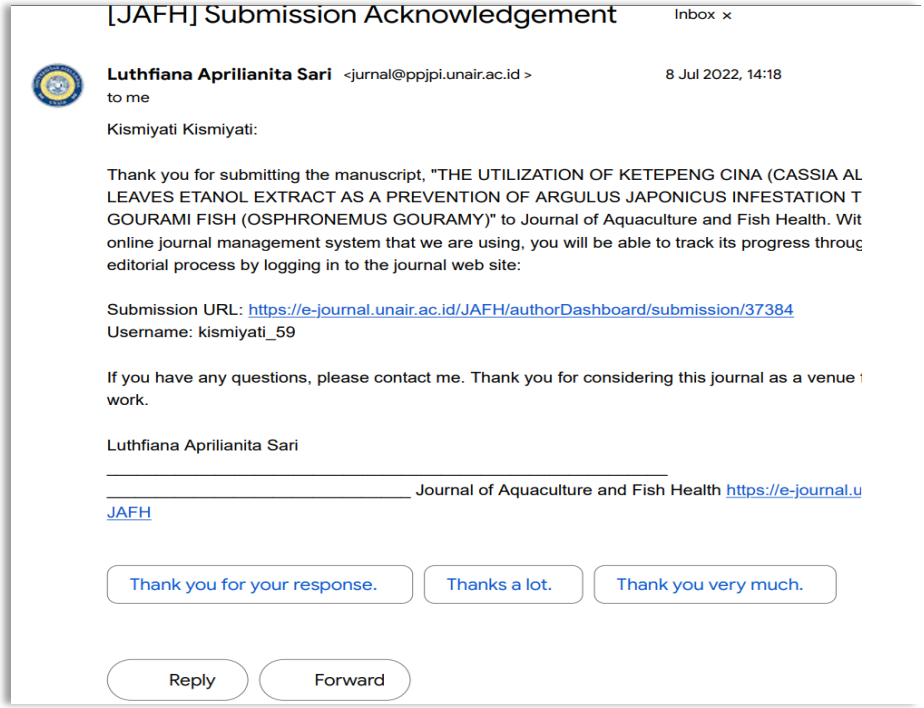
Korespondensi dengan Journal of Aquaculture and Fish Health

1. Pengiriman artikel (8 Juli 2022)
2. Revisi artikel pertama (8 September 2022)
3. Pengiriman revisi artikel pertama (5 November 2022)
4. Revisi artikel ke dua (8 November 2022)
5. Pengiriman revisi artikel ke dua (19 November 2022)
6. Artikel diterima dan akan diterbitkan (9 Desember 2022)

Korespondensi Journal of Aquaculture and Fish Health 12(1) 2023

1. Pengiriman Artikel (8 Juli 2022)

Keterangan: Mengirim artikel tanggal 8 Juli 2022 dengan judul "The Utilization of Ketepeng Cina (*Cassia alata* L.) Leaves Ethanol Extract as a Prevention of *Argulus japonicus* Infestation to Gourami Fish (*Osphronemus gouramy*)" pada website jurnal https://e-journal.unair.ac.id/JAFH dan menerima respon melalui email.


[JAFH] Submission Acknowledgement Inbox ×

Luthfiana Aprilianita Sari <jurnal@ppjpi.unair.ac.id > 8 Jul 2022, 14:18
to me

Kismiyati Kismiyati:

Thank you for submitting the manuscript, "THE UTILIZATION OF KETEPENG CINA (CASSIA AL LEAVES ETANOL EXTRACT AS A PREVENTION OF ARGULUS JAPONICUS INFESTATION T GOURAMI FISH (OSPHRONEMUS GOURAMY)" to Journal of Aquaculture and Fish Health. Wit online journal management system that we are using, you will be able to track its progress throug editorial process by logging in to the journal web site:

Submission URL: https://e-journal.unair.ac.id/JAFH/authorDashboard/submission/37384
Username: kismiyati_59

If you have any questions, please contact me. Thank you for considering this journal as a venue work.

Luthfiana Aprilianita Sari

Journal of Aquaculture and Fish Health https://e-journal.u JAFH

Thank you for your response. Thanks a lot. Thank you very much.

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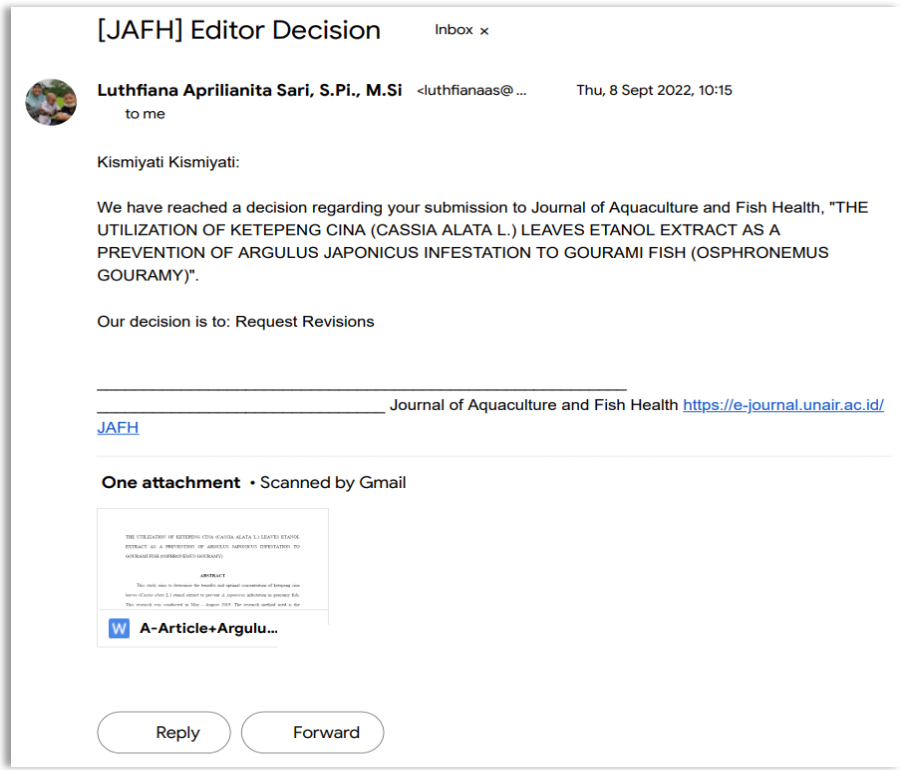
2. Menerima Email Revisi Artikel ke I (8 September 2022)

Keterangan: Menerima email revisi artikel tanggal 8 September 2022. Isi artikel yang direvisi pada penulisan kalimatnya. Pengiriman revisi kembali dikirim melalui website JAFH.


[JAFH] Editor Decision Inbox ×

Luthfiana Aprilianita Sari, S.Pi., M.Si <luthfianaas@... Thu, 8 Sept 2022, 10:15
to me

Kismiyati Kismiyati:

We have reached a decision regarding your submission to Journal of Aquaculture and Fish Health, "THE UTILIZATION OF KETEPENG CINA (CASSIA ALATA L.) LEAVES ETANOL EXTRACT AS A PREVENTION OF ARGULUS JAPONICUS INFESTATION TO GOURAMI FISH (OSPHRONEMUS GOURAMY)".

