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Deep Mycoses in Certain Freshwater Fishes of Eastern Uttar Pradesh (India) Caused by Watermoulds

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Abstract: Fungi are known to attack eggs, fry, fingerlings and adults of fishes and as a general rule the fungal infection starts when the host gets injured either mechanically or as a result of infections other than fungal. Besides the surface infections (dermatomycoses), the fungal pathogens penetrate into deeper tissues and cause deep mycoses damaging the internal tissues and organs. *Anabas testudineus*, *Hypophthalmichthys molitrix*, *Labeo rohita*, *Labeo calbasu*, *Mystus mystus* have been studied for the deep mycoses caused by watermoulds. The present investigations have shown several symptoms from tissue damage to the spinal curvature and an attempt has been made to assess the extent of deep dermal tissue destruction in *Anabas testudineus* parasitized by *Pythium oligandrum* Dreschler. The proteolytic enzyme production by the watermoulds has been found to be directly proportional to the intensity of fungal infection in fish.

Keywords: Deep mycoses, Watermoulds, *Anabas testudineus*, *Hypophthalmichthys molitrix*, *Labeo rohita*, *Labeo calbasu*, *Mystus mystus*, Proteolytic enzyme

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Introduction

Fish is one of the man's oldest foods, and because of its importance as a rich source of protein, it is of great nutritional value. At present more than half of the world's population does not get enough protein and the entire Asia, Africa and Latin America

suffer from malnutrition. In view of the rapid growth of the world's population, it seems advisable to be economical with this important source of very rich, palatable and easily digested protein (Prabhuji and Srivastava, 2014).

Fungi attack eggs, fry, fingerlings and adults of fishes and as a general rule the fungal infection starts when the host gets injured either mechanically or as a result of infections other than fungal. Fungal infection in fishes was first reported in 1777 (Ramsbottom, 1916). Since then a large number of fishes have been reported to be parasitized by different aquatic fungi from different parts of the world. Earlier workers have merely noted the occurrence of these fungi on various fishes and their eggs (Bennet, 1842; Huxley, 1882 a, b; Humphrey, 1893; Patterson, 1903; Lund, 1934). The first significant work on fish mycoses was conducted by Tiffney (1939) who demonstrated the ability of *Saprolegnia parasitica* Coker to parasitize a wide range of fish and amphibians. Vishniac and Nigrelli (1957) demonstrated the ability of fourteen saprophytic species of Saprolegniaceae to infect Mexican Platyfish, *Xiphophorus maculatus*. Other noteworthy works are that of Scott and O'Bier (1962), Scott and O'Warren (1964), Sprague (1965, 1966), Wolke (1975) and Al-Saadi *et al.* (1979). The latest researches in this field includes those of Czczuga and Muszynska (1997, 2001); Czczuga *et al.* (2002); Kiziewicz (2004); Al-Rekabi *et al.* (2005); Udomkusonsri *et al.*, (2007); Refai *et al.* (2010); Eli *et al.* (2011) and Hussian *et al.* (2013).

Bhargava *et al.* (1971) were the first to report the fungal infection of *Anabas testudineus* in India. Later, Srivastava and Srivastava (1975; 1976 a, b, c; 1977 a, b, c, d, e, f), Srivastava (1976), Prabhuji *et al.* (1983, 1984, 1986, 1988), Sinha (1985) and Srivastava *et al.* (1984, 1986, 1992) have reported several watermoulds causing diseases on a wide range of freshwater fish

species. The latest Indian contribution in this field include the research contributions of Prabhuji (2010), Mastan *et al.* (2011), Prabhuji *et al.* (2012 a, b), Prabhuji and Srivastava (2014), Chauhan (2014) and Pachade *et al.* (2014 a, b).

Srivastava (1976) has done an exhaustive work on fish-mycopathology and, besides reporting several diseases of fish caused by aquatic fungi; he suggested various chemical control measures to combat with these diseases. Sinha (1985), Prabhuji and Sinha (2009) and Prabhuji *et al.* (2012 b) have reported deep mycoses in freshwater fishes together with the dermatomycoses and branchiomycoses. Prabhuji *et al.* (1988) reported from India, for the first time, the deep mycoses to the level of the vital organs of fish. Prabhuji and Sinha (2009) have given exhaustive account and the life-cycle pattern of *Ichthyophonus hoferi* causing deep mycoses in *Carassius carassius*, the gold fish. However, the Indian work regarding the watermoulds causing diseases in fish and their control are very meagre and there is an apt need of extensive as well as intensive investigations in this field of research related with the masses.

