Int. J. Biol. Res., 7(1): 47-55, 2019.

FUNGAL INFECTION IN COMMERCIALLY IMPORTANT FISHES OF BALLOKI HEADWORKS, RIVER RAVI, PUNJAB, PAKISTAN

Zafar Iqbal[1] and Zakia Khatoon[2*]

[1]Fish Disease and Health Management Laboratory, Department of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan
[2]Food & Marine Resources Research Centre, PCSIR Labs. Complex, Karachi, Pakistan
[*]Corresponding author's email: zakia.khatoon@gmail.com

ABSTRACT

Over one hundred specimens of fish were sampled from River Ravi in district Kasur to observe fungal infections. The samples comprised of nine species (*Oreochromis aureus, Labeo rohita, Cirrhinus mrigala, Wallago attu, Channa marulius, Ctenopharyngodon idella, Cyprinus carpio, Hypophthalmichthys molitrix, Gibelion catla*). The infected fish showed serious clinical signs such as deep skin ulcers, eroded scales and tips of fins, lesion at the base of fins. The fungal infection was confirmed by isolating the fungi from skin, fins and gills of fish and culturing it on three different media, sabdourd dextrose agar (SDA), Potato dextrose agar (PDA) and malt extract agar (MEA). Six fungal genera, *Aspergillus* (40.92%), *Penicillium* (11.55%), *Fusarium* (6.93%), *Alternaria* (1.98%), *Trichoderma* (1.65%) and *Helminthosporium* (0.66%) were isolated. *Wallago attu* was the most affected species with five types of fungal growth. *Helminthosporium* has been reported for the first time from *W. attu* in Pakistan. There is need to focus attention on the diseases of wild and commercially important fishes. This can be achieved by controlling and lowering of water pollution in Ravi, conducting periodic surveys of fish fauna and application of modern disease diagnostic techniques.

KEYWORDS: Fungal infection, Fishes, Balloki Headworks, River Ravi, Pakistan.

INTRODUCTION

Rivers, Ravi is considered as the smallest tributary of the Indus River System of Pakistan. Its main origin is from Himalaya's mountains, flows through Jammu & Kashmir then enters into Shakargarh (Tehsil of Sialkot), Pakistan (covers about 640 km distance). Bulloki Headwork was constructed on River Ravi originally in 1913 and later extended to link up with Sulemanki Headwork in 1956 to feed the canal network of River Sutlej during the water scarcity season. The Balloki Headworks (Lat. 31^0 $29^/$ N, Long. 73^0 $85^/$ E) is located around 65 km from Lahore (district Kasur), Punjab. The untreated municipal and industrial wastes from Lahore and nearby industrial areas are discharged directly into the River Ravi (Yasar *et al.*, 2010). Various studies were conducted to assess the level of water pollution at various points of Balloki Headworks, e.g., water quality (Javed and Hayat, 1995; Ahmad and Ali, 1998), metal toxicity (Javed and Hayat, 1999; Javed and Mahmood, 2001; Javed, 2004; Rauf and Javed, 2007), heavy metals (Rauf *et al*., 2009; Jabeen *et al*., 2012), pesticides (Akhtar *et al*., 2014). The fish fauna in the aquatic environment also indicates the status of pollution (Javed and Hayat, 1998; Gernhofer *et al*., 2001). Balloki Headworks is considered as one of the main commercial fishing areas of River Ravi with more than 56 native and

alien freshwater fish fauna were reported (Ahmad and Mirza, 2002; Khan *et al*., 2011; Hussain *et al*., 2014). The population of these fishes had drastically declined during the past few years owing to the increasing level of pollution in River Ravi (Hussain *et al*., 2014). The impact of pollution on the growth of fishes in the surrounding waters of Balloki Headworks was studied by Jabeen *et al.* (2012), Shakir and Qazi (2013), Tabinda *et al*. (2013), Shakir *et al.* (2014, 2015). According to More *et al.* (2003) the municipal and industrial toxicants are cytotoxic, mutagenic and carcinogenic for the aquatic life including the fish fauna. Jabeen *et al.* (2012) also stated that health of the people immensely affected by consuming the fish of the polluted area. The importance of fish resources of River Ravi for its provision as a source of food and employment can suggest responsible fisheries management and sound policy development at the national level for the maintenance of food security. Likewise, knowledge on the health status of inland fisheries is the key to better decision-making for its management (de Graaf *et al*., 2012). Fungal diseases in freshwater fishes are increasing due to organic pollutants; they are vector to produce infections which limit the fish production (Kumari and Kumar, 2015). Considering the alarming level of aquatic pollution in River Ravi, it was aimed to investigate the status of fungal infection in fishes and to isolate fungi from infected fishes of the study area.

