Effect of Mengkudu’s (*Morinda citrifolia*) distillation with differential fruit ripeness to control *Argulus* on *Carassius auratus auratus*.

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Abstract. One parasite known to infect is *Argulus*. Noni (*Morinda citrifolia*) is one of the traditional medicinal plants that can be used as an alternative to control parasitic infections. Noni fruit has four levels of ripeness; each of them contains a different antiparasitic. This research aims to determine whether the noni fruit ripeness level differences provide different effects on the control of *Argulus* on *Carassius auratus auratus*. This research is experimental, using Completely Randomized Design. The data obtained in this research were analyzed using statistical test ANOVA (Analysis of Variance) to determine whether there are differences among the treatments, and then followed by Duncan's Multiple Range Test. The result showed value F count < F Table 0,01 and 0,05 which means there is no significant difference in treatment of giving noni juice with different ripeness levels. While Duncan's multiple range test showed that there is a significant difference between P0 (control treatment) and P3 (treatment using noni fruit ripeness level C). This means that noni juice has an impact on the release of *Argulus* on *Carassius auratus auratus* and the most effective treatment is by giving noni juice in the ripeness level C.

1. Introduction

Ornamental fish production in Indonesia, especially freshwater aquaculture, has become an important livelihood for some community and is an alternative to increase the export production of Indonesian ornamental fish [1]. In January 2011 the demand for comet carp in the Denpasar area has increased, which is usually only 2000 fish per month to 2,500 fish per month (Department of Fisheries and Maritime Affairs of the Province of Bali, 2011). Comet fish cultivation to meet consumer demand is often hampered by disease attacks, one of which is a disease caused by a parasitic attack [2]. One of the parasites that often attacks ornamental fish is Argulus. According to a report by [3], Argulus attacks 54-52% of koi carp (*Cyprinus carpio*) in the ornamental fish center of Blitar Regency, East Java.

Chemicals are often used to control argulus in which will cause side effects such as environmental pollution, parasitic resistance, changes in certain hematological parameters and organ contamination. Noni (*Morinda citrifolia*) is one of the traditional medicinal plants that can be used as an alternative to controle parasitic infestations. In [4] study, it was stated that noni juice with a certain dose can be used as a control for Argulus infestation, this is because the noni fruit has antibacterial, antifungal and
antiparasitic properties [5]. Noni contains scopoletin, a chemical compound that inhibits the action of smooth muscle and nerves. Scopoletin can also increase the activity of the peneal gland which can produce serotonin in the brain [6].

Serotonin is one of the ingredients contained in platelet granules and acts as a neurotransmitter nerve signaling agent in the brain and the precursor of the hormone melatonin, in addition, serotonin is a chemical compound that inhibits the work of smooth muscle and nerves so that it can result in vasoconstriction (narrowing of blood vessels) heart and brain membranes [7]. Vasoconstriction caused by serotonin can inhibit the nutritional intake of Argulus in the host. Noni also has alkaloid which is a group of organic compounds, can be highly toxic and some can be used as a treatment and as a vegetable insecticide [8] [9].

There are several opinions regarding on noni fruit as a medical treatment plant [10]. According to [11] the noni fruit which is still green is the most efficacious, while in the ASEAN standards of herbal medicine (1993), the young to old noni fruit can be used for traditional medicine. The amount of scopoletin content is different at each maturity level of noni fruit. The content of scopoletin on noni which is still green and has no seeds is 10.72 ± 0.45 ppm; Noni fruit is green and has seeds containing 19.19 ± 0.68 ppm of scopoletin; Noni fruit that has a white color and hard texture has a scopoletin content of 57.94 ± 0.79 ppm while the ripe noni fruit with white or brownish white rind and soft texture containing 14.11 ± 0.39 ppm scopoletin [10]. Based on the explanation above, this study was conducted to determine the effect of the level of maturity on noni fruit (Morinda citrifolia) for the control of the Argulus parasite in comet goldfish (Carassius auratus auratus).

2. Material and Methods

2.1. Place and time conducted
This research was carried out at the Educational Laboratory of the Faculty of Fisheries and Marine Airlangga University in April 2016.

2.2. Tools and material
The tools used in this research are 20 aquariums (15x15x25) cm³, 1 aquarium size (100x40 x40) cm³, 20 pieces of plastic cups, hose, aerator, aeration hose, aeration stone, measuring cup, filter, blender, 4 glass bottles, thermometers, analytical scales, rulers, label paper, books and stationery.