Our decision is to: Request Revisions

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THE UTILIZATION OF KETEPENG CINA (CASSIA ALATA L.) LEAVES ETANOL EXTRACT AS A PREVENTION OF ARGULUS JAPONICUS INFESTATION TO GOURAMI FISH (OSPHRONEMUS GOURAMY)

ABSTRACT

This study aims to determine the benefits and optimal consentration of ketepeng cina leaves (*Cassia alata* L.) etanol extract to prevent *A. japonicus* infestation in gouramy fish. This research was conducted in May - August 2019. The research method used is the experimental method. This research design used *Complete by Randomized Design* (CRD) with four concentration : Control (P0), 209 ppm (P1), 418 ppm (P2), and 627 ppm (P3). Data was analyzed using *Analysis of Variance* (Anova) and then followed by *Duncan's Multiple Range Test* (DMRT). The results showed that adding ketepeng cina leaves (*Cassia alata* L.) etanol extract can prevent *Argulus japonicus* infestation on gourami fish at the concentration 627 ppm.

Key words: Etanol extract, ketepeng cina leaves, different concentration, gourami fish, *Argulus japonicus* infestation.

Introduction

Gouramy fish is one of the consumable freshwater species in aquaculture (Khairyah *et al.,* 2012). This fish has a lot of meat and relatively easy to culture, so many gourami fish cultured in Indonesia. Gouramy fish has a high selling point and demand (Barkah, 2014). However, gouramy seeds is not in accordance with market needs, caused by high mortality in seeds up to about 50% caused by disease (Nugroho, 2012).

*Argulus japonicu*s is one type of ectoparasites from the Crustacean class which often attacks in freshwater fish culture. *Argulus* can infest carp, gouramy, tilapia, and catfish with a

prevalence 100% and the highest level of intensity is in carp followed by gouramy, tilapia, and catfish (Nurlaela, 2013). Based on research conducted by Wahyuni *et al.* (2013), the data shows that *A. japonicus* infested on gouramy fish culture at Ngrajek Village, Magelang Regency was 4.16%. The results of Abadiyyah's observations (2014), showed that in Fish Seed Hall Rambigundam Jember culture pond, ectoparasites of *A. japonicus* were found which infested gouramy fish.

Comment [SS1]: Fish Seed Hall

A. japonicus predilection on the fins, skin, gills, and the whole host body. Fish that are infested with *A. japonicus* will rub their bodies on hard objects in their surroundings. Clinical symptoms of fish infested with *A. japonicus* are fish seen with lesions on the fins, skin, head, and body surface causing fish to be thin, stunted growth, and death (Noaman *et al.,* 2010).

Prevention of parasitic infestation of *A. japonicus* can use herbal materials available in nature, but due to lack of information and knowledge of the community, causing more people to use chemicals. The use of chemicals in the culture process can damage the environment and the biota in it. In addition, the use of chemicals in the culture process can cause resistance and leave residue in fish (Tam and Avenant, 2005). To reduce the use of chemicals in the culture process, substitution materials are needed to prevent the parasitic *A. japonicus* which is more environmentally friendly, one of which is ketepeng cina leaves.

Ketepeng cina is one type of traditional plant that is often used as an antiparasitic, antifungal, and antibacterial. Ketepeng cina leaves have important ingredients which are alkaloids, saponins, tannins, steroids, anthraquinones, flavonoids, and carbohydrates (Triana *et al.,* 2016). The presence of antiparasitic compounds such as alkaloids, saponins, tannins, anthraquinone glycosides and flavonoid content that can be used as immunostimulants (Kusmardi *et al.,* 2007) on ketepeng cina leaves encourages to commence research on the effectiveness of ketepeng cina leaves extract (*Cassia alata* L.) to prevent *A. japonicus* infestation in gouramy fish (*Osphronemus gouramy*).

Materials and Methods

The study was conducted in February until August 2019 at the Faculty of Fisheries and Marine Airlangga University's Laboratory. The method used in this research is the experimental method. While the research design used is Completely Randomized Design (CRD) using 4 treatments.

Results

The identification of *Argulus* used during the research was carried out after the research finished to keep the *Argulus* used stay in good condition throughout the study. The key to identifying *Argulus* can most easily be seen through the shape of the respiratory area and also the spur on the 4th leg. Respiratory area in *Argulus japonicus* are divided into 2 large and small parts (Cesare, 1986).

Based on the results of observations and identification on several *Argulus*, it was found that all *Argulus* observed were *Argulus japonicus* species. The body part that characterizes the *Argulus japonicus* species and is the key to identification as in the form of carapace, maxilla, abdomen, respiratory area, and legs (Cesare, 1986).

A. japonicus infestation in gouramy fish was calculated after the immersion treatment using ethanol extract of ketepeng cina leaves. The data obtained is the percentage of the amount of *A. japonicus* that can still stick to the surface of the fish's body after 60 minutes of treatment. The percentage of *A. japonicus* infestation in gouramy can be seen in Table 1.

Based on the results of the Analysis of Variance (ANOVA), it was shown that the ketepeng cina leaves ethanol extract had an effect ($p < 0.01$) on the infestation of *A. japonicus* in gouramy fish. The results of further tests using *Duncan's Multiple Range Test* showed that the P0 (control) treatment was not significantly different from P1 and very significantly

different (p <0.01) with P2 and P3 treatments. P1 treatment was not significantly different from P0 and P2 but very significantly different (p <0.01) with P3 treatment. Meanwhile, P2 treatment was not significantly different from P3. From table 1. it is known that the P3 treatment has the best results of all treatments, although based on the results of the analysis of *Duncan's* further tests showed that the P3 treatment did not have a significant difference with the treatment P2. A chart of the percentage of *A. japonicus* infestations in gouramy at the 60th minutes can be seen in Figure 1.

From the chart in Figure 1. shows that the lowest number of *A. japonicus* in infesting fish in the 60th minutes is P3 at 35% and *A. japonicus* that cannot infest fish is 65%. The number of *A. japonicus* infested in the P3 treatment was lower than the P0, P1, and P2 treatments.

Based on the results of the study, there were no significantly different between P0 and P1 treatments. Where in P0, there is no solution of ketepeng cina leaves ethanol extract (*Cassia alata* L.) so that *A. japonicus* is able to infest gourami with an average infestation of 3.58 parasites/fish or 71.67% from the total *A. japonicus*. Whereas in the treatment of P1, the average *A. japonicus* which is able to infest the gouramy fish is still relatively high at 2.92 parasites/fish or 58.33% of the total *A. japonicus*. P2 treatment and P3 treatment were not significantly different (p> 0.05), but P3 was very significantly different (p <0.01) with P0 treatment and also P1 treatment. The average of *A. japonicus* infestation in P2 and P3 treatments were 2.33 parasites/fish and 1.75 parasites/fish respectively. While the percentage of *A. japonicus* infestation in P2 and P3 treatments were 46.67% and 35% respectively

Based on Table 2, observations of behavior in fish were carried out before, during and after treatment. The observed fish behavior was fish movement and fish metabolism. Changes in behavior that occur are in the form of fish experiencing excessive mucus production, breathing faster, and movements become abnormal like rubbing its body into hard objects

Comment [SS2]: Very significantly different

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such as aquarium walls (Noaman *et al.,* 2010). Almost all of the fish's behavior in each treatment had similar behavior both before, during, and after the treatment.

Water quality is one of the main factors that support success during the research because it plays an important role in fish survival. In this study the measured water quality includes temperature, pH, and DO. Water quality measurement data obtained during the study were averaged and then compared with the Indonesian National Standard (SNI) regarding gouramy culture or more precisely in SNI 01-7241-2006. Water quality data during the study and the Indonesian National Standard (SNI) can be seen in Table 3.