MATERIALS AND METHODS

The fish samples were collected from Balloki Headworks, River Ravi and brought live to laboratory of Fish Disease and Health Management in aerated plastic bags under sterile conditions during the month of December 2013. In the laboratory taxonomic identification of fish species were carried out by the help of Mirza (1970) and Misra (1962), the total length (TL), body weight (BW) and health status of each fish was recorded. Visually the infected fishes have white fluffy appearance and bloody spot at the site of infection. Prevalence of fungal infection was calculated by using the following formula:

$$\text{P.O.I.} = \frac{\text{Number of fungal infected fish}}{\text{Total number of fish}} \times 100$$

For fungal culture media were prepared by using three kinds of agar; malt extract agar (MEA) (13 g/200 mL), sabouraud dextrose agar (SDA) (10 g/200 mL) and potato dextrose agar (PDA) (7.8 g/200 mL).

The fish body was arbitrary divided into three parts; skin, fins and gills. Tissues from infected body parts of fish were removed, sterilized with 1% alcohol for 5 minutes, rinsed with sterilized water and later the fungal isolates with the help of sterile needle were inoculated on prepared agar plates. The inoculated plates were incubated at 28-30^{0}C in incubator, observed and in the mid of 4th to 7th day fungal growth was noted. Colonies with different colors were observed in agar plates; microscopic slides were prepared from each colony and stained with 0.05% trypan blue in lactophenol. The slides were observed under Digiopro-labomed microscope and photographed. The fungi were identified with the help of fungal identification key and literature (Willoughby, 1994; Klich, 2002).

RESULTS

In the present study, 101 fish samples of nine different species (*Oreochromis aureus*, *Labeo rohita*, *Cirrhinus mrigala*, *Wallago attu*, *Channa marulius*, *Ctenopharyngodon idella*, *Cyprinus carpio*, *Hypophthalmichthys molitrix* and *Gibelion catla*) were examined for fungal infection. Visually the infected fish showed clinical signs such as deep skin ulcers, eroded scales and fin tips, lesions at base of fins, dermal lesions, damaged caudal fins, and heavy mucous production (Figs. 1-3).Table 1 shows the prevalence of fungal infection in seven fish species; *C. carpio* (100%), *C. mrigala* (82%), *W. attu* (80%), *C. marulius* (80%), *C. idella* (75%), *L. rohita* (66.7%), *O. aureus* (48.7%), whereas *G. catla* and *H. molitrix* did not show any infection. A total of six fungal genera (*Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria*, *Trichoderma* and *Helminthosporium*) were isolated from the infected fish species. Five fungal genera were isolated from *W. attu*; four fungal genera were isolated from *O. aureus*, while from the rest of five each fish species have growth of three fungal genera. The incidence of different fungal genera show that *Aspergillus* was isolated from seven, *Penicillium* was isolated from six, *Alternaria* was isolated from five, *Fusarium* was isolated from three, *Trichoderma* was isolated from two and *Helminthosporium* was isolated from a single fish species, respectively (Table 2). Collectively the fungal genera isolated from skin, fins, and gills of these seven fish species verify that highest prevalence was found with *Aspergillus* (40.92%), followed by *Penicillim* (11.55%) and *Fusarium* (6.93%), whereas *Altenaria* (1.98%), *Trichoderma* (1.65%), and *Helminthosporium* (0.66%) were isolated in low occurrence (Table 3). The data of Table 3 also showed that fins were the most affected body part (74.25%) followed by skin (66.33%) and gills (50.49%).

