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Submission date: 07-Mar-2023 02:08PM (UTC+0800)
Submission ID: 2030999593
File name: JAFH_12_1_135-143_37384.pdf (580.12K)
Word count: 4836
Character count: 25853

Journal of Aquaculture and Fish Health Vol. 12(1) - February 2023
DOI : 10.20473/jafh.v12i1.37384

The Utilization of Ketepeng Cina (*Cassia alata* L.) Leaves Ethanol Extract as a Prevention of *Argulus japonicus* Infestation to Gourami Fish (*Osphronemus gouramy*)

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Received : 2022-07-08
Accepted : 2022-12-04

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

Abstract

This study aims to determine the benefits and optimal concentration of ketepeng cina leaves (*Cassia alata* L.) ethanol extract to prevent A. *japonicus* infestation in gourami fish. This research was cond[17]ed in May - August 2019. The research method used is the experimental method. This research design used Complete R[25]omized Design (CRD) with four concentrations i.e., Control ([9]0), 209 ppm (P1), 418 ppm (P2), and 627 ppm (P3). Data were 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.) to ethanol extract can prevent *A. japonicus* infestation on gourami fish at the concentration of 627 ppm.

INTRODUCTION

Gourami fish (*Osprhronemus gouramy*) is one of the consumption freshwater fish which was known in Indonesia and had so many enthusiasts (Pratama *et al.*, 2018). This fish has a lot of meat and is relatively easy to culture, so many gourami fish are cultured in Indonesia. Gourami fish has a high selling point and demand (Barkah, 2014). However, gourami seeds are not following market needs, caused by high mortality in seeds up to about 50% caused by disease (Nugroho, 2012).

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

The predilection of *A. japonicus* in the gills, fins, and skin (body surface) (Kismiyati *et al.*, 2018). The symptoms usually exhibited by fish infested with this parasite include spot or pinpoint hemorrhages, anemia, fin, and scale loss, increased mucus production, lethargy, erratic swimming, and poor body condition. (Steckler and Yanong, 2012). Host tissue damage is through their

attachment and feeding behavior and is further due to secondary infection with bacteria and fungus (Kumari *et al*., 2019). Heavy infestations of this parasite can cause significant morbidity and mortality (Wafer *et al*., 2015).

Prevention of parasitic infestation of *A. japonicus* can use herbal materials available in nature, but due to a lack of information and knowledge of the community, causes more people to use chemicals. The chemical parasiticides result in the development of resistance, risk of residue formation, environmental contamination, and toxicity to the host (Kumari *et al*., 2019). 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, and flavonoids, and carbohydrates (Triana *et al.,* 2016). The presence of antiparasitic compounds on ketepeng cina leaves encourages to commence research on the effectiveness of ketepeng cina leaves extract (*C. alata* L.) to prevent *A. japonicus* infestation in gourami fish (*O. gouramy*).

METHODOLOGY

Place and Time

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

Research Materials

The materials for this research were 48 tails of gourami seeds size 5-7, 240 adult A. *japonicus*, 400 gram ketepeng cina leaves, ethanol 70%, and fish feed (pellets). Gourami seeds were obtained from the Gunungsari fish market in Surabaya, East Java. *Argulus* was obtained from fish cultivators in Tulungagung district, East Java. Ketepeng Cina leaves were obtained from Sidoarjo, East Java, which was not too old. The tools used in this research consisted of 24 aquariums with size 15x15x30 cm, aerator, water quality test (thermometer, DO meter, pH meter), blender, analytical scales, Erlenmeyer, rotary evaporator vacuum, beaker glass, petri dish, measuring glass, Buchner funnel.

Research Design

The method used in this research is the experimental method. While the research design used is a Completely Randomized Design (CRD) using 4 treatments and 6 replicates. Determination ketepeng cina leaves ethanol extract concentration in this study by determining LC100 (multiplication 0.5x; 1x; and 1.5x) from preliminary research. The concentration of ketepeng cina leaves extract used in treatment were: P0 (0 ppm), P1 (209 ppm), P2 (418 ppm), and P3 (627 ppm), with an observation time of 60 minutes.