Based on the results of water quality measurements in Table 3. an increase in temperature in the treatment P0 to P3 treatment ranged from 28.23 to 28.6° C. In addition, there was also a decrease in pH and also dissolved oxygen (DO). The decrease in pH that occurred ranged from 7.45 to 7.17 and a decrease in DO which ranged from 2.9 to 2.4 mg/L.

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Discussion

The results of this study indicate that the use of ketepeng cina leaves ethanol extract (*Cassia alata* L.) has an effect ($p < 0.01$) on the infestation of *A. japonicus* in gouramy fish. The results of this treatment were able to prove that the use of ketepeng cina leaves ethanol extract (*Cassia alata* L.) with a concentration of 209 ppm, 418 ppm, and 627 ppm was able to prevent the infestation of *A. japonicus* in gouramy with 60 minutes soaking time.

The ability of ketepeng cina leaves ethanol extract (*Cassia alata* L.) in P2 and P3 treatments to prevent *A. japonicus* infestation was higher than the P0 and P1 treatments. Thus, the higher the concentration of ketepeng cina leaves ethanol extract (Cassia alata L.) given, the concentration of antiparasitic compounds contained in ketepeng cina leaves ethanol extract (*Cassia alata* L.) will be higher and will cause fewer *A. japonicus* infestations in gouramy fish. In the P0 treatment, there is still *Argulus japonicus* which does not infest the

gouramy until the end of the treatment which is thought to be caused by several factors such as the unhealthy condition of *A. japonicus* or because *A. japonius* does not want to infest the gouramy. P3 treatment did produce the average *A. japonicus* infestation in gouramy fish at the least, but still relatively high percentage of *A. japonicus* infestations in gouramy fish. Even though P2 and P3 treatments were not significantly different, the percentage of *A. japonicus* infestation in P2 treatment was still high up to 46.67% so that the P3 treatment was a more optimal concentration. To see the maximum results in this study, it is better if the use of ethanol extract concentration of ketepeng cina leaves is improved so that more optimal results can be seen or even until all *A. japonicus* can no longer manifest gouramy fish.

In the P3 treatment, the percentage of the *A. japonicus* infestation was less than the P0, P1, and P2 treatments because the antiparasitic compound contained in the ketepeng cina leaves ethanol extract (*Cassia alata* L.) at the P3 treatment was higher so that it could react and cause an effect in *A. japonicus*, where these antiparasitic compounds will have a synergistic effect (Princess and Milanda, 2016) and can cause *A. japonicus* cannot infest gouramy fish.

The method used in this study is dipping or soaking fish and *A. japonicus* parasites in a solution of ketepeng cina leaves ethanol extract (*Cassia alata* L.). The choice of dipping method is because by using the dipping method, *A. japonicus* which infects gouramy fish can come into direct contact with the ethanol extract solution of ketepeng cina leaves (*Cassia alata* L.) which contains antiparasitic compounds such as alkaloids, anthraquinone glycosides, saponins, and tannins (Cassia alata L.) (Sule *et al,*. 2010).

Alkaloid compounds in ketepeng cina leaves ethanol extract (*Cassia alata* L.) can act as antiparasites for *A. japonicus*. Alkaloids are salts that dissolve easily in water and can degrade cell membranes to enter and damage cells. In addition, alkaloids can also disrupt the nervous system by inhibiting the action of the enzyme astylcholinesterase where the alkaloid

will bind to the enzyme acetylcholinesterase which has the functions to hydrolyze acetylcholine (a neurotransmitter compound) (Cania and Setyaningrum, 2013). Acetylcholine which serves to deliver nerve impulses will undergo hydrolysis by the enzyme acetylcholinesterase into choline and acetic acid. With the binding of the acetylcholinesterase enzyme, there will be an accumulation of acetylcholine which causes nerve impulses to not be sent to the receptors and will cause muscle contractions to not occur (Kurnia, 2012). If acetylcholine cannot join the receptors, muscle contraction will not occur. Muscles and nerves will become the second organ that is damaged because it is located directly under the cuticle (Faith *et al.,* 2015).

Apart from alkaloids, there are also other antiparasitic contents in ketepeng cina leaves ethanol extract (*Cassia alata* L.) such as anthraquinone glycosides, saponins, and tannins. Anthracinone glycosides can work to inhibit protein synthesis (Puteri and Milanda, 2016). Saponins also have the ability to interfere with cell membrane stability by lowering surface tension (Iman *et al.,* 2015). Tannin is a polyphenol compound that can form complex compounds with proteins that interfere with protein absorption.

The content of flavonoids in ethanol extract of ketepeng cina leaves has anti inflamation ability that can close wounds in fish (Gultom *et al.,* 2018). Flavonoids are also known to have the potential to work as immunostimulants where flavonoids can work with lymphokines produced by T cells so that they will stimulate phagocyte cells to respond to phagocytosis (Kusmardi *et al.,* 2007). Antiparasitic compounds contained in the ketepeng cina leaves ethanol extract (*Cassia alata* L.) are indeed in small amounts, but have a synergistic effect between compounds so that they can provide a greater total effect (Puteri and Milanda, 2016).

Comment [SS8]: anti inflamation

The behavior of gouramy fish before treatment looks more silent and clustered, but when there are stimuli such as movement it will move actively to avoid the source of

movement. The behavior of fish during the treatment can be seen if the gouramy is more active in rubbing its body against the aquarium wall due to the infestation of *A. japonicus*, excessive mucus production and the respiration of the gouramy is faster. However, over time the fish will start to calm down until the end of the treatment. After the treatment is complete, *Argulus japonicus* which is still attached to the gouramy is released and the gouramy is returned to the aquarium which contains clean water and strong aeration to remove the remnants of the ketepeng cina leaves ethanol extract (*Cassia alata* L.) which is still attached to the body of the gourami fish suspected of being able to poison gourami fish (Husnawati, 2006).

Nearly all fish treatments exhibited the same behavior both before, during, and after the treatment. There are changes in fish behavior as in Nurlaela's research (2013) which includes responses to abnormal body movements, passive swimming, often at the bottom of the water, fish have a slow reaction or absolutely no reaction when touched by hands, fins often damaged and look bleeding in certain parts, there are injuries both on the surface of the body and fish fins and decreased appetite. Fish behavior is thought to be related to the condition of fish stress due to infestation of *A. japonicus* (Gultom *et al.,* 2018). *Argulus japonicus* will release simultaneously releasing toxic anticoagulant substances that cause the blood of fish sucked by *A. japonicus* not to freeze easily and this is the door for pathogens (Secondary pathogens) for fish (Ogata, 2012). This substance is also what causes the fish to be stressed so that changes in gouramy behavior. Bleeding in gouramy caused by *A. japonicus* infestation is caused by irritation from the mechanical hazards of hooks and stylets from *A. japonicus* (Steckler *et al.,* 2012). Stress affects the body's protective system such as mucus, damage to the skin or mucus layer will facilitate secondary infections (Irianto, 2005).

Water quality in this study was measured at the end of the study to be compared with the literature, whether the water quality used at the time of the study was appropriate or not.

The water quality measured in this study are DO, temperature, and pH. Based on Table 3, it shows that the measured water quality (temperature, pH, and DO) are still within the safe limits for the maintenance of gouramy fish when compared to national standards (SNI, 2006).