The water moulds are basically the facultative parasites and, therefore, it is imperative to understand the life cycle patterns, distribution, effects of various physico-chemical factors on the life-cycle of these microorganisms (Prabhuji, 2011; Prabhuji *et al.*, 2012 a, c). In the present study an attempt has been made to assess the types of deep mycoses symptoms in some diseased fish species. Also observations were made to evaluate the damaged dermal tissue of *Anabas testudineus* parasitized by *Pythium oligandrum*.

Materials and Methods

Fish species (*Anabas testudineus*, *Hypophthalmichthys molitrix*, *Labeo rohita*, *Labeo calbasu*, *Mystus mystus*) were caught from various water reservoirs and observations were made to study the types of deep mycoses symptoms. In order to study the development of pathogen in different body parts of the host fish and also to determine the extent of damage caused by the pathogen, the fish, *Anabas testudineus*, was selected for histopathological studies (Srivastava *et al.*, 2017 a).

Results

During the course of the present investigation following variations in the types of deep mycoses symptoms were observed in the diseased fish species collected from different places:

(a) *Labeo calbasu*: Some specimens collected showed deep tissue damage on mid-dorsal body region (Figs. 1 A, B).

(b) *Anabas testudineus*: The collected specimens showed exfoliation of scales (the primary symptom of deep mycoses) and deep tissue damage (muscular necrosis) on mid-dorsal body region with fungal hyphal mat (Figs. 2 A, B).

(c) *Mystus mystus*: One of the collected specimens indicated distinct tissue degeneration (muscular necrosis) in caudal peduncle region (Figs. 3 A, B).

(d) *Labeo rohita*: A few specimens collected showed the spinal curvature which probably indicates the deep tissue damage in and around the spinal-cord (Fig. 4 A).

(e) *Hypophthalmichthys molitrix*: A few specimens collected showed the spinal

curvature which probably indicates the deep tissue damage in and around the spinal-cord (Fig. 4 B).

Figs. 1 A-B: Mature *Labeo calbasu* showing deep mycoses. A: Symptoms of deep tissue damage on mid-dorsal body region. B: Same; enlarged view.

Figs. 2 A – B: Mature *Anabas testudineus* showing deep mycoses. A: Showing exfoliation of scales, primary symptoms indicating deep mycosis. B: Symptoms of deep tissue damage on mid-dorsal body region with fungal hyphal mat.

Figs. 3 A – B: Mature *Mystus mystus* showing deep-mycoses. A: Infection and tissue destruction in caudal peduncle region; and infection spots in snout region. B: Infection spots on whole middle body region.

Figs. 4 A–B: Mature *Labeo rohita* and *Hypophthalmichthys molitrix* showing symptoms of spinal curvature due to deep internal mycoses. A: *Labeo rohita* B: *Hypophthalmichthys molitrix*.

Isolation of pathogenic watermoulds:

Infections related with deep mycoses were observed on some of the common edible fishes of eastern Uttar Pradesh (India) (Table 1). These fish species were: (i) *Labeo rohita* (ii) *Labeo calbasu* (iii) *Anabas testudineus* (iv) *Hypophthalmichthys molitrix* and (v) *Mystus mystus*. The diseased fish specimens have been collected from the mentioned sites (five

districts of eastern U.P., viz., Gorakhpur, Deoria, Maharajganj, Ballia and Jaunpur) during November, 2013 to December 2014. The host fish individuals were identified using the keys provided by Srivastava (2010).

Pathogenicity test of watermoulds

To ascertain the parasitic ability of the watermoulds isolated, controlled re-infection experiments were conducted using the healthy individuals of *Labeo rohita*, *L. calbasu*, *Anabas testudineus*, *Hypophthalmichthys molitrix*, and *Mystus mystus*.

Hyphae of the watermoulds were observed protruding out from the experimentally injured areas of all the test fishes within 10 – 24 h of the start of the experiment. Such infected individuals died of dermal ulceration within 20 – 48 h of exposure to the watermould inoculum (Table 2). The watermoulds, growing on the artificially infected fish, were isolated and compared with the cultures of the original inocula and was found to be identical. For maintaining the control for the experiment three test fishes of each type were kept under identical conditions, but were not exposed to the watermould inocula.

Histopathological studies

During the present study, observations have been made on the damaged dermal tissues of *Anabas testudineus* by *Pythium oligandrum* (Srivastava *et al.*, 2017 a). On comparison with the healthy dermal tissues (Plate I; Fig. 1) it has been recorded that in the diseased regions the epidermis, stratum spongiosum, stratum compactum and the underlying muscle fibres were seriously damaged and the affected areas were covered with fungal fragments (Plate I, II; Figs. 2 – 6).