The materials used in this study were noni (Morinda citrifolia), 20 healthy comet carps (Carassius auratus auratus) with a total length of 8-10 cm (age ± 2 months), fish feed (pellets), 100 argulus, DO (dissolved oxygen) test, chlorine and pH paper.

2.3. Work procedure
The treatment aquarium is 15x15x25 cm³, filled with 4 liters of clean fresh water per aquarium, stocking one tail per aquarium. Comet goldfish are obtained from traders at the Gunungsari Fish Market with an average size of 8-10 cm, and the Argulus are taken from Blitar. Artificial infestation is carried out for 15 minutes. After Argulus sticks to the fish then the fish is put into a maintenance aquarium. Maintenance is carried out for 3 days. Treatment was given on day 3. Noni fruit was washed with running water, blended and squeezed. The juice of each noni fruit is collected in a clean glass bottle and labeled with paper according to the level of the noni fruit. The concentration of noni juiced used is 3.5% of the volume of water (100% noni concentration). The release of Argulus attached to the comet goldfish is the main parameter of the study. Supporting parameters include water quality including temperature, pH and dissolved oxygen.
2.4. Data analysis

The data obtained were analyzed using ANOVA statistical analysis (Analysis of Variance), then followed by Duncan's Multiple Range Test with a significance level of 5% to find out the best treatment [12].

3. Result and discussion

3.1. Percentage of infestation and Argulus release

Based on the ANOVA test results showed that the soaking of Noni fruit juice affected the Argulus infestation in comet goldfish (calculated F value <F table 0.01 and F table 0.05). The results of observations of the percentage of Argulus release can be observed in Table 1.

Table 1. Release Percentage of Argulus in comets after immersion using noni juice

| Treatment | Average ±SD(%) | Average ±SD Root Transformation (√y+0,5) |
|-----------|----------------|-----------------------------------------|
| P0        | 0b ± 0.00000   | 0,709 ± 0.00145                         |
| P1        | 20ab ± 0.40000 | 2,775 ± 4,13000                         |
| P2        | 30ab ± 1.88326 | 4,847 ± 3,06408                         |
| P3        | 50a ± 0.34641  | 6,315 ±3,77784                         |
| P4        | 15ab ± 0.19149 | 3,077 ± 2,83400                         |

Note: a, b Different superscripts in the same column show very significant differences (p <0.05). PO = control treatment; P1 = treatment of fruit A maturity level; P2 = treatment of fruit B maturity level; P3 = treatment of fruit maturity level; P4 = treatment of fruit maturity level D.

Data analysis using ANOVA test showed, the calculated F value < F table 0.01 and F table 0.05, this means that there are no significant differences in the treatment of noni juice with different levels of maturity while the follow-up test using Duncan's multiple range test showed the results contained significant difference between P0 (control treatment) and P3 (treatment using noni fruit maturity level C).

Argulus control using the soaking of *Morinda citrifolia* is carried out based on the results of [4] which states that the Noni juice extract can be used as Argulus control with the most effective dose of 3.5%. [4] used the Noni juice extract at one level of fruit maturity. In this study, control of Argulus uses noni juice with different levels of fruit maturity. The results showed that the immersion using noni juice with different levels of maturity had an effect on the degree of release of Argulus from the comet fish (*Carassius auratus auratus*) but the average release of Argulus for each treatment showed results that were not significantly different, even directly (directly Table 1) can be seen that the third treatment (P3) has the highest percentage of Argulus release compared to other treatments.