Oxygen in water has an important role in fish survival because it plays a role in the breathing and metabolism of fish. In water containing dissolved oxygen (DO) of 1-5 mg/L, fish can survive but fish growth will be slow (Boyd, 1982). In this study, there was a decrease in DO starting from treatment P0 to treatment P3 along with an increase in water temperature. An increase in temperature of 1° C can cause an increase in oxygen consumption by as much as 10% (Effendi, 2003). Changes in temperature up to 4° C do not have much effect on gouramy, but gouramy will experience stress when changes in water temperature reach 5° C and above (Hastuti, 2005). The degree of acidity (pH) that is too low/acidic can cause fish death, and if it is too high/alkaline it will cause very slow fish growth (Boyd, 1982). In this study, the results of pH measurements for each treatment showed that the pH was still at a safe standard for the life of gouramy.

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Table 1. Percentage of the amount of *A. japonicus* infestation in gouramy fish

Treatment	Percentage of the *A. japonicus* infestation (%) ± SD
P0 (control)	71,67 ^{a} ± 11,67
P1 (209 ppm)	58,33 ^{ab} ± 17,22
P2 (418 ppm)	46,67 ^{bc} ± 112,11
P3 (627 ppm)	35 ^{c} ± 8,37

Note: SD = Standard Deviation, Different Superscripts in the same column show a significantly difference between each treatment ($p < 0.01$).

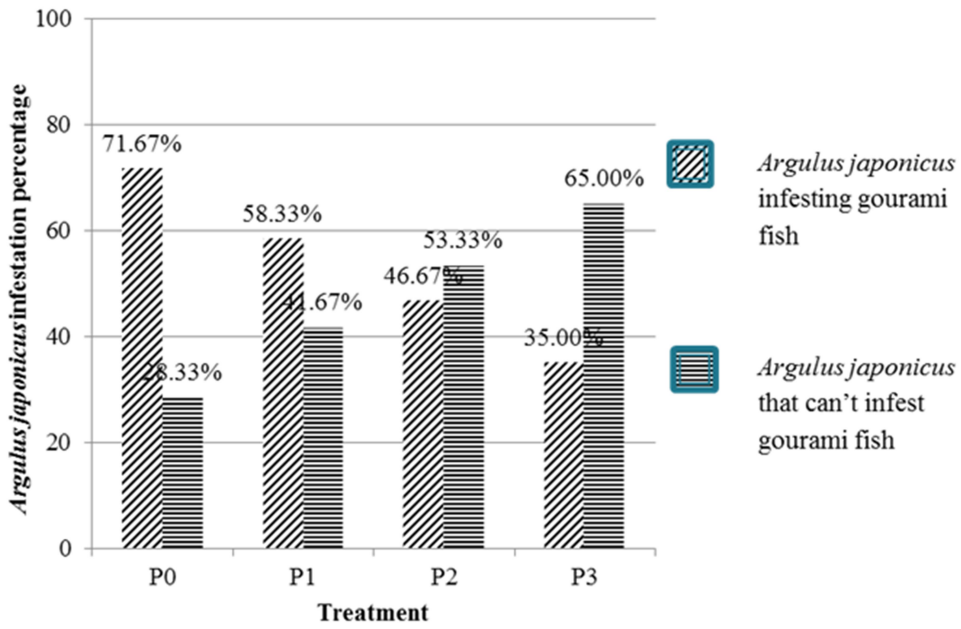


Figure 1. *Argulus japonicus* infestation percentage chart.

Table 2. Fish Behavior Before, During and After Treatment

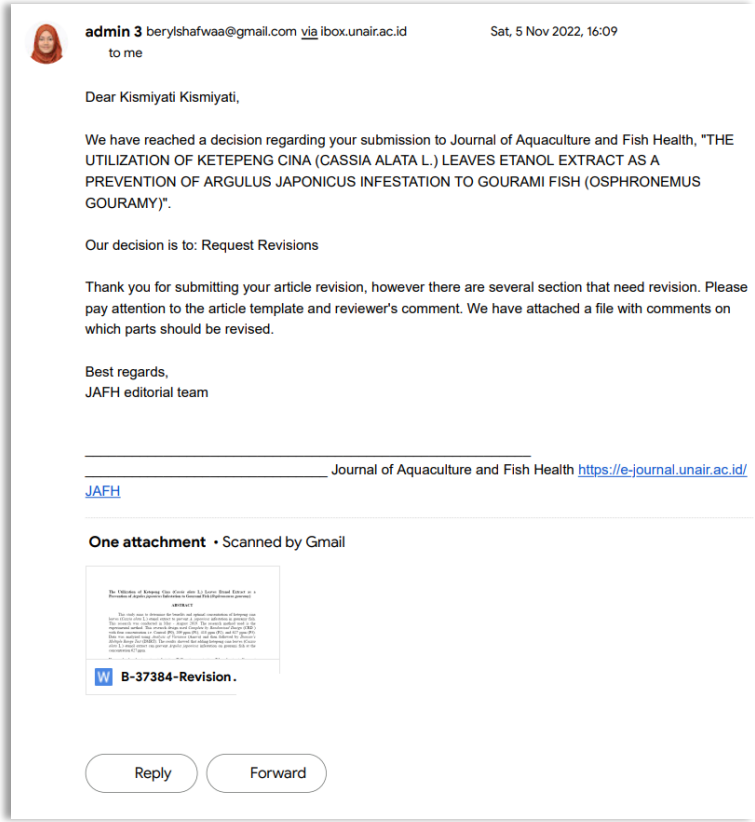
No.	Treatment	Fish Behavior		
		Before Treatment	During Treatment	After Treatment
1	P0 (control)	Fish more silent and clustered, moving actively when there are stimuli such as movement or food	Fish are more active in rubbing their bodies on the walls of the aquarium, excessive mucus production and faster respiration	The fish becomes calmer and respiration gradually normal
2	P1 (209 ppm)	Fish more silent and clustered, moving actively when there are stimuli such as movement or food	Fish are more active in rubbing their bodies on the walls of the aquarium, excessive mucus production and faster respiration	The fish becomes calmer and respiration gradually normal
3	P2 (418 ppm)	Fish more silent and clustered, moving actively when there are stimuli such as movement or food	Fish are more active in rubbing their bodies on the walls of the aquarium, excessive mucus production and faster respiration	The fish becomes calmer and respiration gradually normal
4	P3 (627 ppm)	Fish more silent and clustered, moving actively when there are stimuli such as movement or food	Fish are more active in rubbing their bodies on the walls of the aquarium, excessive mucus production and faster respiration	The fish becomes calmer and respiration gradually normal

Table 3. Data on Average Water Quality in Each Treatment

Treatment	Water quality		
	Temperature (° C)	pH	DO (mg/L)
P0 (control)	28,23	7,45	3,34
P1 (209 ppm)	28,43	7,28	3,03
P2 (418 ppm)	28,58	7,22	2,98
P3 (627 ppm)	28,6	7,17	2,90
SNI, 2006	25-30	6,5-8,5	>2

3. Menerima Email telah Mengirim Revisi Artikel I (5 November 2022)

Keterangan : Menerima email bahwa pengiriman revisi artikel diterima tanggal 5 November 2022.



admin 3 berylshafwaa@gmail.com via ibox.unair.ac.id Sat, 5 Nov 2022, 16:09
to me

Dear Kismiyati Kismiyati,

We have reached a decision regarding your submission to Journal of Aquaculture and Fish Health, "THE UTILIZATION OF KETEPENG CINA (CASSIA ALATA L.) LEAVES ETANOL EXTRACT AS A PREVENTION OF ARGULUS JAPONICUS INFESTATION TO GOURAMI FISH (OSPHRONEMUS GOURAMY)".