Table 1: Collection of infected fish specimens and the watermoulds isolated

S. No.	Name of Fish	Symptoms	Watermoulds isolated
1.	<i>Labeo rohita</i> (G/M)	Lesion on dorsal side	<i>Saprolegnia diclina</i> <i>Aphanomyces laevis</i> . <i>Achlya diffusa</i>
2.	<i>Labeo calbasu</i> (G/B/D)	Lesion on dorsal side	<i>Aphanomyces laevis</i> , <i>Achlya diffusa</i>
3.	<i>Anabas testudineus</i> (J)	Lesion on lateral side	<i>Pythium oligandrum</i>
4.	<i>Labeo rohita</i> (D/M)	Lesion on snout	<i>Saprolegnia diclina</i> , <i>Allomyces recurvus</i>
5.	<i>Hypophthalmichthys molitrix</i> (G)	Spinal curvature	<i>Allomyces recurvus</i>
6.	<i>Labeo rohita</i> (G)	Spinal curvature Whole body infection	<i>Achlya diffusa</i>
7.	<i>Mystus mystus</i> (G)	Whole body infection	<i>Aphanomyces laevis</i>

B: Surha Lake, Ballia

D: Government Fish Farm, Khukhundu, Deoria

G: Ramgarh Lake, Gorakhpur

J: Gujar Tal, Jaunpur

M: Private Fish Farm, Maharajganj

Table 2: Controlled re-infection studies demonstrating the parasitic ability of different watermoulds

Name of Pathogen	Mycosis evident Within hour	Death occurred Within hour
<i>Saprolegnia diclina</i>	10 – 12	20 – 22
<i>Aphanomyces laevis</i>	10 – 12	22 – 24
<i>Achlya diffusa</i>	12 – 15	20 – 22
<i>Allomyces recurvus</i>	18 – 22	24 – 28
<i>Pythium oligandrum</i>	12 – 15	26 – 30

Plate I: Figs. 1-4: Histopathological investigation on dermal tissues of *Anabas testudineus* parasitized by *Pythium oligandrum* X 100. Fig. 1: Healthy dermal tissues. Fig. 2: Diseased dermal part showing completely damaged epidermis, damaged stratum spongiosum and stratum compactum regions indicating detachment of both layers. Arrows indicate the fragments of hyphae of *Pythium oligandrum*. Fig. 3: Diseased dermal part showing damaged epidermis, partially damaged and hypertrophied stratum spongiosum and negligibly damaged stratum compactum. Damaged muscles are evident. Arrows indicate the fragments of hyphae of *Pythium oligandrum*. Fig. 4: Diseased dermal part showing damaged epidermis, partially damaged and hypertrophied stratum spongiosum and negligibly damaged stratum compactum. Arrows indicate the fragments of hyphae of *Pythium oligandrum*. (epi: epidermis; sts: stratum spongiosum; stc: stratum compactum; m: muscle fibres bundles; Hf: hyphal fragments; de: damaged epidermis; dm: damaged muscle fibre bundles). (After Srivastava *et al.*, 2017 a)

Discussion

The water moulds are known to attack the body surface of the fish (dermatomycoses) and progressively grow deeper in the tissues causing deep mycoses (Huxley, 1882 b; Vishniac and Nigrelli, 1957; Egusa 1963; Sinha 1985; Prabhuji *et al.*, 1988; Prabhuji and Sinha, 2009; Srivastava *et al.*, 2017 a).

The observations suggest that the pathogenic water moulds are not tissue specific and capable of attacking virtually any tissue. This conclusion agrees with the observations with other workers (Bootsma,

Plate II: Figs. 5-6: Histopathological investigation on dermal tissues of *Anabas testudineus* parasitized by *Pythium oligandrum*. X 100. Fig. 5: Diseased dermal part showing completely damaged epidermis, partially damaged and hypertrophied stratum spongiosum and negligibly damaged stratum compactum. Damaged muscles are evident. Arrows indicate the fragments of hyphae. Fig. 6: Diseased dermal part showing completely damaged epidermis and stratum spongiosum. Stratum compactum region is also seriously damaged. Arrows indicate the fragments of hyphae. (sts: stratum spongiosum; stc: stratum compactum; m: muscle fibres bundles; Hf: hyphal fragments; dm: damaged muscle fibre bundles). (After Srivastava *et al.*, 2017 a)