Work Procedure

Making of Ketepeng Cina Leaves Ethanol Extract

The making ketepeng cina leaves ethanol extract according to Yacob and Endriani (2010). Ketepeng cina leaves are washed and then blended. Powder of ketepeng cina leaves soaked with ethanol 70% with a ratio of 1 liter of 70% ethanol to 100 mg of leaf powders for 72 hours in a dark glass bottle. The mixture of leaves and ethanol is filtered and the liquid is taken. Then do the evaporator process with a rotary evaporator vacuum. The extract is placed in a petri dish covered with aluminum foil and lubricated so that the extract becomes drier and can be used for a long time.

Identification of *Argulus japonicus*

The identification of the *Argulus* used during the research was carried out after the research finished to keep the *Argulus* used to 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. The respiratory area in *A. japonicus* is divided into 2 large and small parts. The body part characterizes the *A. japonicus* species and is the key to identification in the form of the carapace, maxilla, abdomen, respiratory area, and legs (Cesare, 1986).

Implementation of Research

The research procedure for the utilization of ketepeng cina extract in the prevention of the *Argulus japonicus* infestation on gourami fish was carried out according to the artificial infestation procedure (Kismiyati, 2009). A. *japonicus* is fasted separate from the host for 24 hours so that the artificial infestation process is faster to stick to fish. The artificial infestation is by mixing gourami seeds and *Argulus* in a container of as many as 10 *Argulus* to 2 gourami seeds. Wait about 2 hours or wait for the *Argulus* to stick to the fish. The treatment aquarium is filled with extract with a predetermined concentration. Then enter the gourami seeds that have been infested with *Argulus* into the treatment aquarium (each treatment aquarium contains 2 gourami seeds and 10 *Argulus*). Observations were made for 60 minutes. Observation of fish behavior were carried out before, during, and after treatment. Observation of water quality was carried out before and after treatment.

Data Analysis

This study about the utilization of ketepeng cina leaves ethanol extract was analyzed using a described method. The calculated infested A. *japonicus* on gourami fish after treatment dipping ketepeng cina leaves extract with a predetermined extract concentration. Data analysis in this study was carried out using Analysis of Variance (ANOVA) and then carried out using Duncan's multiple range test for knowing the difference between one treatment with another.

RESULTS AND DISCUSSION

A. japonicus Infestation

A. japonicus infestation in gourami 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 gourami can be seen in Table 1.

Table 1. Percentage of the amount of *A. japonicus* infestation in gourami 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 gourami 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 ($p < 0.01$) from P2 and P3 treatments. P1 treatment was not significantly different from P0 and P2 but very significantly different ($p < 0.01$) from the 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 posthoc tests showed that the P3 treatment did not have a significant

DOI : 10.20473/jafh.v12i1.37384

difference with the treatment P2. A chart of the percentage of *A. japonicus* infestations in gourami at 60 minutes can be seen in Figure 1.

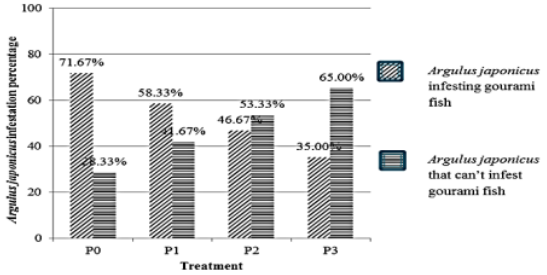

Figure 1. *Argulus japonicus* infestation percentage chart.

The chart in Figure 1 shows that the lowest number of *A. japonicus* infesting fish in the 60 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 in 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 (*C. alata* L.) so *A. japonicus* can infest gourami with an average infestation of 3.58 parasites/fish or 71.67% of the total *A. japonicus*. Whereas in the treatment of P1, the average *A. japonicus* which can infest the gourami 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 (*C. alata* L.) has an effect ($p < 0.01$) on the infestation of *A. japonicus* in gourami fish. The results of this treatment were able to prove that the use of ketepeng cina leaves ethanol extract (*C. alata* L.) with a concentration of 209 ppm, 418 ppm, and 627 ppm was able to prevent the infestation of *A. japonicus* in gourami with 60 minutes soaking time.

The ability of ketepeng cina leaves ethanol extract (*C. alata*) in P2 and P3 treatments to prevent *A. japonicus* infestation was higher than in the P0 and P1 treatments. Thus, the higher the concentration of ketepeng cina leaves ethanol extract (*C. alata*) given, the concentration of antiparasitic compounds contained in ketepeng cina leaves ethanol extract (*C. alata*) will be higher and will cause fewer *A. japonicus* infestations in gourami fish. In the P0 treatment, there is still *A. japonicus* which does not infest the gourami 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. japonicus* does not want to infest the gourami. P3 treatment did produce the average *A. japonicus* infestation in gourami fish at the least, but a still relatively high percentage of *A. japonicus* infestations in gourami fish. Even though the 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 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 gourami fish.