Our decision is to: Request Revisions

Thank you for submitting your article revision, however there are several section that need revision. Please pay attention to the article template and reviewer's comment. We have attached a file with comments on which parts should be revised.

Best regards,
JAFH editorial team

Journal of Aquaculture and Fish Health https://e-journal.unair.ac.id/JAFH

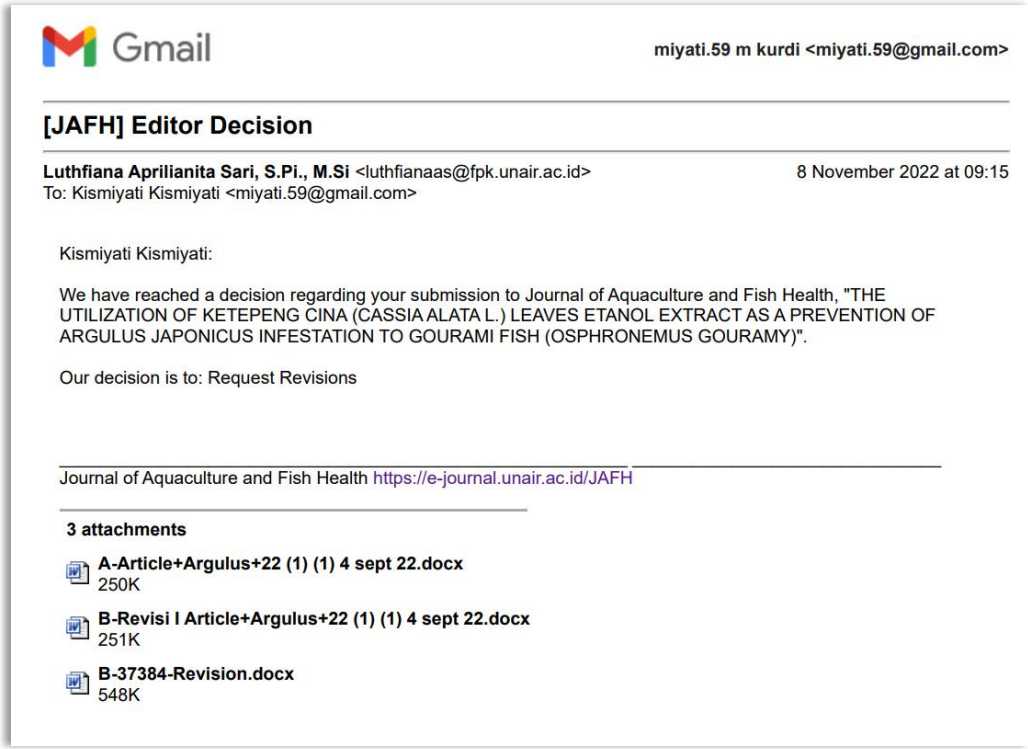
One attachment • Scanned by Gmail

B-37384-Revision.

Reply Forward

4. Menerima Email Revisi Artikel ke 2 (8 November 2022)

Keterangan: Menerima balasan email untuk revisi artikel tanggal 8 September 2022. Bagian yang direvisi pada penulisan kalimat, pemilihan referensi harus 10 tahun terakhir, metodologi, dan analisa data.



Gmail **miyati.59 m kurdi <miyati.59@gmail.com>**

[JAFH] Editor Decision

Luthfiana Aprilianita Sari, S.Pi., M.Si <luthfianaas@fpk.unair.ac.id> 8 November 2022 at 09:15
To: Kismiyati Kismiyati <miyati.59@gmail.com>

Kismiyati Kismiyati:

We have reached a decision regarding your submission to Journal of Aquaculture and Fish Health, "THE UTILIZATION OF KETEPENG CINA (CASSIA ALATA L.) LEAVES ETANOL EXTRACT AS A PREVENTION OF ARGULUS JAPONICUS INFESTATION TO GOURAMI FISH (OSPHRONEMUS GOURAMY)".

Our decision is to: Request Revisions

Journal of Aquaculture and Fish Health https://e-journal.unair.ac.id/JAFH

3 attachments

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B-Revisi I Article+Argulus+22 (1) (1) 4 sept 22.docx
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The Utilization of Ketepeng Cina (*Cassia alata* L.) Leaves Etanol Extract as a Prevention of *Argulus japonicus* Infestation to Gourami Fish (*Osphronemus gouramy*)

ABSTRACT

This study aims to determine the benefits and optimal consentration of ketepeng cina leaves (*Cassia alata* L.) etanol extract to prevent *A. japonicus* infestation in gouramy fish. This research was conducted in May - August 2019. The research method used is the experimental method. This research design used *Complete by Randomized Design* (CRD) with four concentration i.e. Control (P0), 209 ppm (P1), 418 ppm (P2), and 627 ppm (P3). Data was analyzed using *Analysis of Variance* (Anova) and then followed by *Duncan's Multiple Range Test* (DMRT). The results showed that adding ketepeng cina leaves (*Cassia alata* L.) etanol extract can prevent *Argulus japonicus* infestation on gourami fish at the concentration 627 ppm.

Keywords: *Argulus japonicus* infestation, Different concentration, Ethanol extract, Gourami fish, Ketepeng cina leaves

INTRODUCTION

Gouramy fish is one of the consumable freshwater species in aquaculture (Khairyah *et al.,* 2012). This fish has a lot of meat and relatively easy to culture, so many gourami fish cultured in Indonesia. Gouramy fish has a high selling point and demand (Barkah, 2014). However, gouramy seeds is not in accordance with market needs, caused by high mortality in seeds up to about 50% caused by disease (Nugroho, 2012).

*Argulus japonicu*s is one type of ectoparasites from the Crustacean class which often attacks in freshwater fish culture. *Argulus* can infest carp, gouramy, tilapia, and catfish with a prevalence 100% and the highest level of intensity is in carp followed by gouramy, tilapia, and catfish (Nurlaela, 2013). Based on research conducted by Wahyuni *et al.* (2013), the data shows that *A. japonicus* infested on gouramy fish culture at Ngrajek Village, Magelang Regency was 4.16%. The results of Abadiyyah's observations (2014), showed that in Fish Seed Hall Rambigundam Jember culture pond, ectoparasites of *A. japonicus* were found which infested gouramy fish.

A. japonicus predilection on the fins, skin, gills, and the whole host body. Fish that are infested with *A. japonicus* will rub their bodies on hard objects in their surroundings. Clinical symptoms of fish infested with *A. japonicus* are fish seen with lesions on the fins, skin, head, and body surface causing fish to be thin, stunted growth, and death (Noaman *et al.,* 2010).

Prevention of parasitic infestation of *A. japonicus* can use herbal materials available in nature, but due to lack of information and knowledge of the community, causing more people to use chemicals. The use of chemicals in the culture process can damage the environment and the biota in it. In addition, the use of chemicals in the culture process can cause resistance and leave residue in fish (Tam and Avenant, 2005). To reduce the use of chemicals in the culture process, substitution materials are needed to prevent the parasitic *A. japonicus* which is more environmentally friendly, one of which is ketepeng cina leaves.

