Identification and the prevalence of fungal gouramy (Osphronemus gouramy) in modern market Surabaya

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Abstract. Gouramy (Osphronemus gouramy) is the one type of fish consumption that are quite popular in Indonesia. Modern market provides a wide range of needs such as fresh fish. Sales of fresh fish in modern markets in Surabaya could reach 80% annually. Required more careful handling against high transaction fresh fish in modern market including whether there is disease in fresh fish like fungus. This research aims to learn about identification and the prevalence of fungal infecting gouramy at modern market Surabaya. Results of the study showed that of the 10 samples taken from 5 location, 7 fish showed positive fish infacted by the fugus. The fugus are Trichoderma, Rhizopus oryzae, Saprolegnea, Aspergillus flavus, and Fusarium. The value of the prevalence of fungus which infects fish gouramy is 70%.

1. Introduction

Gouramy (Osphronemus gouramy) is a type of cultivated fish included in 10 types of fish that are targeted to increase aquaculture production by 353% in 2009-2014 which was proclaimed by the Indonesian Ministry of Maritime Affairs and Fisheries [1].

Data from the Director General of Cultivation shows that gouramy production from 2010 to 2013 showed a positive performance with an average annual increase of 15.74%, but did not reach the target [2]. The unsatisfactory results of gouramy production are caused by bad water conditions that lead to disease [3]. One of the obstacles in cultivating gouramy (O. gouramy) is the attack of pests and diseases, both at the hatchery level and at its enlargement [4].

One of the diseases that attacks ready-to-consume gouramy is fungus [5]. Clinical symptoms of fungal infection are the presence of fine threads resembling cotton sticks to the eggs or wounds on the external parts of the fish such as changes in the color of the fins and the rednedd of fish’s body. The fungus is quickly transmitted to other fish in the same pond so that the potential losses incurred are quite large [6]. Several types of fungi in gouramy, namely Penicillium glabrum, Rhizopus oryzae, Aspergillus flavus, A. niger, A. candidus, Saprolegnia, Fusarium and Curvularia lunata [5].

Fungi in fish are dangerous because they produce mycotoxins as a result of their metabolites. Mycotoxins in Aspergillus sp. namely aflatoxins are harmful to animals and humans [7]. Aflatoxins in high concentrations can cause acute illness and death, while low concentrations in the long term can cause necrosis of liver and kidney cells [8]. Based on this background, it is necessary to know the types of fungi that attack gouramy in modern markets in the Surabaya area.

2. Materials and methods

2.1. Method

This research was conducted using a survey method through direct on-site data collection. The location for taking fish samples was determined deliberately. The sampling area was carried out in five designated
Surabaya areas, namely 'A' as Central Surabaya, 'B' as North Surabaya, 'C' as East Surabaya, 'D' as South Surabaya, and 'E' region as West Surabaya.

The sampling method was carried out randomly (random sampling) to the modern markets in each area of Surabaya. Samples from the Central Surabaya area were taken from Carrefour Kapasan with the code "Th". Samples from the North Surabaya area were taken from Giant Rajawali with the code "Ua". Samples from the East Surabaya area were taken from Superindo Mulyosari with the code "Tr". Samples from the South Surabaya area were taken from Giant Ahmad Yani with the code "Sn". Samples from the West Surabaya area were taken from the Pakuwon Hypermarket with the code "Bt". From each modern market, two gouramy fish were taken as samples to be examined.

2.1.1. Tools sterilization
The equipment that is carried out by the sterilization process is laminary flow, refrigerator, sectio kit, tray, cover glass, object glass, microscope, bunsen, ose needle, and petri dish.

2.1.2. Media
Media for the isolation and identification of fungi, namely Sabouraud Dextrose Agar (SDA) and Lactophenol Cotton Blue.

2.1.3. Fungsi isolation from gouramy
The fungus was isolated using a loop needle then implanted on SDA media. The media was incubated at 25 °C for 2-7 days and then identified in the laboratory. Samples grown on SDA media are a mixture of various kinds of fungal isolates and are often contaminated with bacteria so that they need to be purified. The purification process begins by taking one type of colony using an ose on old SDA media that has a similar texture, then isolating it on new SDA media and incubating it at 25 °C for 2-7 days to get pure isolates.

2.1.4. Examination of samples and identification of fungi
The purified fungi are ready for identification. The identification technique used to observe fungal isolates was the slide culture method. Start by placing a bent U-shaped plate on a Petri dish. Object glass can be placed on the plate. Give a block so that the size of 1x1 cm above the object glass then closed the glass cover. To maintain moisture, add a little water to the petri dish. Cover the petri dishes and store for incubation at 25 °C for 3-7 days.

