

# Prevalence and Identification Fungal of Fungal Disease on Catfish (*Clarias gariepinus*) Juvenile at Kendal CPrevalence and Identification Fungal of Fungal Disease on Catfish oastal Region, Central Java

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**Submission date:** 26-Feb-2021 03:28AM (UTC+0700)

**Submission ID:** 1518195852

**File name:** s\_gariepinus\_Juvenile\_at\_Kendal\_Coastal\_Region,\_Central\_Java.pdf (683.12K)

**Word count:** 3669

**Character count:** 19189

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## Prevalence and identification fungal of fungal disease on Catfish (*Clarias gariepinus*) juvenile at Kendal Coastal Region, Central Java

To cite this article: Fauza Alfisyahrin *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **246** 012048

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## Prevalence and identification fungal of fungal disease on Catfish (*Clarias gariepinus*) juvenile at Kendal Coastal Region, Central Java

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**Abstract.** Fungal disease on fish frequently as secondary infection and can cause death. It found on wound of fish. The aim of this study was to identify fungi that appeared on catfish hatchery at Kendal Coastal area. Methodology of this research was experimental at exploratory. Based on the method, 10 fungal infected catfish consisted of fish at 5-7 cm and another 5 fish at 8-10 cm length. Prevalence obtained from 80 fish observed in every pond, from 4 ponds. Body wound such as lesions and fin rot suspected of fungal infection were streaked on to Sabouraud Dextrose Agar (SDA) and incubated in room temperature for 2 to 7 days. nine fungi found infected fish sample with various character. The fungi then purified and identified. Based on the morphology characters, the fungi were identified as K.21 (*Microsporum* sp.), K.22 (*Fusarium* sp.), K.31 (*Aspergillus flavus*), K.33 (*A. fumigatus*), K.71 (*A. niger*), K.72 (*A. nidulans*), K.73 (*Fusarium oxysporum*), K.8 (*Trichophyton* sp.), and K.9 (*Rhizopus stolonifer*) the prevalence was 30% (Pond A), 20% (Pond B), 25% (Pond C), 22,5%(Pond D) and fungal dominant rate between 8 and 16%. Postulate Koch were conducted with twice repetition for each fungi to 10 fishes indicated. *A. flavus* was the most pathogenic fungal species to catfish with mortality 85% while the lowest of causing mortality was *R. stolonifer* with only 15%.

### 1. Introduction

Catfish (*Clarias gariepinus*) is a profitable aquaculture organism, a fast growing fish, and has a good economic value as it's excellences. Catfish conveniently culture with a simple or traditional technologies [1]. Increasing production require good juvenile in quality and quantity. Kendal is a region in Central Java which produced catfish with amount reached 14,018,800 juvenile in 2010 [2]. Fungal disease is a problem aquaculture have to deal with [3]. This matter could detained production. Degrade of environment could impact to imbalance the interaction between fish and environment, and may triggered disease [4].

Fungi can easily infecting fish by disperse through the water. Fungi infected fish when immune system of the fish deteriorate, this could be happened due to inappropriate water condition or happened as secondary infection [5]. Some fungi infected fish potent to occurring fish mortality. The fungi were *Aphanomyces invandans*, *A. astaci*, *Branchiomyces sanguinis*, and *B. Demigrans* [6], *Aspergillus flavus* [7]. Furthermore, zoospore and fungi conidium were able to associated with fish disease, they were *Aspergillus*, *Fusarium*, *Penicillium* spp., *Alternaria* spp., *Mucor*, and *Rhizopus* [8].

The aim of this study is to discover the fungi infected catfish (*Clarias gariepinus*) juvenile, to find out the prevalence of fungi infected catfish (*Clarias gariepinus*) juvenile at Kendal Coastal Region, Central Java, and to ascertain the fungi which potential to infect fish. The study was conducted during April to July 2018, fish sample taken from hatchery at Sukorejo, Kendal, Central Java. Koch's Postulate test conducted at Aquaculture Department Laboratory, Fishery and Marine Science Faculty, Diponegoro University, and fungi isolation and identification was conducted in Tropical Marine Biotechnology Laboratory, Diponegoro University