Ketepeng cina is one type of traditional plant that is often used as an antiparasitic, antifungal, and antibacterial. Ketepeng cina leaves have important ingredients which are alkaloids, saponins, tannins, steroids, anthraquinones, flavonoids, and carbohydrates (Triana *et al.,* 2016). The presence of antiparasitic compounds such as alkaloids, saponins, tannins, anthraquinone glycosides and flavonoid content that can be used as immunostimulants (Kusmardi *et al.,* 2007) on ketepeng cina leaves encourages to commence research on the

effectiveness of ketepeng cina leaves extract (*Cassia alata* L.) to prevent *A. japonicus* infestation in gouramy fish (*Osphronemus gouramy*).

METHODOLOGY

Time and Place

The study was conducted in February until August 2019 at the Faculty of Fisheries and Marine Airlangga University's Laboratory.

Research Materials

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Research Design

The method used in this research is the experimental method. While the research design used is Completely Randomized Design (CRD) using 4 treatments.

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Working Procedure

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Identification of *A. japonicus*

The identification of *Argulus* used during the research was carried out after the research finished to keep the *Argulus* used stay in good condition throughout the study. The key to identifying *Argulus* can most easily be seen through the shape of the respiratory area and also the spur on the 4th leg. Respiratory area in *Argulus japonicus* are divided into 2 large and small parts (Cesare, 1986). The body part that characterizes the *Argulus japonicus* species and is the key to identification as in the form of carapace, maxilla, abdomen, respiratory area, and legs (Cesare, 1986).

Data Analysis

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RESULTS AND DISCUSSION

A. japonicus Infestation

A. japonicus infestation in gouramy fish was calculated after the immersion treatment using ethanol extract of ketepeng cina leaves. The data obtained is the percentage of the amount of *A. japonicus* that can still stick to the surface of the fish's body after 60 minutes of treatment. The percentage of *A. japonicus* infestation in gouramy can be seen in Table 1.

Table 1. Percentage of the amount of *A. japonicus* infestation in gouramy fish

Treatment	Percentage of the *A. japonicus* infestation (%) ± SD
P0 (control)	71,67 ^{a} ± 11,67
P1 (209 ppm)	58,33 ^{ab} ± 17,22
P2 (418 ppm)	46,67 ^{bc} ± 112,11
P3 (627 ppm)	35 ^{c} ± 8,37

Note: SD = Standard Deviation, Different Superscripts in the same column show a significantly difference between each treatment ($p < 0.01$).

Based on the results of the Analysis of Variance (ANOVA), it was shown that the ketepeng cina leaves ethanol extract had an effect ($p < 0.01$) on the infestation of *A. japonicus* in gouramy fish. The results of further tests using *Duncan's Multiple Range Test* showed that the P0 (control) treatment was not significantly different from P1 and very significantly different ($p < 0.01$) with P2 and P3 treatments. P1 treatment was not significantly different from P0 and P2 but very significantly different ($p < 0.01$) with P3 treatment. Meanwhile, P2 treatment was not significantly different from P3. From table 1. it is known that the P3

treatment has the best results of all treatments, although based on the results of the analysis of *Duncan's* further tests showed that the P3 treatment did not have a significant difference with the treatment P2. A chart of the percentage of *A. japonicus* infestations in gouramy at the 60th minutes can be seen in Figure 1.

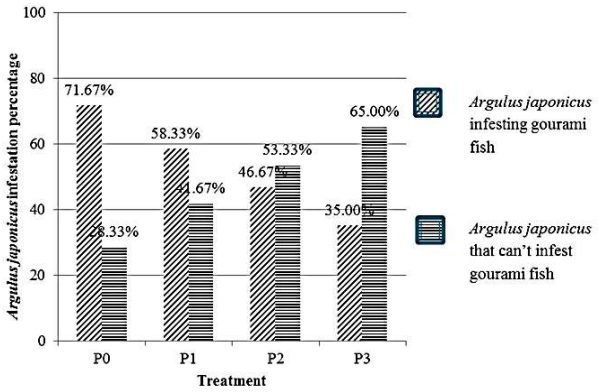

Figure 1. *Argulus japonicus* infestation percentage chart.

From the chart in Figure 1. shows that the lowest number of *A. japonicus* in infesting fish in the 60th minutes is P3 at 35% and *A. japonicus* that cannot infest fish is 65%. The number of *A. japonicus* infested in the P3 treatment was lower than the P0, P1, and P2 treatments.

Based on the results of the study, there were no significantly different between P0 and P1 treatments. Where in P0, there is no solution of ketepeng cina leaves ethanol extract (*Cassia alata* L.) so that *A. japonicus* is able to infest gourami with an average infestation of 3.58 parasites/fish or 71.67% from the total *A. japonicus*. Whereas in the treatment of P1, the average *A. japonicus* which is able to infest the gouramy fish is still relatively high at 2.92 parasites/fish or 58.33% of the total *A. japonicus*. P2 treatment and P3 treatment were not significantly different ($p > 0.05$), but P3 was very significantly different ($p < 0.01$) with P0 treatment and also P1 treatment. The average of *A. japonicus* infestation in P2 and P3 treatments were 2.33 parasites/fish and 1.75 parasites/fish respectively. While the percentage of *A. japonicus* infestation in P2 and P3 treatments were 46.67% and 35% respectively.

The results of this study indicate that the use of ketepeng cina leaves ethanol extract (*Cassia alata* L.) has an effect ($p < 0.01$) on the infestation of *A. japonicus* in gouramy fish. The results of this treatment were able to prove that the use of ketepeng cina leaves ethanol extract (*Cassia alata* L.) with a concentration of 209 ppm, 418 ppm, and 627 ppm was able to prevent the infestation of *A. japonicus* in gouramy with 60 minutes soaking time.

The ability of ketepeng cina leaves ethanol extract (*Cassia alata* L.) in P2 and P3 treatments to prevent *A. japonicus* infestation was higher than the P0 and P1 treatments. Thus, the higher the concentration of ketepeng cina leaves ethanol extract (Cassia alata L.) given, the concentration of antiparasitic compounds contained in ketepeng cina leaves ethanol extract (*Cassia alata* L.) will be higher and will cause fewer *A. japonicus* infestations in gouramy fish. In the P0 treatment, there is still *Argulus japonicus* which does not infest the gouramy until the end of the treatment which is thought to be caused by several factors such as the unhealthy condition of *A. japonicus* or because *A. japonius* does not want to infest the gouramy. P3 treatment did produce the average *A. japonicus* infestation in gouramy fish at

the least, but still relatively high percentage of *A. japonicus* infestations in gouramy fish. Even though P2 and P3 treatments were not significantly different, the percentage of *A. japonicus* infestation in P2 treatment was still high up to 46.67% so that the P3 treatment was a more optimal concentration. To see the maximum results in this study, it is better if the use of ethanol extract concentration of ketepeng cina leaves is improved so that more optimal results can be seen or even until all *A. japonicus* can no longer manifest gouramy fish.

In the P3 treatment, the percentage of the *A. japonicus* infestation was less than the P0, P1, and P2 treatments because the antiparasitic compound contained in the ketepeng cina leaves ethanol extract (*Cassia alata* L.) at the P3 treatment was higher so that it could react and cause an effect in *A. japonicus*, where these antiparasitic compounds will have a synergistic effect (Puteri and Milanda, 2016) and can cause *A. japonicus* cannot infest gouramy fish.