A non-significant statistical result is thought to be caused by several things. One of which is the amount of antiparasitic compounds in noni at each level of maturity has a value that is not much different. According to the research of [13] states that noni fruit at each maturity level has a different amount of scopoletin content but does not show a significant difference because scopoletin at each level of noni fruit has almost the same amount. The tendency of the percentage of argulus release at each fruit maturity level can be seen in this study, as in treatment P1 (fruit maturity level A) with the characteristic of green fruit and no seeds. The release of Argulus only occurs in the second test with a percentage of 80%. This means that four Argulus are separated from the fish comet after immersion using noni juice maturity level A for 15 minutes with a dose of 3.5% so that the treatment P1 only has an average release of Argulus of 20.04% of the number of four replications.
The release of Argulus in the P1 treatment only occurred in the second replication test, while the first, third and fourth tests did not show the release of Argulus. This could be due to the small antiparasitic content such as alkaloids and scopoletin in noni fruit with maturity level A. Whereas the P2 treatment gave an increasing result of argulus release compared to P1 treatment although the average release of Argulus in this treatment was only 30.01%. There were three replications of treatment which caused the release of Argulus: on the second test as much as 60% (three Argulus off), the third test was 20% (one loose Argulus) and 40% fourth replication (two loose Argulus tails). The first test of P2 treatment did not lead to the release of Argulus from comet fish. This P2 treatment uses the Noni juice extraction level of maturity B with green fruit skin characteristics and has seeds in the fruit.

The next treatment is P3, this treatment uses the Noni juice level of C maturity with white or yellowish-white fruit characteristics and hard fruit flesh texture. In this P3 treatment the average loose Argulus is the highest value which is 50%. The last treatment is P4, soaking comet infested with Argulus using Noni juice maturation level D with the characteristics of white or brownish white fruit and the texture of aqueous soft fruit as well as releasing the distinctive odor of noni. This treatment produces the lowest percentage of Argulus release compared to treatments P1, P2 and P3. Argulus in P4 treatment is only released by 15.03%. The small percentage of loose Argulus is caused by the content of scopoletin in noni fruit will decrease in ripe noni fruit [10].

P3 treatment has the highest argulus release from fish, so that it can be said that the most effective control. This treatment uses Noni fruit juice with a level of maturity C that has white or yellowish-white fruit characteristics and hard fruit flesh texture. This is consistent with the study of [10] which states that the levels of scopoletin on noni fruit increases with align with age or the maturity of noni fruit and reaches its peak at the third maturity level (hard fruit flesh, yellowish yellowish fruit). The high percentage of argulus release in P3 treatment can be caused by the high content of scopoletin in fruits with a maturity level of C when referring to research by [10].

Scopoletin itself is a chemical compound found in noni fruit (Morinda citrifolia) which functions to inhibit the work of smooth muscles and nerves, in this case it inhibits the nerve work of Argulus and comet fish. But to avoid fish death, it is determined that the optimal soaking time for the release of Argulus from comet fish is 15 minutes, because immersion exceeding 15 minutes will cause the death of comet fish [4]. Scoopoletin can also increase the activity of the peneal gland which can produce serotonin in the brain [6]. Serotonin is one of the ingredients contained in platelet granules and acts as a neurotransmitter / nerve signaling in the brain and the hormone melatonin precursor. In addition, serotonin is a chemical compound that inhibits nerve work so that it can cause vasoconstriction (narrowing of blood vessels) of the heart and brain membranes [7]. Vasoconstriction caused by serotonin can inhibit the nutritional intake of Argulus in the host so that Argulus will release its grip on the host. Whereas in comet fish, side effects of the process of inhibiting the work of smooth muscles and nerves can be seen from the behavior of fish during the treatment.

3.2. Observation of fish behavior
Observation of fish behavior is important especially to see changes in fish responses due to the changing of environments. The results of observations of the fish's behavior are before the fish is infested with Argulus, before the treatment / after the fish is infested with Argulus, during the treatment, and after the treatment. Before Argulus infestation, the fish moved actively and normally, then showed signs of irritation and moved quite aggressively after being infested with Argulus. This is caused by Argulus which has begun to stick to the body surface and fish fins causing itching on the surface of the body and fins, causing changes in the behavior of the fish from initially moving normally to moving quite aggressively such as turning the body, rubbing the body against the plastic glass wall where artificial infestation done and sometimes trying to jump out of the surface of the water. In accordance with the opinion of [14] which states that fish infested with Argulus sp. will become restless, glide to and fro or sometimes jump off the surface of the water and rub its body and severe attacks can cause the fish to become lazy / more silent, lose appetite, and change color due to excessive mucus production. Same
with [14], [15] also stated that fish that were attacked by Argulus experienced abnormal behavior, including irregular swimming fish and rubbing their bodies against the aquarium wall. Observation of fish behavior during the treatment showed a physiological response to changes in the water environment. P0 treatment, which is a control treatment, is a treatment without noni juice given so that the response of fish behavior is not much different from before the treatment took place. In the treatment of P1, the fish begin to have difficulty breathing and tend to be silent, as well as in the P2 treatment although at the beginning of the treatment the fish move aggressively for a while but then tend to be quiet and occasionally return aggressive. Difficulty in breathing experienced by fish can be caused by the content of alkaloids found in noni fruit, as [8] explains that the influence of toxins caused by alkaloids mainly attacks the nervous system and breathing.