### 2. Research Methods

#### 2.1. Fish Sampling



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Fish sample were catfish (*Clarias gariepinus*) taken from catfish hatchery in Kendal Coastal Area, Central Java. Fish collected with purposive sampling method, fish sample were 10 catfish juvenile infected with fungi, consisted of 5 fish with 5 – 7 cm length and 5 fish with 8 – 10 length which taken from two ponds. Prevalence calculation obtained from 80 fishes observed in every pond, with total 4 ponds. Fish sample showed infected fungal clinical signs such as wounded on fin, head, or mouth, and cotton like grew on the wound. Fungi isolation conducted at Tropical Marine Biotechnology, Diponegoro University. Wound isolated aseptically, the wound or part of the fish body placed to Sabouraud Dextrose Agar (SDA) as the growth fungi media. Fungi isolates then incubated in room temperature with 24 h lighting for 2 – 7 days until the fungi grow.

## 2.2. Procedure

Implementation procedure were tools sterilization for tools used during the research. Sterilization done by using autoclave, meanwhile sterilization for laminary air flow, section kit, object glass and cover glass cleaned using alcohol. Fish basin sterilization done by washed with soap and dry in the sun. Sabouraud Dextrose Agar (SDA) needed to make media as much as 65 grams for every 1000 ml water, then sterilized with autoclave for 15 minutes in 121°C and 1 atm pressure.

## 2.3. Identification, prevalence, and dominance.

Fish infected with fungi observed by its clinical signs appeared on the body surface and fin. Fungi isolates identified by the morphology character. Isolate identification done by observation macroscopically and microscopically. Obtained result from observation for fungi identification were shape, texture, color, and growth of fungi colony, as well as the shape of hyphae, conidium, spore to identified the genus or species of the fungi. The prevalence value of fungi infected fish in catfish (*Clarias gariepinus*) hatchery gained from proportion of the amount of infected fish to the sum of fish observed. The formula of prevalence calculation [9] were as follows,

$$\text{Prevalence} = \frac{\text{the amount of infected fish}}{\text{the amount of fish observed}} \times 100\%$$

The dominance value showed the existence of the fungi in infection. Dominance of the fungi infected fish calculated with formula [9] explained as follows,

$$\text{Dominance} = \frac{\text{the amount of one kind of fungi infected the fish}}{\text{the sum of fungi infected fish}} \times 100\%$$

## 2.4. Koch's Postulate

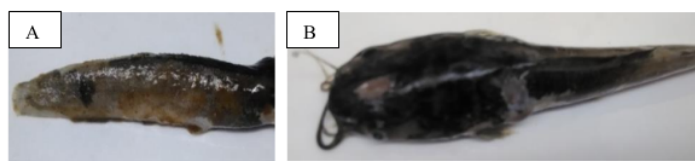
Koch's Postulate is a procedure to prove if an organism could lead an infection or disease [10]. Tested fish were catfish (*Clarias gariepinus*) juvenile size of 5 – 10 cm. The fish placed in basins with 10 litre water and 10 fish in each basin. Fish acclimated in the basin prior to re-infection within 7 days. Firstly, the fish were hurt on the body surface and fin prior to infected with fungi. The fungi diluted in physiology NaCl with 10<sup>4</sup> density and pour 2, 5 ml to each the basin. Water quality observed at the hatchery and during Koch's Postulate test were temperature and pH.

## 3. Results and Discussion

### 3.1. Result

#### 3.1.1. Clinical Signs

The clinical signs occurred on infected fish were wound covered by cotton like growth on fin, mouth, head, and dorsal. The same clinical signs appeared on infected fish in Koch's Postulate test, there were cotton wool like growth on body surface. The clinical signs on infected fish show on figure 1 as follows



**Figure 1.** Fish infected with fungi (A) cotton wool like growth on body surface, (B) eroded skin wound

Clinical sign observation and mortality of fish in Koch's Postulate test after 14 days show on table 1 as follows,