1973; Nolard-Tintigner, 1971, 1973, 1974; Wolke, 1975; Dukes, 1975; Neish, 1977; Papatheodorou, 1980; Sinha, 1985; Prabhuji, 2013; Prabhuji and Sinha, 2009; Prabhuji and Srivastava, 2013; 2014). The infection of water moulds may not be treated as a simple superficial infection, but, a cutaneous invasion of deeper tissues. They may invade the vital tissues of the fish. Nolard-Tintigner (1971) concluded that the fatal invasion of tissues might depend on the speed of fungal growth, the type of tissue destroyed and the distance between the point of infection and the vital organ. A simple change in the superficial tissues may not justify the death of fish. However, it seems reasonable to think that the actual cause of death may be related to the

impaired osmoregulation and the inability of the fish to maintain its body fluid balance (Gardner, 1974; Hargens and Perez, 1975). The biochemical analysis may provide a more convincing explanation for the process. Various stressors which include crowding, injury, sub-optimal water temperature, handling and presence of noxious chemicals in sub-lethal concentration etc. acting singly or synergistically operate through pituitary-interrenal axis to produce an increase in the level of plasma-corticosteroids which can impair the inflammatory responses (Mc Leay, 1975) and lead to an increase in corticosteroid regulated protein catabolism and gluconeogenesis (Woodhead, 1975). This may ultimately result into a protein deficiency which contributes to the wasting of skeletal muscles and leads to decrease in antibody production and collagen synthesis. Lack of collagen, in turn, impairs the ability of the fish to heal wounds and ulcers (Prabhuji *et al.*, 2012 b) and results into mortality.

Srivastava *et al.* (2017 a) have reported deep dermal mycosis caused by *Pythium oligandrum* Dreschler on *Anabas testudineus* Bl. Sinha (1985) have earlier reported similar tissue damage in deep dermal tissues in *Anabas testudineus* Bl., *Channa punctatus* Bl., *Chela laubuca* Ham. and *Colisa lalius* Ham. caused by *Dictyuchus sterile*, *Saprolegnia diclina* and *Saprolegnia ferax*. Srivastava *et al.* (1994) have also described deep dermal mycoses in *Chela laubuca* Ham. caused by *Achlya orion* Coker and Couch. The lesions were completely dominated by the profuse growth of fungal mycelia. The epidermis and the dermis were completely missing and the subcutis (hypodermis) layer also showed disintegration exposing the underlying musculature. Some of the hyphal fragments,

penetrating into the muscle tissue, were also observed.

Roberts *et al.* (1970), Pickering and Willoughby (1977), Roberts (1978), and Prabhuji *et al.* (2012 b) have reported deep dermal mycoses deeper enough up to destruction of musculature to the level of bones. The pathogens were watermoulds together with bacteria and probably viruses too and therefore, designated to be the "Dermal Ulcerative Syndrome". The present investigation has recorded the destruction of only upper dermal tissues from epidermis up to hypodermis with mild to moderate damage in upper muscle layers in *Anabas testudineus* by *Pythium oligandrum*. Although, it does not appear likely that watermoulds produce any toxins within the fish tissues (Rucker, 1944; Nolard-Tintigner, 1973; Peduzzi *et al.*, 1976; Neish, 1977), the biochemical stimulants behind the tissue necrosis, hypertrophy or hyperplasia cannot be ignored. There remains the possibility that toxic materials, if secreted by the pathogen, is of quite transient nature in the host and thus can be detected with difficulty though it may still be effective. Therefore, the possible activity of toxins or pectolytic and proteolytic enzymes cannot be ruled out. Srivastava *et al.* (2017 b) have studied in detail the role of pectolytic and proteolytic enzymes affecting the virulence of watermoulds during pathogenesis on fish. Furthermore, they found a direct effect of physico-chemical factors on the production of proteolytic enzymes by watermoulds and elucidated the directly proportional correlation between the amount of proteolytic enzyme produced and the severity of infection.

Conclusion

The pathogenic watermoulds are responsible for severe infection at various levels in fishes from egg stage to the adult and cause heavy damage to pisciculture. The injuries at body surface is the major factor in developing a number of disease symptoms like dermal mycoses, deep mycoses and spinal curvature etc. The present work has provided a detail study on deep mycoses in fishes. The production of proteolytic enzyme by the pathogen has been found to be directly proportional to the intensity of fungal infection i.e. higher the concentration of protease enzyme more will be the tissue destruction.

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