DISCUSSION

According to an estimate about 729 tons/day untreated industrial and domestic wastes are being dumped into the River Ravi (Yasar *et al.*, 2010). Various studies have been carried out on the severe pollution of River Ravi related to heavy metal (Javed and Hayat, 1999; Rauf *et al*., 2009; Jabeen *et al*., 2012), pesticides (Akhtar *et al*., 2014), toxicity (Javed and Mahmood, 2001), water quality (Javed and Hayat, 1995; Javed, 2004) and aquatic life (Javed and Hayat, 1998; Tabinda *et al.*, 2013). The intensity of negative factors also cause stresses on the lives of aquatic organisms (Shakir and Qazi, 2013; Shakir *et al*., 2014, 2015).Ogbonna (1989) indicates that in the aquatic ecosystem there are certain fungal infection which occur in brood stock and all life stages of fish and eggs. They are ubiquitous in nature and are source of fish diseases under favorable conditions which ultimately cause poor growth, loss of fecundity, minimize production and mortality of fishes (Naik and Minhas, 1992).

The aim of present study was to investigate fungal infection in commercially important fishes of Balloki Headworks, River Ravi in Kasur District. Six fungal genera (*Aspergillus, Penicillium, Fusarium, Alternaria, Trichoderma* and *Helminthosporium*) were isolated from 7 different fish species. From the species which caught >10 in numbers, higher infection level was observed in *C. mirigala* (82.35%), followed by *W. attu* (80%), *L. rohita* (66.7%), and *O. aureus* (48.7%). Five other fish species were collected <10 specimens in number, from these *H. molitrix* and *G. catla* were collected in single specimen each with no indication of fungal growth.

Fig.1. Partly eroded caudal and anal fins of *Wallago attu*.

Fig. 2.*Channa marulius* having damaged and eroded caudal fin tips.

Fig.3. *Cyprinus carpio* with skin lesions (red circles) and partly damaged caudal fin.

Table 1. Prevalence of fungal infection in fish samples examined.

S. No.	Fish species	Number of fishes	No. of infected fishes	Prevalence of fungal infection %
1.	*Oreochromis aureus* (Steindachner, 1864)	39	19	48.7
2.	*Labeo rohita* (Hamilton, 1822)	21	14	66.7
3.	*Cirrhinus mrigala* (Hamilton, 1822)	17	14	82.35
4.	*Wallago attu* (Bloch & Schneider, 1801)	10	8	80.0
5.	*Channa marulius* (Hamilton, 1822)	5	4	80.0
6.	*Ctenopharyngodon idella* (Valenciennes, 1844)	4	3	75.0
7.	*Cyprinus carpio* Linnaeus, 1758	3	3	100.0
8.	*Hypophthalmichthys molitrix* (Valenciennes, 1844)	1	-	-
9.	*Gibelion catla* (Hamilton, 1822)	1	-	-
	Total	101	65	64.34

Table 2. Occurrence of various fungal genera in different fish species.

S. No.	Fish species	*Aspergillus*	*Penicillium*	*Alterniaria*	*Fusarium*	*Tricho derma*	*Helminthsporium*
1.	*O. aureus*	+	+	+	+	-	-
2.	*C. idella*	+	+	+	-	-	-
3.	*W. attu*	+	+	+	-	+	+
4.	*L. rohita*	+	+	-	-	+	-
5.	*C. mrigala*	+	-	+	+	-	-
6.	*C. marulius*	+	+	-	+	-	-
7.	*C. carpio*	+	+	+	-	-	-

Note: In the Table (+) denotes presence and (-) shows absence of fungal infection

Table 3. Incidence of fungal genera isolated from different organs of fish species.