To observe the fungi, take a clean glass object then add one drop of lactophenol cotton blue and cover it with a glass cover that has been overgrown with fungus, then it can be directly observed on an electron microscope.

Fungal identification using conventional identification techniques includes two stages, namely macroscopic and microscopic observation of fungi. Macroscopic observations include colony form and colony color, while microscopic observations include hyphae shape, spore shape, spore location and identification is carried out according to Summerbell's identification procedure [9]. The fungi that have been identified can then be determined in their percentage prevalence.

2.2. Research parameter
The main parameters observed in this study were the type of fungus and the prevalence of fungi. Supporting parameters to be observed include DO, pH, and temperature. The prevalence of fungi is calculated by the following formula:

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\text{Prevalence} = \frac{N}{n} \times 100\% 
\]
3. Results and discussion

3.1. Result

3.1.1. Fungi isolation

Fungi of gouramy (*O. gouramy*) from modern market is isolated from fish scales, fins and gills. Each of these organs was isolated and it was found that form location A, the gouramies with the Th 1 isolation code has fungi grown on the fin organs, as well as the Th 2 isolate code. Fish samples at location B with code Ua 1 isolate were not found to have fungal infestation (negative) while code Ua 2 fungi isolate has fungi frown on the fin organ. The sample of gouramy in location C with isolate code Tr 1 was found to be grown by fungi on the scales organ, while isolate code Tr 2 was free from any fungal infestation (negative). Sampling of gouramy at location D with isolate code Sn 1 was found to be grown fungi on the scales, while isolate code Sn 2 was grown by fungi on the fins. In the sampling of location E with isolate Bt 1 code, it was found that the fungus grew on the fins, while the Bt 2 isolate code was not found to have a fungal infection (negative).

3.1.2. Fungi identification

Based on the results of the identification of fungi in gouramy (*O. gouramy*) in the modern market of Surabaya, it is known that the sample of gouramy in location A with isolate code Th1 is overgrown with fungi with the characteristic yellowish white colony and flat surface. While the microscopic features are curved macroconidia and in it has 3 cells. These characteristics are the same as those possessed by the *Fusarium fungus* [10], which is white to yellowish, with jagged edges, and a flat surface. *Fusarium* produces macroconidia and microconidia from phialids [11]. The hyaline macroconidia are sickle-shaped with three bulkheads. While the microconidia is ranging from one to two cells, hyaline, ovoid. Samples from region A with isolate code Th 2, region B with isolate code Ua 2, and sample region D with code Sn 2 had similar characteristics, namely; the colonies are yellowish green in color and have a microscopic feature of long conidophores, with rounded viscicles at the ends and spore chains that spread over the surface of the viscicles. The characteristics of *Aspergillus flavus*, namely greenish yellow colonies and microscopic characteristics of *A. flavus*, which have a long conidophore reaching 400-800 μm, vesicles and conidia that are round in shape with a diameter of 25-45 μm [5].

The sample of gouramy in location C with isolate code Tr 1 has the characteristics of a blue-green colony with white fur on it. The microscopic features are branched conidophores, solitary or clustered phialids, and round conidia. These characteristics are the same as the characteristics of the fungus *Trichoderma harzianum* which has a turquoise color with white fur and grows very fast [9]. The microscopic appearance of *T. harzianum* has septate and hyaline hyphae. Conidophores are hyaline and branched. Phialid *T. Harzianum* hyaline, solitary or in groups, and attached to conidophore. Conidia attached to phialids are round, smooth-walled, and green.

Sampling of gouramy at location D with isolate code Sn 1 contained a fungus with a characteristic of white cotton colony that grew thinly on the surface of the media. The microscopic appearance is a long rhizoid, a long conidophore with a round sporangiophore at the end. Conidia are round in the sporangiophores. These characteristics are the same as those of *Rhizopus oryzae* with colony characteristics such as white cotton rhizoid. *R. oryzae* is threadlike, hyphae that are not septa, and stolons are smooth and brown. The length of the hyphae of *R. oryzae* reaches 18 μm to 1.5 μm [5].
In the sampling location E with isolate code Bt 1, a fungus was overgrown with a characteristic of white cotton colony that grew to fill the petri dish. The microscopic features are filamentous mycelium, unseptual branches, and branched hyphae. These characteristics are the same as those of *Saprolegnia* which have white colonies such as cotton threads [12]. Microscopic characteristics of the genus *Saprolegnia* sp. are unseptic branches and has branching hyphae, also can live in a wide temperature range, namely 3-33 °C [12]. Samples from region B with isolate code Ua 1, samples from region C with isolate code Tr 2, and samples from region E with isolate code Bt 2 were not found to have any fungal infections (negative).