**Table 1.** Clinical Sign Observation and Mortality of Fish In Koch's Postulate Test

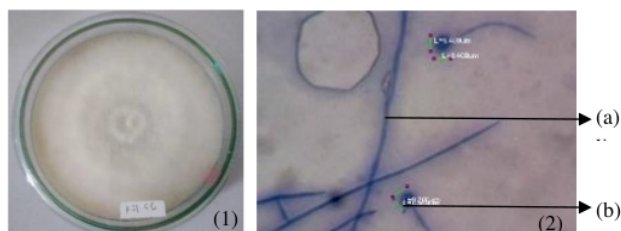
No.	code	Numbers of the dead fish (individual)	Numbers of live fish (individual)	Mortality (%)	Clinical signs
1.	K. 21 a	2	8	20	White fibers like cotton on the fin
	K. 21 b	2	8		
2.	K. 22 a	1	9	35	Wound on the caudal and on the dorsal fin
	K. 22 b	6	4		
3.	K. 31 a	0	10	50	Brownish fibers that covers the fish body
	K. 31 b	10	0		
4.	K. 33 a	8	2	85	Wound on the fin
	K. 33 b	9	1		
5.	K. 71 a	8	2	45	Wound on the fin and caudal
	K. 71 b	1	9		
6.	K. 72 a	0	10	20	Fibers on the fish body
	K. 72 b	4	6		
7.	K. 73 a	3	7	35	White wound on the caudal
	K. 73 b	4	6		
8.	K. 8 a	6	4	40	Wound on the dorsal fin
	K. 8 b	2	8		
9.	K. 9 a	1	9	15	Fibers like cotton on the wound
	K. 9 b	2	8		

Koch's Postulate test result showed that K.33 (*A. flavus*) causing the highest mortality with number 85% and the lowest of causing mortality were K.9 (*Rhizopus stolonifer*) with number 15%.

### 3.1.2. Identification

The result of fish identification with fungal infection was 9 isolates identified fungi (K.21, K.22, K.31, K.33, K.71, K.72, K.73, K.8, K.9) from 17 isolates. The fungi were identified as *Microsporum* sp. (K.21), *Fusarium* sp. (K.22), *Aspergillus fumigatus* (K.31), *Aspergillus flavus* (K.33), *Aspergillus niger* (K.71), *Aspergillus nidulans* (K.72), *Fusarium oxysporum* (K.73), *Trichophyton* sp. (K.8), dan *Rhizopus stolonifer* (K.9). Each fungi shown in figures as follows,

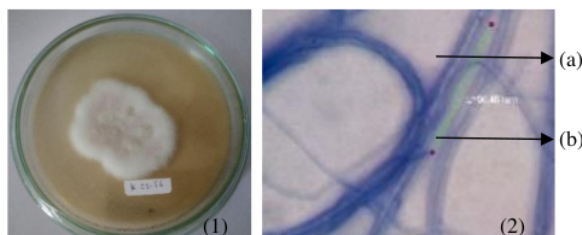
*Microsporum* sp (K.21) was a fast growing fungi, white-cream colored, cotton and downy textured on both sides of the colony and has hyphae with no septate and round conidium.



**Figure 2.** *Microsporum* sp. (1) Macroscopic observation (2) Microscopic observation with 400x magnification

Information: (a) Hyphae, (2.2.b) Microconidium (8,4 – 9,28  $\mu\text{m}$ )

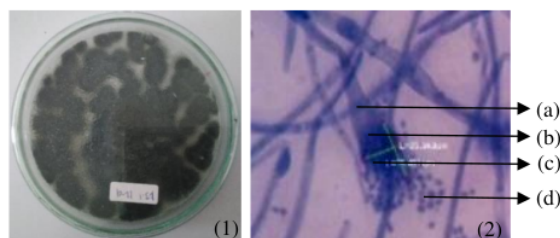
*Fusarium* sp (K.22) was a slow growth fungi, creamy reddish and bony white colored on the edge of colony and white-pink on the reverse. The colony textured was deep cottony, and has septate hyphae



**Figure 3.** *Fusarium* sp. (1) Macroscopic observation (2) Microscopic observation with 400x magnification

Information: (3.2.a) Hyphae, (3.2.b) Septate

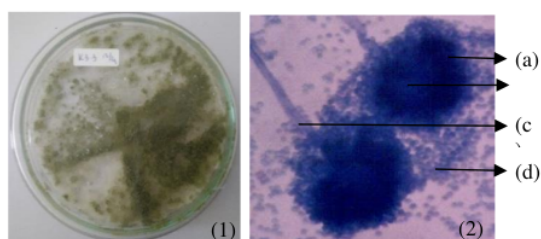
*Aspergillus fumigatus* (k.31) was a fast-growing fungi, blue grayish colored and orange colored in reverse. Powdery texture and wrinkle in reverse.