S. No.	Identified fungal genera	No. of infected / inoculated plates			Total no. of infected plates (out of 303)	Incidence %
		Skin (out of 101)	Fins (out of 101)	Gills (out of 101)		
1.	*Aspergillus* sp.	42	45	37	124	40.92
2.	*Penicillium* sp.	13	16	6	35	11.55
3.	*Fusarium* sp.	7	10	4	21	6.93
4.	*Alternaria* sp.	2	1	3	6	1.98
5.	*Trichoderma* sp.	2	3	0	5	1.65
6.	*Helminthosporium* sp.	1	0	1	2	0.66
	Total	67	75	51	193	
	Percentage	66.33%	74.25%	50.49%	63.7%	

The results presented in Tables 2 and 3 show that amongst the seven infected fish species, *Aspergillus* was the most prevalent fungus with occurrence of 40.92% found in the skin, fins and gills. High prevalence of *Aspergillus* species was also reported by Iqbal and Saleemi (2013) in *Catla catla* and Chauhan *et al.* (2014) in *Labeo calbasu*, where it caused granulomas formations and necrotization in internal organs of experimental fish resulting 100% mortality. The second next fungal genus observed was *Penicillium* (11.55%) isolated from fins, skin and gills of six fish species. Iqbal *et al.* (2012a & b) also found *Penicillium* in economically important carps. Chauhan (2013) in India reported low (2.1%) incidence of *Penicillium* in freshwater fishes whereas Gholampourazizi *et al.* (2014) isolated *Penicillium* (12.5%) from *Salmo trutta caspius* in Iran. The third next fungal genus isolated was *Fusarium* with 6.9% incidence in the skin, fins and gills of three fish species. Fadaeifard *et al.* (2011) isolated *Fusarium* from the eggs and brood stock of rainbow trout; Malathi and Rajendran (2012) also isolated *Fusarium* from *Channa punctatus,* whereas Gholampourazizi *et al.* (2014) reported 8.33% incidence of *Fusarium* in *S. trutta caspius*; Chauhan (2014) also reported prevalence of *Fusarium* in freshwater fishes of Bhopal; Kumari and Kumar (2015) isolated *Fusarium* from *L. rohita, C. catla, C. marulius, Notopterus chitala* and *Channa striatus* from Gandak River, India. The fourth common fungal genus which infects the fish was *Alternaria* (1.98%) isolated from five fish species. Fadaeifard *et al.* (2011) reported *Alternaria* as a common fungal infection found in the fish farms in Iran. The result can also be compared with Iqbal *et al.* (2012a & b); Chauhan (2013), Iqbal and Mumtaz (2013), Haroon *et al.* (2014) and Gholampourazizi *et al.* (2014), who reported the presence of *Alternaria* in different freshwater and ornamental fishes. The fifth fungal growth was genus *Trichoderma* (1.65%) isolated from two fish species. Many species in the genus *Trichoderma* can be characterized as opportunistic virulent plant symbionts, its occurrence in water bodies and effect on fish health still needs to be clarified. The sixth fungus *Helminthosporium* isolated in rare occurrence (0.66%) and severely infected the skin and gills of *W. attu.* It seems to be the first report of *Helminthosporium* sp. isolated from any freshwater fish in Pakistan. The results however are comparable to Gholampourazizi *et al.* (2014) who isolated *Helminthosporium* along with 11 other fungal genera from *S. trutta caspius* and its incidence was 4.16%. It was also observed that five fungal genera were isolated from *W. attu*; four fungal genera were isolated from *O. aureus;* the remaining five fish species carried three fungal genera each with different combination.

The industrial and municipal waste discharged into the River Ravi has increased level of heavy metals in water, sediments and aquatic organisms (Rauf *et al*., 2009; Jabeen *et al.*, 2012). Although due to the addition of freshwater through upstream Q. B. Link canal into Ravi, the effect of water pollution become mild at Head Balloki area (Shakir *et al.*, 2014). There is need to give attention on the diseases of wild but commercially important fish throughout the stretch of River Ravi. This may be achieved through control and lowering of water pollution in Ravi, conducting periodic survey of fish fauna and application of modern disease diagnostic techniques. The significance of pathogenicity of *Trichoderma* sp. and *Helminthosporium* sp. in freshwater fishes of Pakistan need further investigation. The fungal infection in these fish species may be attributed to highly pollutant water in the River Ravi which is deteriorating due to high influx of untreated urban sewage and industrial effluents (Shakir *et al.*, 2015).

REFERENCES

Ahmad, K. and W. Ali. (1998). Variation in the Ravi River water quality. Proc. 24th WEDC Conference. Sanitation and water for all. Islamabad, Pakistan, 153-156.