### 3.1.3. Fungi prevalence

Based on the results of the calculation of the prevalence of fungi identified in the samples of gouramy (*O. gouramy*) in the modern market of Surabaya in all regions, it is known that from a total sample of 10 samples, the prevalence of fish infected with the fungus is 70%. The prevalence of fungi at location A is known to be 100%, location B is known to be 50%, location C is known to be 50%, location D is known to be 100% and location E is known to be 50%.

### 3.1.4. Water quality

Based on the results of water quality measurements at each sampling location of gouramy (*O. gouramy*) from 5 locations, the temperature was 26 °C, with 4 ppm of Dissolved Oxygen (DO), and pH 7.

### 3.1.5. Weight and total length of gouramy (*O. gouramy*)

Based on the measurement results of weight and body length of gouramy (*O. gouramy*), it is known that in location A with sample code Th 1, fish sample has weight of 749 g and has total length of 34 cm. While sample code Th 2, fish sample has weight of 670 g and total length of 31.5 cm. At location B with sample code Ua 1 the weight of the fish sample is 495 g and the total length is 28.5 cm, while the sample code Ua 2 the weight of the fish sample is 489 g and the total length is 28 cm. The weight of the fish sample with the sample code Tr 1 at location C was 550 g and the total length was 28.5 cm, while the weight of the fish sample with the sample code Tr 2 was 500 g and the total length was 28.5 cm. The sample weight of fish at location D with sample code Sn 1 was 490 g and a total length of 28.5 cm, while the weight of the fish sample with sample code Sn 2 was 619 g and the total length was 30 cm. The sample weight of fish at location E with sample code Bt 1 was 650 g and a total length of 31.5 cm, while the weight of fish samples with sample code Bt 2 was 770 g and total length was 32 cm.

### 3.2. Discussion

Based on the results of research conducted in five modern markets in Surabaya, it is known that fungi are found on the scales and fins. There were no fungi found on gill organs of any fish. Fungi can grow on fish scales and fins [13]. Of all the fungi that grew, five types of fungi were identified, namely *Fusarium*, *Aspergillus flavus*, *Trichoderma*, *Rhizopus oryzae*, and *Saprolegnia*. The *Fusarium fungus* identified from isolate location A was one of the fungi that had the widest habitat distribution. The distribution of *Fusarium fungi* is very wide with various target organs [10].

Some *Fusarium* species can be pathogenic in humans and animals because they produce mycotoxins [14]. *Fusarium* fungi species that produce zearalenon-type mycotoxins include *F. graminarum* which has an extrogenic activity that causes reproductive failure and diarrhea. *F. sporotrichiodes* and *F. graminearum* were able to produce trichotesene mycotoxins which caused skin necrosis, indigestion, coagulation and immunological disorders [15].

At the sampling locations of fish B, C and D, it was known that the isolated gouramy was identified to be infected by *Aspergillus flavus*. *A. flavus* is one of the main fungal species that produces aflatoxin type mycotoxins. Aflatoxins produced by *A. flavus* consist of B1 and B2 aflatoxin [15]. Aflatoxin B1 (AFB1)
are the most toxic types and are carcinogenic, hepotoxic and mutagenic. Acute poisoning by aflatoxin B1 in fish liver can cause failure of carbohydrate metabolism, fat and protein synthesis, resulting in decrease of liver function due to clotting of erythrocytes and decreased serum protein synthesis. Meanwhile, chronic poisoning will cause immunosuppression due to decreased vitamin K activity and decreased phagocytic activity in macrophages.

At location C it is known that the isolated gouramy (O. gouramy) samples were identified with Trichoderma fungus which is thought not to be a pathogenic fungus to gouramy (O. gouramy). Trichoderma fungus is considered a non-pathogenic fungus. However, Trichoderma has been found in the lung cavity and liver of patients who have had liver transplants [16].

The Trichoderma harzianum colony has a turquoise color with white fur and grows very fast. The microscopic appearance of Trichoderma harzianum has septate and hyaline hyphae. Conidophores are in form of hyaline and branched. Phialids of Trichoderma harzianum are hyaline, solitary or in groups, and attached to conidiophores. Conidia attached to phialids are round, smooth-walled, green in color [9].