In the P3 treatment, the percentage of the *A. japonicus* infestation was less than in the P0, P1, and P2 treatments because the antiparasitic compound contained in the ketepeng cina leave ethanol extract (*C. alata*) 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 gourami fish.

The method used in this study is dipping or soaking fish and A. *japonicus* parasites in a solution of ketepeng cina leave ethanol extract (*C. alata*). The choice of dipping method is because by using the dipping method, A. *japonicus* which infects gourami fish can come into direct contact with the ethanol extract solution of ketepeng cina leaves (*C. alata*) which contains antimicrobial compounds such as alkaloids, anthraquinones, flavonoid, tannins, saponins, phenolic, steroids, and terpenoid (Vivekanandan and Ajeesh, 2018).

Alkaloid compounds in ketepeng cina leaf ethanol extract (*C. alata*) can act as antiparasitic 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 acetylcholinesterase where the alkaloid will bind to the enzyme acetylcholinesterase which has the function 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 do not occur (Kurnia, 2012).

Anthraquinone compounds probably can limit passive diffusion through the parasite's cell membrane (Dimmer *et al.*, 2019). Flavonoids can increase leukocyte activation, leading to improved lysozyme secretion (Wahjuningrum *et al.,* 2021). Saponins can 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 inflammation ability that can close wounds in fish (Gultom *et al.,* 2018). Antiparasitic compounds contained in the ketepeng cina leaves ethanol extract (*C. alata*) 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 fish infested *Argulus* that occur are fish experiencing excessive mucus production, abnormal behavior, irregular swimming fish and rubbing their bodies against the aquarium wall (Asiseh *et al.*, 2020). Almost all of the fish's behavior in each treatment had similar behavior both before, during, and after the treatment.

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 gourami fish before treatment looks more silent and clustered, but when there are stimuli such as moving it will move actively to avoid the source of movement. The behavior of fish during the treatment can be seen if the gourami 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 gourami is faster. However, over time the fish will start to calm down until the end of the treatment. After the treatment is complete, *A. japonicus* which is still attached to the gourami is released and the gourami is returned to the aquarium which contains clean water and strong aeration to remove the remnants of the ketepeng cina leaves ethanol extract (*C. alata*) which is still attached to the body of the gourami fish suspected of being able to poison gourami fish.

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 the infestation of *A. japonicus* (Gultom *et al.*, 2018). *A. 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 which changes gourami behavior. Bleeding in gourami caused by *A. japonicus* infestation is caused by irritation from the mechanical hazards of hooks and stylets from *A. japonicus* (Steckler and Yanong, 2012).

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 gourami culture or more precisely in SNI 01-7241-2006. Water quality data during the study and the 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 is dissolved oxygen (DO), temperature, and pH. Based on Table 3, shows that the measured water quality (temperature, pH, and DO) are still within the safe limits for the maintenance of gourami fish when compared to national standards (SNI, 2006).

The optimum water temperature can make the fish's response to feeding optimum. The temperature is less or more than the optimum temperature range, the fish's response to feeding will decrease (Nugraha *et al*., 2020). The gourami fish can still live and grow in the low oxygen content because of having tools and additional breathing that is the labyrinth (Jumaidi *et al.*, 2016). In this study, there was a decrease in DO starting from treatment P0 to treatment P3 along with an increase in water temperature. Temperature values are related to DO values in the aquarium water. The higher the temperature of the water, the lower the solubility of oxygen in water, and vice versa. The pH value will affect the growth of fish because the appetite of fish is reduced at low pH. A low pH value can cause clumping of mucus in the gills and the fish will suffocate so that the food consumed is used more as energy to maintain the body than for growth. (Nugraha *et al.*, 2020). In this study, the results of temperature, DO, and pH measurements for each treatment showed that was still at a safe standard for the life of gourami.

CONCLUSION

The results showed that adding ketepeng cina leaves (*Cassia alata* L.) ethanol extract can prevent *Argulus japonicus* infestation on gourami fish at the concentration 627 ppm.

ACKNOWLEDGMENT

The author expresses many thanks to the supervisors who have given a lot of knowledge and energy. The author also thanks the editors and reviewers for their suggestions and input on our manuscript.

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