The behavior of the fish in the P3 treatment showed symptoms of aggressive movements at the beginning. In the next treatment the fish tended to be silent, with faster opening and closing of the operculum and more feces. Symptoms of this behavior change is the physiological response of fish to environmental changes or new media given the treatment of noni juice. [16] explains that in a limited environment, fish will seek balance if stressors start coming. Subsequent reactions have an effect on fish movements that are starting to increase. Whereas the behavior of the P4 treatment fish was the same as the P1 treatment, the fish tended to be weak and had difficulty breathing. The response of fish behavior varies depending on the sensitivity and endurance of fish [17].

Noni (Morinda citrifolia) contains antiparasitic substances. In addition to scopoletin, non-alkaloid in noni also acts as an antiparasitic substance which triggers the release of Argulus from comet fish. Alkaloids are a group of organic compounds that can be highly toxic and some can be used as a treatment and as a vegetable insecticide [8] [9]. [18] also states that alkaloids are nerve poisons. [19] added, the role of this nerve poison is by inhibiting the enzyme cholinesterase, so that there will be disruption of excitatory transmission which causes decreased muscle coordination and death for Aedes aegypty larvae. The enzyme acetylcholinesterase plays a role in controlling neurotransmitters produced in axons near the synaptic cleft. Acetylcholine continues impulses in the synaptic cleft. After the impulse is passed on acetylcholine, the enzyme cholinesterase is hydrolyzed into choline, in the absence of the cholinesterase enzyme, the accumulation of acetylcholine results in impaired impulse transmission, which causes decreased nerve coordination, muscles, convulsions and can even cause death of insect larvae [20].

Different levels of fruit maturity have different contents. [13] mentions that the content of scopoletin in noni fruit increases with increasing level of maturity. This means that the more mature the fruit, it has a higher scopoletin content. In this study, the maturity level of C fruit with white or yellowish-white fruit characteristics and hard fruit flesh texture is the most effective level of maturity to cause the release of Argulus in comet fish when compared with other levels of maturity. This is consistent with the opinion of [10] which states that the level of maturity of C with the same fruit characteristics has the highest content of scopoletin.

3.3 Water quality

Observations on water quality were carried out during comet fish maintenance (3 days) and showed a normal range for comet fish. During the study, the water temperature of comet fish maintenance ranged from 29.5 to 32 °C, the degree of acidity (pH) between 6-7 and dissolved oxygen between 4-8 mg / L. This range of water quality values does not significantly affect the research parameters, namely the release of Argulus because the immersion treatment using noni juice is carried out in a plastic cup of treatment with the same temperature range (the water used is sourced from the same reservoir).

Temperature is the main regulator in various natural processes in the water environment [22]. [21] mentions the optimal temperature for the maintenance of comet fish between 19°-28°C. The temperature range during fish rearing in this study is around 29.5-32 °C and comet fish can still survive in that temperature. Comet can be maintained with a pH range of 7-7.5 (Nugroho, 2008). The pH value during this study was between 7-8. This value is still in the pH range that is suitable for the life of a comet goldfish [4]. Dissolved oxygen measurement results in this study have a value of 4 mg / L - 8 mg / L. This value is a good dissolved oxygen ratio for fish survival, while the minimum dissolved oxygen


content (DO) is 2 mg / L under normal conditions and not contaminated by toxic / toxic compounds [23]. Ideally, dissolved oxygen content should not be less than 1.7 mg / L.

4. Conclusion
Mengkudu’s (Morinda citrifolia) at all levels of maturity can be used to control Argulus infestation in comet carp (Carassius auratus auratus). Fish farmers or the wider community can use noni juice at each level of fruit maturity based on this study in controlling Argulus in comet goldfish (Carassius auratus auratus) but the authors also suggest it is better to use noni juice with maturity level C to see the tendency of the release of Argulus more when compared to other level of maturity. Further research needs to be done on the source of antiparasitic substances in the more specific parts of the noni fruit (in the seeds or flesh of the fruit).

5. References

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