**Figure 4.** *Aspergillus fumigatus* (1) Macroscopic observation (2) Microscopic observation with 400x magnification

Information: (4.2.a) Conidiophore, (4.2.b) Vesicle, (4.2.c) Phialides, (4.2.d) Conidia  
(Vesicle size: 15,45 – 20,45  $\mu\text{m}$ )

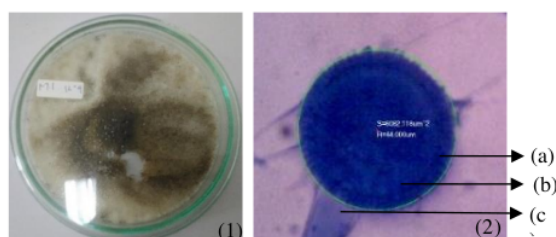
*Aspergillus flavus* (K.33) had downy texture on the base and powdery on the surface of the colony. The colony was white and yellowish green on the surface, pale yellow on reverse. The fungi grew filled the plate within 5 to 6 days.



**Figure 5.** *Aspergillus flavus* (1) Macroscopic observation (2) Microscopic observation with 400x magnification

Information: (5.2.a) Phialides, (5.2.b) Vesicle, (5.2.c) Conidiophore, (5.2.d) Conidium  
(Vesicle size: 30 – 40  $\mu\text{m}$ )

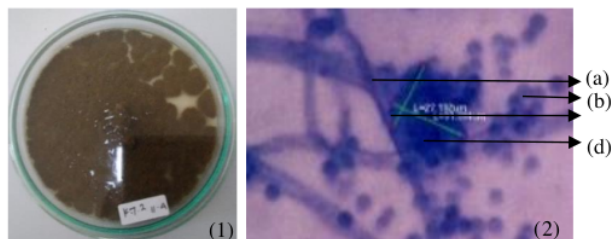
*Aspergillus niger* (K.71) was white colored on the base surface and brown to black on the surface of the colony, pale yellow on surface. Downy and powdery textured and grow fast within 2 to 4 days.



**Figure 6.** *Aspergillus niger* (1) Macroscopic observation (2) Microscopic observation with 400x magnification

Information: (6.1.a) Conidiophore, (6.1.b) Vesicle, (6.1.c) Phialides, (6.1.d) Konidium  
(Vesicle size: 15,45 – 20,45  $\mu\text{m}$ )

*Aspergillus nidulans* (K.72) was grow fast and filled plate in day 5. Old green colored on early days and turn into brown last days, pale brown on the reverse. Downy and powdery colony textured



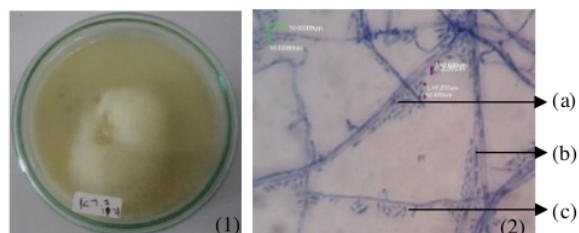
**Figure 7.** *Aspergillus nidulans* (1) Macroscopic observation (2) Microscopic observation with 400x magnification

Information: (7.2.a) Conidiophore, (7.2.b) Vesicle, (7.2.c) Phialides, (7.2.d) Conidium



(Vesicle size: 15,45 – 20,45  $\mu\text{m}$ )

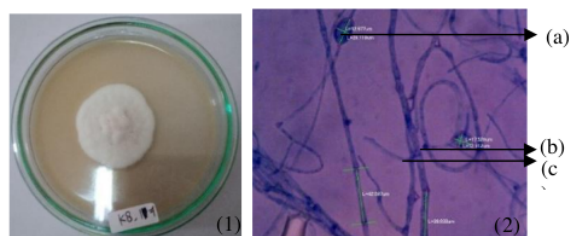
*Fusarium oxysporum* (K.73) was downy and cotton textured, but slow growth. White to pink and purplish on the centre and white on the edge of the colony white same color on reverse.