Ahmad, Z. and M.R. Mirza. (2002). Fishes of River Ravi from Lahore to Head Balloki. *Biologia* (Pakistan), 48: 317-322.

Akhtar, M., S. Mahboob, S. Sultana and T. Sultana. (2014). Pesticides in the River Ravi and its tributaries between its stretches from Shahdara to Balloki Headworks, Punjab-Pakistan. *Water Environ.Res*., 86: 13-19.

Chauhan, R. (2013). Studies on conidial fungi isolated from some fresh water fishes. *Int.J.Adv.Life.Sci.*, 6: 131-135.

Chauhan, R. (2014). Studies on some fresh water fishes found infected with Dermatomycoses, collected from different water bodies in and around Bhopal, India. *Indo Am.J. Pharmaceut.Res*., 4:1591-1596.

Chauhan, R., Z. Nisar and A.H. Baig. (2014). Studies on Aspergillomycosis in *Labeo calbasu* found infected with *Aspergillus flavus* and *A. terreus*. *World J.Phar.Pharmaceut. Sci*., 3:1842-1848.

De Graaf, G., D.M. Bartley, J.V. Jorgensen and G. Marmulla. (2012). The Scale of Inland Fisheries, Can We Do Better? Alternative Approaches. *Fish.Managem.Ecol.,*

Fadaeifard, F., M. Raissay, H. Bahram, E. Rahimi and A. Najafipoor. (2011). Freshwater fungi isolated from eggs and broodstocks with a emphasis on *Saprolegnia* in rainbow trout farms in west Iran. *Afr.J.Microbiol.Res*., 4: 3647-3651.

Gernhofer, M., M. Pawert, M. Schramm, E. Muller and R. Triebskorn. (2001). Ultra structural biomarkers as tools to characterize the health status of fish in contaminated streams. *J.Aquat.Ecosyst.Stress Recov*., 8: 241-260.

Gholampourazizi, I., S.M. Hosseinifard, S. Rouhi and H. Moqhtader. (2014). Isolation and recognition infection fungus of *Salmo trutta caspius* in fish farming of the Mazandaran province, Northern Iran. *J.Anim.Biol*., 6: 45-53.

Haroon, F., Z. Iqbal, K. Pervaiz and A.N. Khalid. (2014). Incidence of fungal infection of freshwater ornamental fish in Pakistan. *Int.J.Agric.Biol*., 16: 411-415.

Hussain, A., A.Q.K. Sulehria, M. Ejaz, A. Maqbool and M R. Mirza. 2014. Temporal variations in commercial fish community of a floodplain of the River Ravi, Pakistan. *Biologia*, 60: 73-80.

Iqbal, Z., M. Asghar and Rubaba. (2012a). Saprolegniasis in two commericially important carps. *Pak. J. Zool*., 44: 515-520.

Iqbal, Z., U. Sheikh and R. Mughal. (2012b). Fungal infections in some economically important freshwater fishes. *Pak.Vet.J*., 32: 422-426.

Iqbal, Z. and R. Mumtaz. (2013). Some fungal pathogens of an ornamental fish, Blackmoor (*Carassius auratus* L.). *Eur.J.Vet.Med*., 2: 1-10.

Iqbal, Z. and S. Saleemi. (2013). Isolation of pathogenic fungi from a freshwater commercial fish, *Catla catla* (Hamilton). *Sci.Int. (Lahore)*, 25: 851-855.

Jabeen, G., M.M. Javed and H. Azmat. (2012). Assessment of heavy metals in the fish collected from the River Ravi, Pakistan. *Pak.Vet.J*., 32:107-111.

Javed, M. and S. Hayat. (1995). Effect of waste disposal on the water quality of River Ravi from Lahore to Head Baloki, Pakistan. *Proc. Pakistan Congr. Zool*., 15: 41-51.

Javed, M. and S. Hayat. (1998). Fish as a bio-indicator of freshwater contamination by metals. *Pak.J.Agri.Sci*., 35: 11-15.

Javed, M. and S. Hayat. (1999). Heavy metal toxicity of River Ravi aquatic ecosystem. *Pak.J.Agri.Sci*., 36: 1-9.

