shipra river of Ujjain, Madhya Pradesh,sporulation temper ature ranging from 17-28 °C were hyaline to light green, sickle-shaped, aseptate dematiaceous conidia bent at right angle, 40-50 µm long and 3-5 µm wide with a characteristic scar in the middle. Setosynnema isthmosporum , Conidia isolated from Shipra river of Ujjain, Madhya Pradesh,sporulation temperature ranging from 17-25 °C were sigmoid 150-200 µm long, 3-4 µm wide, tapering towards the tips, 6-7 septate. Tetrachaetum elegans , Conidia isolated from Shipra river of Ujjain, Madhya Pradesh,sporulation temperature ranging from 15-20 °C were hyaline, tetra-radiate, main axis bent at the insertion of 2 appendages, main axis 130-200 µm long, arms 50-150 µm long and uniform in width with 6-8 septations. Tetracladium marchalianum ,Conidia isolated from Shipra river of Ujjain, Madhya Pradesh,sporulation temperature ranging from 15-30 °C were tetra-radiate, with two spherical knobs (one central and one eccentric), the main axis is 15-20 µm long, while the central knob is 9- 12 µm long, septations not prominent. Tetracladium setigerum , Conidia isolated from Shipra river of Ujjain, Madhya Pradesh,sporulation temperature ranging from 17-20 °C were hyaline, 12-15 µm long and 3-4 µm wide with 3-6 septations, conidial axis, distally digit form. The knobs are 5-12 µm long with 1- 3 septations. Triscelophorus acuminatus , Conidia isolated from Shipra river of Ujjain, Madhya Pradesh,sporulation temperature ranging from 20-32 °C were hyaline, variable in size and shape, the main axis is 30-50 µm long, gradually tapering at tip; the lateral arms are 27-36 µm in length. Thus, in the present study, out of 14 genera, 10 genera were recorded with single species and the rest 4 genera namely, Anguillospora, Lemonniera, Lunulospora, and Tetracladium were found with 2 species each. A perusal of seasonal occurrence of different species in the habitat indicates that most

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| Volume 6, Issue 9, September 2023 |

| DOI:10.15680/IJMRSET.2023.0609015 |

of the waterborne conidial fungi showed a marked fluctuation in their occurrence (Sati and Arya, 2009). In the present study, maximum species (11 species) were recorded in both rainy (July to September) and winter seasons (November to December). [13]A. crassa, B. rhombica, C. chaetocladia, C. aquaticum, H. Roseus and T. setigerum were isolated only during rainy season; A. acuminata, C. aquatica, C. tentacula, L. pseudofloscula, L. terrestris and T. elegans were isolated only during winter season while L. curvula, L. cymbiformis, S. isthmosporum, T. marchalianum and T. acuminatus were isolated in both rainy and winter seasons. Least species (6 species) viz., A. acuminata, A. crassa, A. longissima, C. aquatica, C. aquaticum and T. marchalianum were recorded during summer season (April to June). This may be due to high temperature (> 30 °C) and high pH (8) in summers and moderate to low temperature (15-30 °C) and acidic to neutral pH (6 -7) in rainy and winter seasons. The pH of water bodies differed seasonally from 6-8, being maximum during summer and minimum during the rainy season. The pH condition between 6-7 favoured maximum, growth of these fungi and the sporulation temperature between 15-20°C was found to be optimal . Many investigators have also observed similar results that the water-borne conidial fungi show maximum growth in rainy and winter seasons due to low temperature and pH (6-7) (Barlocher, 1987; Belwal and Sati, 2001; Dang et al., 2009; Krauss et al. 2011). It was interesting to note that altitudinal variations considerably influenced the species composition of water-borne conidial fungi in all the selected sites. A. longissima was isolated only from Ramgaarh stream (≈1000 m); C. chaetocladia, C. tentacula, L. terrestris and T. setigerum were isolated only from Kulgarh stream (≈1100 m); while B. rhombica, L. cymbiformis and T. elegans were isolated only from Devdwar stream (≈1500 m). This may be because of the temperature and altitudinal preferences of different species that they are linked to geographical distribution, and some species are common in temperate regions while some in tropical regions. Some temperate species are found to survive at freezing temperatures, and tropical species may survive at higher temperatures as reported by Sridhar and Barlocher, (1993) and Krauss et al. (2011). Further, out of the 18 species isolated from different streams, T. marchalianum was found to occur throughout the year from all the sites. This may be regarded as temperature tolerant and common species of all the streams. The observed results are also supported by the studies of Duarte et al. (2013) and Bai et al. (2018) in that the environmental variables such as temperature, pH, nutrient availability, altitudinal variations can affect fungal community composition.

IV. CONCLUSION

On the basis of the present study, it is concluded that seasonal and altitudinal variations greatly influence the species composition of water-borne conidial fungi isolated from Shipra river of Ujjain, Madhya Pradesh, The rainy (July to September) and winter seasons (November to December) supported maximum growth (11 species), while minimum growth (6 species) was recorded in summer season (April to June). 13 species were isolated from high altitude stream isolated from Shipra river of Ujjain, Madhya Pradesh. T. marchalianum was found to occur throughout the year from all the sites. Thus it may be regarded as temperature tolerant and common species of all the streams. These fungi were best sporulated at 15-20 °C and preferred pH (6-7) slightly acidic to neutral. This study gives a brief account of the diversity of Hyphomycetous fungi isolated from Shipra river of Ujjain, Madhya Pradesh,and also provided a database for altitudinal impact on species composition of water-borne conidial fungi.[13]

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| ISSN: 2582-7219 | www.ijmrset.com | Impact Factor: 7.54| Monthly Peer Reviewed & Referred Journal |

| Volume 6, Issue 9, September 2023 |

| DOI:10.15680/IJMRSET.2023.0609015 |

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Impact Factor
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