The method used in this study is dipping or soaking fish and *A. japonicus* parasites in a solution of ketepeng cina leaves ethanol extract (*Cassia alata* L.). The choice of dipping method is because by using the dipping method, *A. japonicus* which infects gouramy fish can come into direct contact with the ethanol extract solution of ketepeng cina leaves (*Cassia alata* L.) which contains antiparasitic compounds such as alkaloids, anthraquinone glycosides, saponins, and tannins (*Cassia alata* L.) (Sule *et al,*. 2010).

Alkaloid compounds in ketepeng cina leaves ethanol extract (*Cassia alata* L.) can act as antiparasites for *A. japonicus*. Alkaloids are salts that dissolve easily in water and can degrade cell membranes to enter and damage cells. In addition, alkaloids can also disrupt the nervous system by inhibiting the action of the enzyme astylcholinesterase where the alkaloid will bind to the enzyme acetylcholinesterase which has the functions to hydrolyze acetylcholine (a neurotransmitter compound) (Cania and Setyaningrum, 2013). Acetylcholine which serves to deliver nerve impulses will undergo hydrolysis by the enzyme acetylcholinesterase into choline and acetic acid. With the binding of the acetylcholinesterase enzyme, there will be an accumulation of acetylcholine which causes nerve impulses to not be sent to the receptors and will cause muscle contractions to not occur (Kurnia, 2012). If acetylcholine cannot join the receptors, muscle contraction will not occur. Muscles and nerves will become the second organ that is damaged because it is located directly under the cuticle (Faith *et al.,* 2015).

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Apart from alkaloids, there are also other antiparasitic contents in ketepeng cina leaves ethanol extract (*Cassia alata* L.) such as anthraquinone glycosides, saponins, and tannins. Anthracinone glycosides can work to inhibit protein synthesis (Puteri and Milanda, 2016). Saponins also have the ability to interfere with cell membrane stability by lowering surface tension (Iman *et al.,* 2015). Tannin is a polyphenol compound that can form complex compounds with proteins that interfere with protein absorption.

The content of flavonoids in ethanol extract of ketepeng cina leaves has anti inflamation ability that can close wounds in fish (Gultom *et al.,* 2018). Flavonoids are also known to have the potential to work as immunostimulants where flavonoids can work with lymphokines produced by T cells so that they will stimulate phagocyte cells to respond to phagocytosis (Kusmardi *et al.,* 2007). Antiparasitic compounds contained in the ketepeng cina leaves ethanol extract (*Cassia alata* L.) are indeed in small amounts, but have a synergistic effect between compounds so that they can provide a greater total effect (Puteri and Milanda, 2016).

Fish Behavior

Based on Table 2, observations of behavior in fish were carried out before, during and after treatment. The observed fish behavior was fish movement and fish metabolism. Changes in behavior that occur are in the form of fish experiencing excessive mucus production,

breathing faster, and movements become abnormal like rubbing its body into hard objects such as aquarium walls (Noaman *et al.,* 2010). Almost all of the fish's behavior in each treatment had similar behavior both before, during, and after the treatment.

Table 2. Table 2. Fish Behavior Before, During and After Treatment

Treatment	Fish Behavior		
	Before Treatment	During Treatment	After Treatment
P0 (control)	Fish more silent and clustered, moving actively when there are stimuli such as movement or food	Fish are more active in rubbing their bodies on the walls of the aquarium, excessive mucus production and faster respiration	The fish becomes calmer and respiration gradually normal
P1 (209 ppm)	Fish more silent and clustered, moving actively when there are stimuli such as movement or food	Fish are more active in rubbing their bodies on the walls of the aquarium, excessive mucus production and faster respiration	The fish becomes calmer and respiration gradually normal
P2 (418 ppm)	Fish more silent and clustered, moving actively when there are stimuli such as movement or food	Fish are more active in rubbing their bodies on the walls of the aquarium, excessive mucus production and faster respiration	The fish becomes calmer and respiration gradually normal
P3 (627 ppm)	Fish more silent and clustered, moving actively when there are stimuli such as movement or food	Fish are more active in rubbing their bodies on the walls of the aquarium, excessive mucus production and faster respiration	The fish becomes calmer and respiration gradually normal

The behavior of gouramy fish before treatment looks more silent and clustered, but when there are stimuli such as movement it will move actively to avoid the source of movement. The behavior of fish during the treatment can be seen if the gouramy is more active in rubbing its body against the aquarium wall due to the infestation of *A. japonicus*, excessive mucus production and the respiration of the gouramy is faster. However, over time the fish will start to calm down until the end of the treatment. After the treatment is complete, *Argulus japonicus* which is still attached to the gouramy is released and the gouramy is returned to the aquarium which contains clean water and strong aeration to remove the remnants of the ketepeng cina leaves ethanol extract (*Cassia alata* L.) which is still attached to the body of the gourami fish suspected of being able to poison gourami fish (Husnawati, 2006).

Nearly all fish treatments exhibited the same behavior both before, during, and after the treatment. There are changes in fish behavior as in Nurlaela's research (2013) which includes responses to abnormal body movements, passive swimming, often at the bottom of the water, fish have a slow reaction or absolutely no reaction when touched by hands, fins often damaged and look bleeding in certain parts, there are injuries both on the surface of the body and fish fins and decreased appetite. Fish behavior is thought to be related to the condition of fish stress due to infestation of *A. japonicus* (Gultom *et al.,* 2018). *Argulus japonicus* will

release simultaneously releasing toxic anticoagulant substances that cause the blood of fish sucked by *A. japonicus* not to freeze easily and this is the door for pathogens (Secondary pathogens) for fish (Ogata, 2012). This substance is also what causes the fish to be stressed so that changes in gouramy behavior. Bleeding in gouramy caused by *A. japonicus* infestation is caused by irritation from the mechanical hazards of hooks and stylets from *A. japonicus* (Steckler *et al.,* 2012). Stress affects the body's protective system such as mucus, damage to the skin or mucus layer will facilitate secondary infections (Irianto, 2005).

Water Quality

Water quality is one of the main factors that support success during the research because it plays an important role in fish survival. In this study the measured water quality includes temperature, pH, and DO. Water quality measurement data obtained during the study were averaged and then compared with the Indonesian National Standard (SNI) regarding gouramy culture or more precisely in SNI 01-7241-2006. Water quality data during the study and the Indonesian National Standard (SNI) can be seen in Table 3.

Table 3. Data on Average Water Quality in Each Treatment

Treatment	Water quality		
	Temperature (° C)	pH	DO (mg/L)
P0 (control)	28,23	7,45	3,34
P1 (209 ppm)	28,43	7,28	3,03
P2 (418 ppm)	28,58	7,22	2,98
P3 (627 ppm)	28,6	7,17	2,90
SNI, 2006	25-30	6,5-8,5	>2

Water quality in this study was measured at the end of the study to be compared with the literature, whether the water quality used at the time of the study was appropriate or not. The water quality measured in this study are DO, temperature, and pH. Based on Table 3, it shows that the measured water quality (temperature, pH, and DO) are still within the safe limits for the maintenance of gouramy fish when compared to national standards (SNI, 2006).

Oxygen in water has an important role in fish survival because it plays a role in the breathing and metabolism of fish. In water containing dissolved oxygen (DO) of 1-5 mg/L, fish can survive but fish growth will be slow (Boyd, 1982). In this study, there was a decrease in DO starting from treatment P0 to treatment P3 along with an increase in water temperature. An increase in temperature of 1° C can cause an increase in oxygen consumption by as much as 10% (Effendi, 2003). Changes in temperature up to 4° C do not have much effect on gouramy, but gouramy will experience stress when changes in water temperature reach 5° C and above (Hastuti, 2005). The degree of acidity (pH) that is too low/acidic can cause fish death, and if it is too high/alkaline it will cause very slow fish growth (Boyd, 1982). In this study, the results of pH measurements for each treatment showed that the pH was still at a safe standard for the life of gouramy.