Javed, M. and G. Mahmood. (2001). Metal toxicity of water in a stretch of River Ravi from Shahdera to Baloki Headworks. *Pak.J.Agri.Sci.*, 38: 37-42.

Javed, M. (2004). Studies on metal toxicity and physico-chemistry of water in the stretch of River Ravi from Balloki Headworks to Sidhnai Barrage. *Indus.J.Biol.Sci*., 1: 106-110.

Khan, M.A., Z. Ali, S.Y. Shelly, Z. Ahmad and M.R. Mirza. (2011). Aliens; a catastrophe for native freshwater fish diversity in Pakistan. *J.Anim.Plant Sci.,* 21: 435-440.

Klich, M.A. (2002). *Identification of common Aspergillus* species. Pp. 116. Centraal bureau voor Schimmel cultures, Utrecht, The Netherlands.

Kumari, R. and C. Kumar (2015). Fungal infection in some economically important fresh water fishes in Gandak River near Muzaffarpur region of Bihar. *Int.J.Life Sci.Pharma. Res.,* 5: 1-11.

Malathi, K. and K. Rajendran. (2012). Isolation of fungi and bacteria from various tissues of freshwater fish *Channa punctatus* (BLOCH). *Asian J.Micro.Biotech.Environ.Sci.,* 14:553-556.

Mirza, M.R. (1970). A contribution to the fishes of Lahore including revision of classification and addition of new records. *Biologia* (Pakistan), 16: 71-118.

Misra, K.S. (1962). An aid to the identification of the common commercial fishes of India and Pakistan. *Rec.Indian Mus*., 57: 1-320.

More, T.G., R.A. Rajput and N.N. Bandela. (2003). Impact of heavy metals on DNA content in the whole body of freshwater bivalve, *Lamelleidens marginalis*. *Environ.Sci.Pollut.Res*., 22: 605-616.

Naik, I.U. and I.K. Minhas. (1992). *Bacterial diseases of cultured carps in Punjab*. Punjab Fish.Bull., 2.Fisheries Research and Training Institute, Lahore Pak. pp. 10.

Ogbonna, C.I.C. (1989). Fungi associated with disease of freshwater fishes in Plateau state, Nigeria. *J.Aquat.Sci*., 4: 59-62.

Rauf, A. and M. Javed. (2007). Copper toxicity of water and plankton in the River Ravi, Pakistan. *Int.J.Agric.Biol*., 9: 771-774.

Rauf, A., M. Javed, M. Ubaidullah and S. Abdullah. (2009). Assessment of heavy metals in Sediments of the River Ravi, Pakistan. *Int.J.Agric.Biol*., 11: 197-200.

Shakir, H.A. and J.I. Qazi. (2013). Impact of industrial and muncipial discharges on growth coefficient and condition factor of major carps from Lahore stretch of River Ravi. *J.Ani.Plant Sci.,*23: 167-173.

Shakir, H.A., J.I. Qazi, A.S. Chaudhry and S. Ali. (2014). Metal accumulation patterns in livers of major carps from anthropogenically affected segment of the River Ravi, Pakistan. *Int.J.Adv.Res.Biol.Sci*., 1: 261-268.

Shakir, H.A., J.I. Qazi, A.S. Chaudhry and S. Ali. (2015). Metal bioaccumulation levels in different organs of three edible fish species from the River Ravi, Pakistan. *Int.J.Aqua.Biol*., 3: 135-148.

Tabinda, A.B., S. Bashir, A. Yasar and M. Hussain. (2013). Metals concentrations in the riverine water, sediments and fishes from River Ravi at Balloki Headworks. *J. Anim.Plant Sci*., 23:76-84.

Yasar, A., A. Fawad, A. Fateha, I. Amna and R. Zainab. (2010). *River Ravi potentials, pollution and solutions: an overview*. World Environmental Day: pp 39-48. Pakistan Engineering Congress Building, Lahore. S 4660. Pakistan.

Willoughby, L.G. (1994). *Fungi and Fish Diseases*. Pisces Press, Stirling. UK.pp. 57.

(Received April 2019; Accepted May 2019)