At location D, it is known that from the samples of isolated gouramy (O. gouramy), Rhizopus oryzae is identified as not a pathogenic fungus in gouramy (O. gouramy) [17]. R. oryzae is a fungus that does not produce toxins and its presence does not inhibit fish growth, even the immune system of infected fish is not disturbed [5].

Based on the results of the study, it is known that at location E that the isolated gouramy (O. gouramy) samples were identified by fungus Saprolegnea. Saprolegnea fungus is known to not only attack gouramy (O. gouramy) but also other freshwater fish species such as tilapia (Oreochromis niloticus) [18]. Saprolegnea fungus infestation starts from a wound on the fish’s body and a drastic change in the environment, causing the fish to experience stress and a decrease in the immune system [19].

The clinical symptoms of gouramy (O. gouramy) infected by Saprolegnea appears to swim slowly and irregularly, also there white hyphae are shown on the body. Fish movements also tend to weaken and only move slowly on the edge of the aquarium and are not balanced [20]. Gouramy (O. gouramy) which contains Saprolegnea is also seen to have decreased appetite, being individual, swimming passively, disturbing of balance and experiencing damage to the skin and fins [21].

Based on the research results, it is known that at location A the prevalence of gouramy infected with the fungus sample is 100%. In location B the prevalence value is 50%, in location C the prevalence value is 50%, in location D the prevalence value is 100%, while in location E the prevalence value is 100%.

Of the five types of fungi found in five locations, almost all of them are dangerous for human consumption. However, all types of fungi found had a negative impact on the market. All types of fungi were found growing like cotton on the fish’s body. Rhizopus fungi have a macroscopic appearance that resembles cotton [5]. These fungi have ruined the appearance of the fish, thereby reducing the selling price of the fish.

Of the five sampling locations of gouramy, it is known that the prevalence of the five sampling locations for gouramy (O. gouramy) varies. At location A, the prevalence value of gouramy infected with the fungus sample is 100%. In location B the prevalence value is 50%, in location C the prevalence value is 50%, in location D the prevalence value is 100%, while in location E the prevalence value is 100%.

Of the five types of fungi found in five locations, almost all of them are dangerous for human consumption. However, all types of fungi found had a negative impact on the market. All types of fungi were found growing like cotton on the fish’s body. Rhizopus fungi have a macroscopic appearance that resembles cotton [5]. These fungi have ruined the appearance of the fish, thereby reducing the selling price of the fish.

Of the five sampling locations of gouramy, it is known that the average prevalence value of fish infected with fungi is 70%. The prevalence of gouramy studied was in the usual category (89-70%) [22]. This is thought to have occurred due to the level of cleanliness of the water and excessive population at each sampling location which is not good enough as a living habitat for gouramy. Fungal attacks are opportunistic because they only attack fish when experiencing stress or a decrease in the immune system due to changes in environmental conditions. Fungal infections in gouramy are also caused by secondary effects of bacterial, viral and parasitic infections, post-harvest handling and dense fish populations in the aquarium [19].

The water quality testing result obtained during the study showed that there were no significant differences between the five research locations. This shows that the water quality (temperature, pH and DO) is still classified as meeting the life requirements of gouramy (O. gouramy). The life requirements for
gouramy (*O. gouramy*) are constant temperature of 25-28 °C, water acidity (pH) 6.5-8 and oxygen solubility of more than 4 mg/l [23].

Based on the research results, it is known that the total weight and length of the gouramy (*O. gouramy*) samples in the five locations varies and is in accordance with the standard rules for the weight of consumption fish recommended by the government. The standard weight of consumption of gouramy (*O. gouramy*) is 500 - 750 g [24]. The total length of the standard samples of gouramy (*O. gouramy*) in the five research locations is also in accordance with government recommendations. The total standard length of consumption of gouramy (*O. gouramy*) is 28-40 cm [26]. It is known that all fish samples, both small and large, have the potential to grow in fungus. The fungus attacks gouramy from eggs to adulthood [25].

4. Conclusion

Based on the results of the discussion, it was identified that five species of fungi, namely *Fusarium*, *Aspergillus flavus*, *Rhizopus oryzae*, *Trichoderma* and *Saprolegnea* were found on gourami (*O. gouramy*) from modern markets of Surabaya, with an average prevalence value of 70%. Further research is needed in each area of the modern Surabaya market to determine the level of fungal infestation in fresh gouramy (*Osphronemous gouramy*).

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