**Figure 8.** *Fusarium oxysporum* (1) Macroscopic observation (2) Microscopic observation with 400x magnification

Information: (8.2.a) Microconidium, (8.2.b) Hyphae, (8.2.c) Phialides

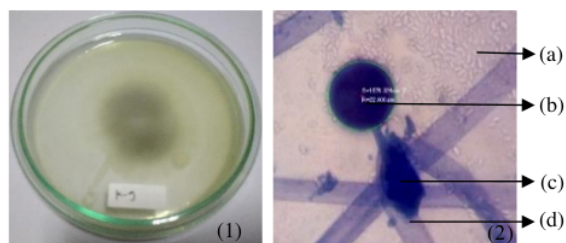
*Trichophyton* sp. (K.8) was a slow growth fungi with downy textured. Pale white to orange brownish and water appeared as the fungi got older. The reverse colony colored orange brownish on the center.



**Figure 9.** *Trichophyton* sp. (1) Macroscopic observation (2) Microscopic observation with 400x magnification

Information: (9.2.a) Chlamidospore, (9.2.b) Microconidium, (9.2.c) Phialides

*Rhizopus stolonifer* (K.9) was grow fast within 2 to 3 days and filled the plate. The texture were white grayish cotton and pale colored on reverse



**Figure 10.** *Rhizopus stolonifer* (1) Macroscopic observation (2) Microscopic observation with 400x magnification

Information: (10.2.a) Sporangiospore, (10.2.b) Sporangium, (10.2.c) Columella, (10.2.d) Sporangiophore



### 3.1.3. Prevalence

The prevalence result of *Clarias gariepinus* from hatchery at Kendal Coastal Area were 30% for pond A, 20% for pond B, 25% for pond C, and 22.5% for pond D, with eighty fish for each pond.

### 3.1.4. Dominance

The result of dominance fungi from infected fish was 9 dominance fungi. The dominance of 9 fungi are show in table 2.

**Table 2.** Dominance of Fungi Infected Catfish (*Clarias gariepinus*) Juvenile at Kendal Coastal Area, Central Java

No.	Fungi	Dominance (%)
1.	<i>Microsporium</i> sp.	16.0
2.	<i>Fusarium</i> sp.	12.5
3.	<i>Aspergillus fumigatus</i>	12.5
4.	<i>Aspergillus flavus</i>	8.3
5.	<i>Aspergillus niger</i>	8.3
6.	<i>Aspergillus nidulans</i>	8.3
7.	<i>Fusarium oxysporum</i>	8.3
8.	<i>Trichophyton</i> sp.	12.5
9.	<i>Rhizopus stolonifer</i>	12.5

*Microsporium* sp. was the highest dominant fungi infected catfish (*Clarias gariepinus*) juvenile at Kendal Coastal Area with number 16% whilst the lowest were *A. flavus*, *A. niger*, *A. nidulans*, and *Fusarium oxysporum*.

### 3.1.5 Water Quality

Water quality represented by pH and temperature measured in catfish (*Clarias gariepinus*) hatchery at Kendal Coastal Area, Central Java and during Koch's Postulate test. The temperature during Koch's Postulate test were between 24 and 27°C and in catfish (*Clarias gariepinus*) hatchery at Kendal Coastal Area, Central Java were between 24 and 26°C. pH measured during Koch's Postulate test were between 5 – 8 and 7 – 8 in catfish (*Clarias gariepinus*) hatchery at Kendal Coastal Area.

### 3.2. Discussion

Based on the study, the fish found infected with fungi showed clinical signs such as wound and covered with growth of cotton wool like. The clinical signs appeared on infected fish cannot represented by fungi species causing infection. [11] found that ulcer and lessions on skin indicated the fungal infection. *Microsporium* sp. identified by its character, cotton wool like textured colored white colony an same on the reverse. *Microsporium* sp. has microconidia and septate hyphae. [12] explained that *Microsporium* genera classification based on sexual reproduction characteristic. Besides, identification mainly based on asexual reproduction (myceliumseptate, microconidium, and macroconidium). *Microsporium* sp. can cause dermatophytosis. Koch's Postulate test result showed that *Microsporium* sp. causing mortality with number 20%. [12] dermatophytosis in an infection caused by fungi from *Microsporium* sp. genera. Infection on wound occurred, and released secondary metabolite which role as toxin for the host.