ACKNOWLEDGEMENT

REFERENCES

Commented [D6]: Mohon tambahkan ucapan terima kasih

Commented [D7]: Mohon gunakan referensi 10 tahun terakhir
Mohon diesuaikan penulisan dengan author guideline JAFH, tambahkan link DOI di setiap akhir referensi

Abadiyyah, R. 2014. Prevalensi dan Intensitas Ektoparasit yang Menyerang Gurami (*Osphronemus gouramy* Lac.) di Balai Benih Ikan Rambigundam Kabupaten Jember. Skripsi. Universitas Jember. 56 hal.

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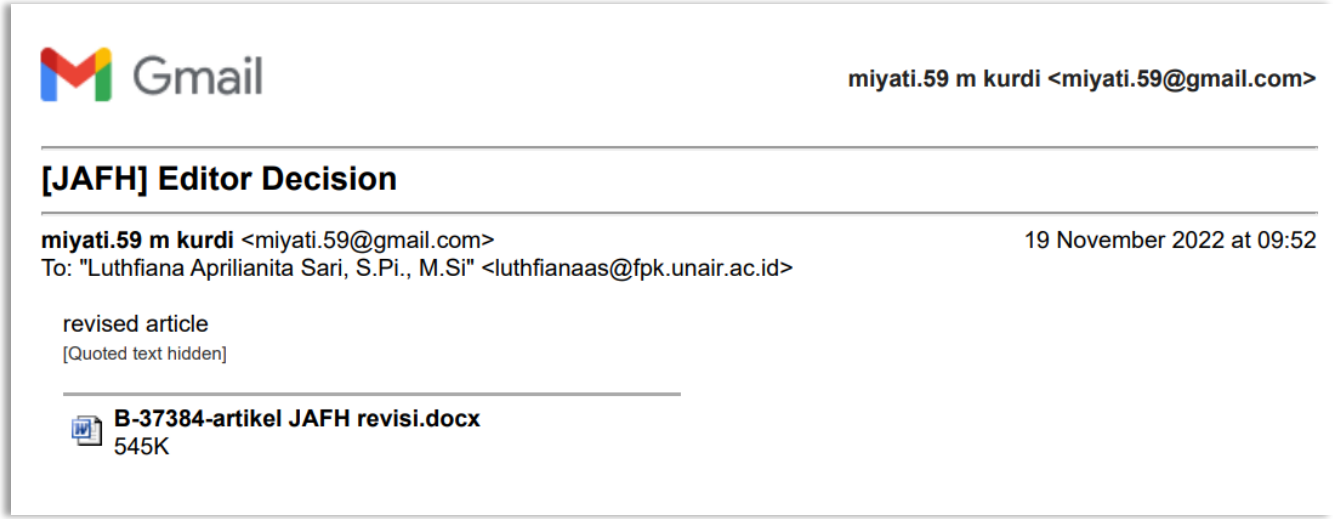
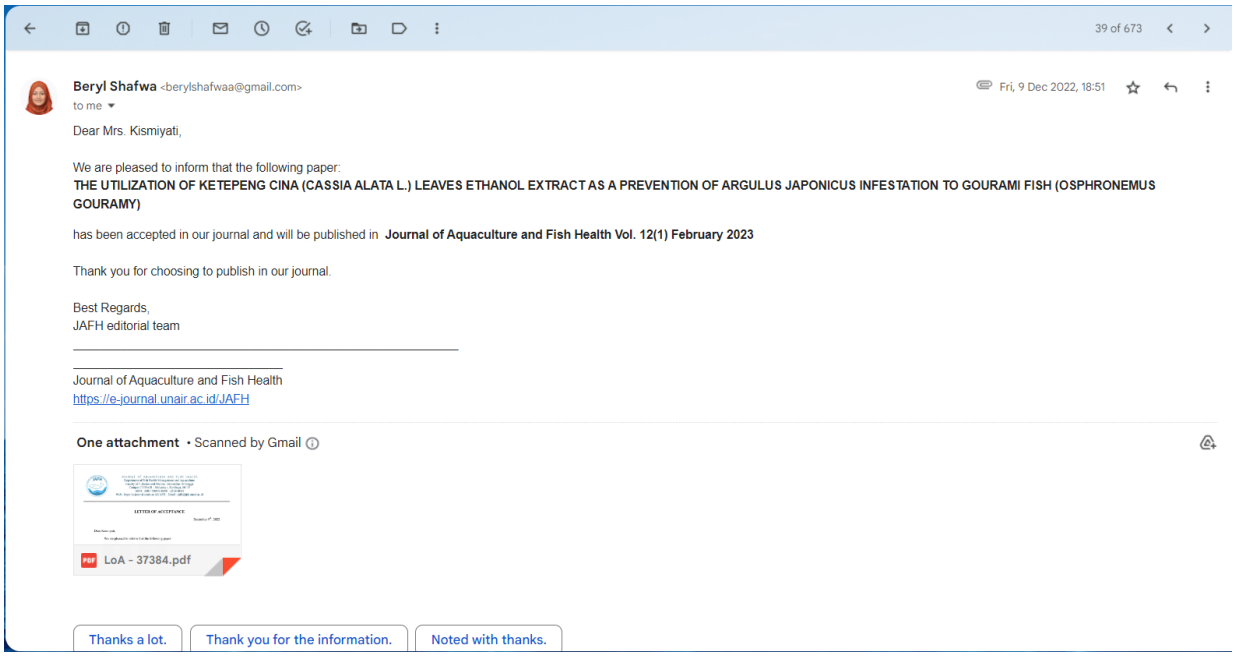
5. Pengiriman Revisi Artikel ke 2 (19 November 2022)

Keterangan: Pengiriman revisi artikel melalui email tanggal 19 November 2022.



6. Informasi Artikel telah Diterima dan akan Terbit pada Bulan Februari 2023 (9 Desember 2022)

Keterangan: Artikel publish pada website pada Februari 2023 dengan link : https://e-journal.unair.ac.id/JAFH/article/view/37384



Letter of Acceptance:

Journal of Aquaculture and Fish Health
Department of Fish Health Management and Aquaculture
Faculty of Fisheries and Marine, Universitas Airlangga
Campus C UNAIR - Mulyorejo, Surabaya, 60115
ISSN : 2301-7309/E-ISSN : 2528-0864
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LETTER OF ACCEPTANCE

December 9th, 2022

Dear Kismiyati,

We are pleased to inform that the following paper :

ID #	Authors	Title
37384	Yudin Ardha Pradipta, Kismiyati, and Putri Desi Wulansari	The Utilization of Ketepeng Cina (*Cassia alata* L.) Leaves Ethanol Extract as a Prevention of *Argulus japonicus* Infestation to Gourami Fish (*Osphronemus gouramy*)

has been **ACCEPTED** in our journal and will be published in **Journal of Aquaculture and Fish Health Volume 12 No. 1 (2023).**

Thank you for choosing to publish in our journal.

Kindest Regards,

Luthfiana Aprilianita Sari, S.Pi., M.Si.
Chief Editor - Journal of Aquaculture and Fish Health