K. 22 were identified as *Fusarium* sp. and K.73 were identified as *Fusarium oxysporum*, causing mortality in Koch's Postulate test with number 35% and 40% respectively. Infection caused by *Fusarium* named *Fusariosis*. [11] *Fusarium* infection on fish causing dermatitis and lession with various development depended on another factors such as water quality and sun light. *Fusarium oxysporum* infection occurred internally and externally.

*Aspergillus* genera found on this study were *fumigatus* (K.31), *A. flavus* (K.33), *A. niger* (K.71), dan *A. nidulans* (K.72). Koch's Postulate test result showed the number of mortality caused by *fumigatus*

(K.31), *A. flavus* (K.33), *A. niger* (K.71), dan *A. nidulans* (K.72) were 50%, 85%, 45%, and 20% respectively. [13] said that, *Aspergillus* conidium easily dispersed on air and soil. The most deadly secondary metabolite produced by *A. flavus*. *A. fumigatus* produced gliotoksin, fumagilin, and helvolic acid that role as toxic for the host.

*Trichophyton* sp. identified because of the characteristic, microconidium, phialides, chlamidospore, and hyphae. Based on Koch's Postulate test, *Trichophyton* sp. causing mortality on fish with number 60%. *Trichophyton* sp. was a causal disease for human, animals, and plants. [12] explained that, *Trichophyton rubrum* synthesized lysine. [14] said that, the most occurrence fungi isolated from eggs, juvenile, and water culture of *Clarias gariepinus*.

*Rhizopus stolonifer* (K.9) has hyphae, sporangiophore, columela, sporangium, sporangiospore. *Rhizopus* sporangium rounded shape with size 20 – 25  $\mu\text{m}$ . Based on Koch's Postulate, *Rhizopus stolonifer* causing mortality with number 20%. [15] explained that, some *Rhizopus* species were infectious disease to human and animals. Infection caused by rhizoxin phytotoxin synthesized by endosymbiotic *Burkholderia* attached to hyphae.

The highest dominance of fungi infected fish was *Microsporium* sp. with number 16% and the lowest were *A. flavus*, *A. niger*, *A. nidulans*, and *Fusarium oxysporum* with number 8,3%. The prevalence obtained from eighty fish observed in 4 ponds were 30% (pond A), 20% (pond B), 25% (pond C), 22, 5% (pond D). High prevalence indicated bad water quality, handling, and infrastructure [16]. The optimum condition for *Aspergillus* to produced aflatoxin were 85% relative humidity, temperature within 25 – 35°C, and pH 6 [7]. Increasing and decreasing fungal infection in catfish production depended on management and maintenance condition. Occurrence of fungal infection dan affected by environment and climate changing [17]

#### 4. Conclusion

Based on the study, fungi isolates obtained from catfish hatchery at Kendal Coastal Area were (*Microsporium* sp.), K.22 (*Fusarium* sp.), K.31 (*Aspergillus flavus*), K.33 (*A. fumigatus*), K.71 (*A. niger*), K.72 (*A. nidulans*), K.73 (*Fusarium oxysporum*), K.8 (*Trichophyton* sp.), dan K.9 (*Rhizopus stolonifer*). The highest dominance of fungi infected fish was *Microsporium* sp. with number 16% and the lowest were *A. flavus*, *A. niger*, *A. nidulans*, and *Fusarium oxysporum* with number 8, 3%. The highest prevalence was 30% (pond A) and the lowest was 20% (pond B).

#### Acknowledgement

The authors would like to thank the head of the Tropical Marine Biotechnology, Aquaculture Laboratory, Faculty of Fisheries and Marine Science. Also Ephistalia who have helped this research. We are also grateful to the Head of Integrated Laboratory, Diponegoro University which provides an opportunity to conduct this research. This research was partly funded by PNPB of Faculty Fishery and Marine Science Faculty contract number 1501-9/UN 7.5.10/LT/2018.

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# Prevalence and Identification Fungal of Fungal Disease on Catfish (Clarias gariepinus) Juvenile at Kendal C

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